Rheumatic Heart Disease in Egypt

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Abstract

Rheumatic Heart Disease remains one of the most neglected cardiac conditions in children and young adults around the world. The pathogenesis is complex and remains elusive, and the clinical characteristics vary around the world. This thesis concentrates on different aspects of the disease in Egypt, where it is known to have a high incidence.

The methodology included epidemiological studies in school children in Aswan and investigation of RHD in a population with history of RF, using newly developed echocardiographic criteria. Concomitantly, the pattern of immune response in RF and RHD was determined in serum and excised valves.

In this series RF presents in children and young adults, as well as adults, (0.2-44 years, 10.69 ± 6.24) with polyarthritis being the most common clinical presentation (87.9%) and recurrences of RF being very common (98.2%). RHD affected 23 in 1000 school children in Aswan with over 90% of the cases being subclinical and developed in up to 69.2% of the individuals with history of RF, predominantly as mitral regurgitation. Risk factors for the development and severity of RHD were shown to be low disease awareness, non-compliance to penicillin prophylaxis or a regimen of longer than 15-days. Resistance to antibiotic regimens, including Penicillin and Vancomicin seems to lead to development and recurrences of RF in Egypt. This series showed the presence of immune activation and ongoing immunological reaction in an apparently quiescent phase of the disease with distortion of normal valvular architecture, histology and composition.

This work has served to define the epidemiology, pattern of disease, immune reponse and predisposing factors in a population with no previous data, also contributing to the improvement of the echocardiographic diagnostic criteria. Standardization of the criteria will allow comparison of prevalence in different areas and improve case detection.

“*We don't believe in Rheumatic Fever or true love until the first attack...*”

(Adapted from: Marie Von Ebner-Eschenbach (Austrian novelist, 1830-1916))

This work is dedicated to my beloved father and mother,

Mohamed and Maria do Céu Kotit



Forgive me

Forgive me for all missed occasions
Holidays, birthdays, anniversaries
Forgive me for not being there
Every moment, every second, every day

Forgive me for not sharing your joy and happiness
And for not always showing how much I care

Forgive me for not drying your tears
Or talk you out of your fears
Forgive me for not holding your hand
When you most needed

Forgive me for all missed kisses
And the phone good night wishes
But only my pillow knows
How much my heart misses

You're always in my heart
I'll carry you with me all my life
You are my safe port
My shining star
My home
You are everything and more

Susy Natália

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Abbreviations

RF: Rheumatic Fever

RHD: Rheumatic Heart Disease

CBC: complete blood cell count

CRP: C-reactive protein

ESR: Erythrocyte sedimentation rate

ASO: antistreptolysin O

ASOT: antistreptolysin O titer

ECG: Electrocardiogram

WHO: World Health Organization

GAS: Group A Streptococcus

GCS: Group C Streptococcus

GGS: Group G Streptococcus

HLA: Human leukocyte antigen

Th: T-helper cell

MHC: Major histocompatibility complex

GAGs: Glycosaminoglycans

IL: Interleukin

TNF-α: tumor necrosis factor-α

IFN-γ: Interferon

APCs: antigen-presenting cells

PMNs: polymorphonuclear leukocytes

AFM: Atomic force microscopy

FIB: focused ion beam

PARF: peptide associated with rheumatic fever

CHAPTER 1

INTRODUCTION

1.1 Background

Summary

Rheumatic heart disease (RHD) is estimated to affect around 15,6 to 19,6 million people worldwide, of whom 2,4 million are children (Carapetis 1999, 2004, 2005[1]). The disease is responsible for 25–50% of cardiac conditions in children and young adults in some areas (Woo 1983, Markowitz 1981). However, the true disease burden is certainly substantially higher and the true prevalence of RHD has been underestimated, owing to the almost total lack of echocardiographic screening for this disease.

RHD starts with a pharyngeal infection, caused by group A (GAS), group C (GCS) or G (GGS) streptococci leading to Rheumatic Fever (RF), which presents in a variety of ways after a latent period of two to three weeks following the initial pharyngitis. Importantly, the geographical variety in types of streptococcal strains and their rheumatogenicity has not been clarified till date.

Carditis occurs in up to 80% of patients with RF, and at least 60% of untreated patients develop chronic progressive and permanent valvular damage and dysfunction known as RHD (Steer 2009[2]) affecting predominantly the mitral valve (Carapetis 2008, Ramakrishnan 2009, Reddy 2004) with appearent difference in the exact type of valve affection in different parts of the world. Subclinical RHD shows a prevalence of 90%, for which echocardiography is a successful screening tool (Marijon 2007). However, there has been debate concerning the echocardiographic signs of early RHD and the diagnostic criteria to be used.

The pathogenesis of RHD is complex and involves both microbial and host factors that predispose and influence the development of the clinical features by triggering the autoimmune humoral and cellular response that causes inflammation leading to the establishment of chronic RHD. A major role is attributed to inflammatory cytokines in mediating heart lesions (Guilherme 2009) as streptococcal throat infection triggers an inflammatory reaction that involves several proinflammatory cytokines which can be detected in peripheral blood as well as valvular tissue of RF and RHD patients (Guilherme 2009). Nevertheless, RHD valves also show signs of cellular infiltration in combination with specific structural and biochemical features (Sampaio 2007).

RHD causes high morbidity and mortality and remains a major public health problem among most of the world's population. However, RHD remains being badly neglected, with very few important initiatives and little research and many gaps in our knowledge of its pathogenesis and mechanisms. In Egypt, a high percentage of schoolchildren have clinical evidence of cardiac valvular damage due to RHD and the course is found to be severe and aggressive (Kassem 1982, Abdin 1968, El Sherif 1975), nevertheless, the prevalence and determinants which seem to differ from other areas are still unknown while data remains lacking.

1.2 Rheumatic Fever

The worldwide incidence is estimated to reach up to 700 cases per 100,000 among children and young adults between ages 5 and 20 leading to around 10 to 20 million new cases every year (Carapetis 2005[1], Carapetis 2005[2], Tibazarwa 2008, Carapetis 2000, Soto Lopez 2001) in certain parts of the world of whom 60% to 90% will develop RHD (Ganguly 2004, Carapetis 2005[1], Carapetis 2005[2]). Importantly, the epidemiology of RF varies between countries and regions (Bisno 1997, Dinkla 2003), and there is great concern about the high prevalence of streptococcal infection and RF (WHO 1986, Steer 2009) which remains endemic in different areas. The decline of the incidence of RF in industrialized countries over the past four decades has been attributed to more hygienic and less crowded living conditions, improvement of access to medical care and introduction of antibiotics. However, focal outbreaks of RF have been reported, often in combination with the reappearance of certain rheumatogenic serotypes (Veasy 1987). The acute illness causes considerable morbidity and mortality, however, the major clinical and public health effects derive from RHD (Olivier 2000).

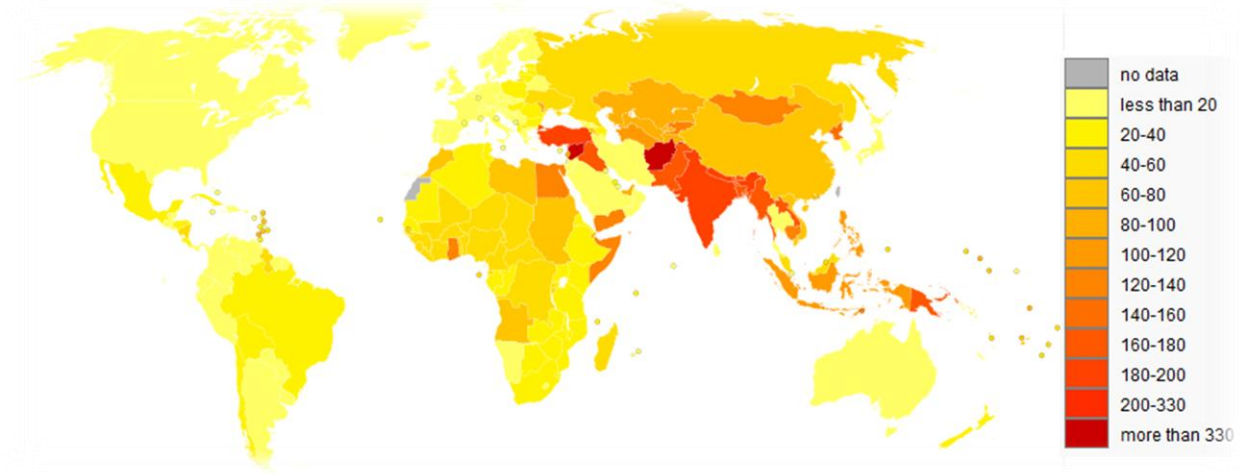


Figure 1.1 Age-standardised disability-adjusted life year (DALY) rates from RHD by country (per 100,000 inhabitants)

1.2.1 Presentation

The first symptoms appear after a latent period of two to three weeks following the initial pharyngitis, presenting in a variety of ways, including arthritis, carditis, chorea, subcutaneous nodules, and erythema marginatum. Polyarthritis is the earliest as well as the most common manifestation of RF, present in 60% to 80% of patients (Guilherme 2009) starting characteristically in the joints of the lower extremities early in the rheumatic attack when streptococcal antibodies are at peak elevation (Ilia 1992).

Carditis, the most serious complication, occurs a few weeks after the infection in 40% to 80% of patients with RF and leads to chronic damage to the heart valves, known as Rheumatic Heart Disease (RHD), characterized by progressive and permanent valvular lesions (Guilherme 2009, Sanyal 1982, Sanyal 1974, Steer 2009[2], Bland 1951).

Sydenham chorea occurs in 10 to 30% of patients with RF. Unlike arthritis or carditis, symptoms of chorea present late, usually months after the initial pharyngitis and the process is self-limiting and reversible (Jones Criteria 1992). Erythema marginatum, the characteristic rash, also known as erythema annulare, occurs in 5 to 13% patients (Massell 1958). Subcutaneous nodules are currently an infrequent manifestation of RF with a small frequency of 0 to 8% (Massell 1958) varying in different series and countries (Behera 1994).

History of an antecedent sore throat 1-5 weeks prior to onset of RF is present in 70% of older children and young adults. However, only 20% of the younger children can recall an antecedent sore throat. Although individuals of any age group may be affected, most cases are reported in persons aged 5 to 15 years (Steer 2009) as streptococcal pharyngitis is mainly found in this age group (Bassili 2000, Dajani 1992, Bharani 2010). RF is therefore principally a disease of childhood, with a median age of 10 years, being rare in children younger than 3 or above 18 years (Carapetis 2005[1]). While pre-school children may frequently acquire stretococcal infections, overt disease is usually mild or absent although outbreaks in child care settings have been reported (Majeed 1986). The low incidence of less than 6 percent of streptococcal throat infection in children below 2 years of age may be the result of the low adherence rate of streptococci to buccal mucosal epithelia during the first years of life (Guggenbichler 1994). Streptococcal pharyngitis remains an uncommon cause of pharyngitis in pre-school children. However, approximately 40 percent of streptococcal infections are believed to occur in children

2 to 6 years of age (Markowitz 1963) and it is not clear why RF is uncommon in children below 5 years and rare in children younger than 3 years (Guggenbichler 1994, Rosenthal 1968). Furthermore, although less commonly seen in adults compared with children, rheumatic fever in adults accounts for 20% of cases.

Rheumatic fever occurs equally in males and females, however, with a worse prognosis for females (Sanyal 1982, Cole 1976, Longo-Mbenza 1998, Mahmudi 2006). Patients with previous RF are at risk of recurrence, which is the highest within 5 years and younger age at the time of the initial episode. Generally, the recurrent attacks are similar to the initial attack, however, risk of carditis and severity of valve damage increase with each attack (Sanyal 1982).

1.2.2 Diagnosis

The main clinical features of RF are outlined in the Jones Criteria (Stollerman 2001), which are constantly updated (Stollerman 1965, Stollerman 2001, Jones Criteria 1992) (Table 1.1). The diagnosis is made upon the presence of two major, or one major and two minor manifestations, plus evidence of previous GAS infection. Chorea and indolent carditis do not require evidence of previous GAS infection and recurrent episode requires only one major or several minor manifestations, plus evidence of previous GAS infection. The minor criteria lack diagnostic specificity than the major criteria and arthralgia without objective findings is common in RF. The pain usually involves large joints, maybe mild or incapacitating, and may be present for days to weeks, often varying in severity (Agarwal 1986, Nair 1990).

Although, every revision increases the specificity but decreases the sensitivity of the criteria, in response to the steadily declining incidence of RF in developed countries (WHO 2004). Furthermore, the diagnosis of a recurrent RF attack can be less obvious than that of a first episode (Jones Criteria (1992 update)), therefore the Jones Criteria is applied more readily to initial attacks (Stollerman 2001).

Table 1.1 Guidelines for the diagnosis of rheumatic fever (Jones Criteria, 1992 update)

Major manifestations
-Carditis
-Polyarthritis
-Chorea
-Erythema marginatum
-Subcutaneous nodules
Minor manifestations
Clinical findings:
-arthralgia
-Fever
Laboratory findings:
-Raised Erythrocyte sedimentation rate or C-reactive protein concentrations
-prolonged PR interval on electrocardiogram
Supporting evidence of antecedent GAS infection:
-Positive throat culture or rapid antigen test for GAS
-Rased or rising streptococcal antibody titer

*****Criteria**:** Two major, or one major and two minor manifestations must be present, plus evidence of previous GAS infection. Chorea and indolent carditis do not require evidence of previous GAS infection. Recurrent episode requires only one major or several minor manifestations, plus evidence of previous GAS infection.

Alternatively, the 2002-03 WHO criteria (Table 1.2) (WHO 2004) specify less stringent requirements for the diagnosis of recurrent RF in patients with established RHD and are thought to be more sensitive than the Jones criteria (Olivier 2000, WHO 2004).

Table 1.2 WHO Criteria (2002-03) (WHO 2004)

First episode
-as per Jones criteria (Guidelines for the diagnosis of rheumatic fever. Jones Criteria, 1992 update)
Recurrent episode
-In a patient without established RHD: as per first episode
-In a patient with established RHD: requires two minor manifestations, plus evidence of antecedent GAS infection. Evidence of antecedent GAS infection as per Jones Criteria, but with addition of recent scarlet fever.

*Chorea and indolent carditis do not require evidence of antecedent GAS infection

1.2.3 Diagnostic tools

There are different additive diagnostic tools for the detection of streptococcal infection and the diagnosis of RF.

Streptococcal isolation

Streptococci can be isolated by culture of throat swab, but they are only found in 15-20% of patients. This may be due to both the latency period between infection and the onset of RF symptoms and the prior use of antibiotic. Nevertheless, non-invasive carriage of group A streptococcus contributes to the low sensitivity of throat culture in diagnosing a preceding infection and rapid antigen detection tests from throat swabs have the same limitations as cultures with specificity of 95% but lower sensitivity.

Laboratory tests

Laboratory tests cannot independently determine the diagnosis of RF, although some provide supporting evidence of illness or are used to follow the progress of treatment. Different infection parameters are used to confirm the suspicion of RF differentiating between general infection parameters and streptococcal specific parameters.

The initial routine laboratory assessment should consist of a complete blood cell count (CBC), including a white blood cell and differential count, and the determination of acute phase indicators such as ESR and CRP, the major inflammatory parameters. These acute phase reactants are plasma proteins that increase during acute phase of inflammation. They are produced by the liver under regulation of circulating cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), released by monocytes and macrophages. Importantly, both ESR and CRP are affected by anti-inflammatory medications (Lane 2002, Wagner-Weiner 2002).

Evidence of preceding streptococcal infection is set with the presence of increased antistreptolysin O (ASO), the antibody produced against an antigen produced by streptococci or other streptococcal antibodies as anti-DNAse B or antihyaluronidase, positive rapid direct group A strep carbohydrate antigen test or recent scarlet fever (Stollerman 2001).

Complete blood count (CBC)

A complete blood cell count (CBC) includes a white blood cell and differential count. Leukocytosis with neutrophilia and mild to moderate anaemia are found in RF. Haemologlobin levels below 90g/l are usually associated with severe carditis. Lymhocytosis and severe anaemia suggest a differential diagnosis that includes leukemia and sickle cell anaemia.

Erythrocyte sedimentation rate (ESR)

Acute phase reactants are always elevated at the onset of acute RF. ESR is one of the major monitoring tests for acute phase inflammation because it correlates with increased levels of acute phase reactants, in particular, fibrinogen, which is one of the acute phase reactants produced by the liver in response to IL-6, IL-1, and TNF. About 60 to 70% of ESR depends on fibrinogen because of its neutralizing effect on erythrocyte syalic acid, which inhibits red blood cell aggregation (Kowalczyk 2006, Sox 1986, Paulus 2004).

The erythrocyte sedimentation rate (ESR) is elevated in the first weeks of the disease, and higher levels are found among patients with cardiac involvement.

C-reactive protein (CRP)

CRP is an acute-phase protein which recognizes pathogens and mediates the complement system and phagocytic cells. It activates neutrophils, monocytes, and platelets, upregulates adhesion molecules, and appears to play a role in the clearance of apoptotic and necrotic host cells. These latter properties of CRP have been suspected to contribute to inflammation and autoimmune diseases. CRP is a member of the pentraxin family of proteins, made in the liver under the control of cytokines such as IL-6, IL-1, and TNF-α (Volanakis 2001, Du Clos 2004, Szalai 2004).

CRP usually appears in the sera of patients in the acute stages of a number of inflammatory conditions such as most bacterial as for example acute RF with or without carditis. CRP is considered to be a sensitive indicator of inflammation. Changes in the serum level of CRP with time from the same patient can be used as an index of recovery. The use of the CRP test to measure the effectiveness of therapy is of great clinical significance in cases such as acute

rheumatic fever. C-reactive protein (CRP) is elevated at the onset of the acute phase and tends to disappear at the end of the second or third week (Yeh 2004).

Anti-streptolisin O (ASO)

The ASO titer (ASOT) demonstrates the body's reaction to an infection caused by streptococci which produces the enzyme streptolysin O, which can lyse red blood cells. Because streptolysin O is antigenic containing a protein foreign to the body, the body reacts by producing antistreptolysin O (ASO), which is a neutralizing antibody. Serial ASO testing is often performed and recommended at 15-day intervals to determine the difference between an acute or convalescent blood sample. The normal values are 160 Todd units/ml for adults and 170-330 Todd units/ml for children from 5 to 12 years. The sensitivity of ASO titer (adults with >240 Todd U and children with >320 Todd U) is 80%. Streptococcal antibodies are the most useful infection parameters because they reach a peak titer at about the time of onset of RF, they indicate true infection rather than transient carriage and any significant recent streptococcal infection may be detected by making several tests of different antibodies. Approximately 20% of patients with RF may not have this antibody. In these cases, determination of anti-hyaluronidase, ant-deaoxyribonuclease B (Anti-Dnase B) and/or anti-streptokinase antibodies may be essential for the diagnosis of recent infection. Negative infection parameters should be followed by a throat culture (Bisno 1997, Hahn 2005, Roodpeyma 2005).

Chest Radiograph and Electrocardiogram (ECG)

The chest radiograph and ECG may be abnormal in only 30% of patients with carditis. The chest radiograph usually shows cardiomegally only in patients with myocarditis or moderate to severe pericardial effusion.

Electrocardiograms may reflect the effect of the inflammatory process on the conduction system and repolarization abnormalities characterized by prolonged PR and QT intervals can be observed. The most characteristic feature in acute RF is conduction disturbances most commonly in the form of 1st degree heart block (a prolonged PR interval) which occurs in 24- 40%. Repeat tracings are recommended to demonstrate a variation in atrioventricular conduction which is more valuable. The PR interval usually returns to normal after the disease becomes inactive and

it can occur with or without carditis. In acute rheumatic pericarditis, ST elevation or inversion is present. Low-voltage QRS complexes and abnormalities of the ST interval may be seen with pericarditis (WHO 2004).

Echocardiography

Echocardiography improves the diagnosis and is successfully used for the detection of carditis without overt evidence of valvular or other cardiac involvement (Marijon 2007, Carapetis 2008). Therefore, ultrasound should be considered as the primary diagnostic modality for RHD survey in children and should be used to diagnose subclinical acute rheumatic carditis and silent indolent rheumatic carditis (Olivier 2000, WHO 2004, Carapetis 2005[2]). The echo criteria is an important issue for consensus definition, and there is an increased interest in new and comprehensive criteria to enforce the diagnostic criteria for RHD.

1.3 Rheumatic Heart Disease

It is believed that there are 15,6 to 19,6 million people affected with RHD worldwide, of which 2,4 million children aged between 5 to 14 years (Carapetis 2005[1], Carapetis 2004). Importantly, estimates suggest that over 6 million of the 10 to 20 million individuals who acquire RF every year go on to develop RHD with most deaths occurring in childhood and early adulthood (Carapetis 1999, Carapetis 2005[1]). RF and RHD are therefore one of the major and leading causes of cardiovascular disease as they are responsible for 25 to 50% of cardiac conditions in children and young adults in endemic areas (Dale 1985, Woo 1983, Markowitz 1981). However, the true disease burden is likely to be substantially higher as these estimates are based on conservative assumptions, and the overall quality of epidemiological data from developing countries is poor particularly with respect to epidemiologic studies and the lack of screening (Carapetis 2004).

School children

Echocardiography has resulted in prevalence rates of 62 in 1,000 in Kenya (Anabwani 1996), 48 in 1,000 in Nicaragua (Paar 2010) and 30.4 in 1,000 in Mozambique (Marijon 2007) providing an average point prevalence of 40 in 1,000 in school-aged children (Paar 2010). Importantly, this

prevalence increases significantly with age, peaking up to 42.6 per 1,000 in children aged 10–12 years (Carapetis 2008). These results confirm that the prevalence of RHD in school-aged children is far greater than earlier estimates and extend the observations to show a high prevalence of subclinical disease in young adults who may be at great risk for developing chronic progressive RHD. Importantly, on the basis of the few studies that have estimated age-specific prevalence in a broad range of ages (Carapetis 2000, Agarwal 1995, Aung 1992, Brown 2003), it has been previously calculated that the number of RHD cases in children aged 5-14 years should be increased fivefold to sevenfold in order to reflect the true number of prevalent cases in the general population (Carapetis 2005[1]) as the number of cases of RHD in school-aged children represents less than 20% of the cases in the whole population (Steer 2009).

In Egypt, a high percentage of schoolchildren have clinical evidence of cardiac valvular damage due to RHD and the course is found to be severe and aggressive (Kassem 1982, Abdin 1968, El Sherif 1975), nevertheless, the true prevalence and determinants are still unknown and while data from several other places is available, current accurate data from Egypt is lacking. However, a prevalence of 5.1 per 1000 schoolchildren has been previously estimated in the early 1990's (Alwan 1993, WHO 1992).

1.3.1 Clinical presentation

RHD is usually the result of cumulative cardiac valve damage from recurrent or persistent episodes of RF, which are sometimes asymptomatic but leading to significant rheumatic valve disease in later life after just one carditis episode, as confirmed by echocardiography. Subclinical carditis or valvular affection, without clinical murmurs but documented functional changes is relatively common in RF (Tubridy-Clark 2007, Figueroa 2001) and approximately 90% of cases of RHD are clinically silent, occurring in asymptomatic individuals without audible murmurs (Marijon 2007). Therefore, many people with RHD do not have a known history of RF and are unaware of their disease until it is detected incidentally during a medical examination, or until their disease worsens and they develop symptoms of cardiac failure (Carapetis 2005[1]).

Cardiac involvement is often established by finding of a new murmur of mitral or aortic insufficiency. Mitral regurgitation, heard best at the apex, is generally of moderate-to-high

intensity throughout systole. Aortic insufficiency is a basal diastolic murmur that is usually high-pitched and blowing and decreases in intensity toward the end of diastole.

RHD predominantly affects the mitral valve and, less commonly, the aortic valve. Mitral valve incompetence is the most common valvular lesion with a prevalence ranging up to 94% (Carapetis 2008, Ramakrishnan 2009), particularly in the early stages of the disease. Importantly, patients with mitral incompetence can remain relatively asymptomatic for up to 10 years, as a result of compensatory left atrial and left ventricular dilatation before the onset of left ventricular systolic dysfunction. Mitral stenosis is believed to develop later as a result of persistent or recurrent valvulitis, although rapid progression to mitral stenosis has been described in some areas. Tricuspid regurgitation can occur as a result of volume overload, usually caused by mitral stenosis (Jaffe 1988, Vasan 1996, Carapetis 2000).

Similar prevalence of subclinical carditis and permanent RHD features suggests that acute valve lesions induced by subclinical rheumatic carditis persist to about the same extent as the lesions in clinically evident acute RHD (Marijon 2007). Importantly, progression of the rheumatic valvular lesions to chronic subclinical valvular disease is around 82% (Meira 2005) and the prevalence of persistence or deterioration of subclinical carditis is around 60% after five years of follow up in spite of continuous penicillin prophylaxis and no evidence of recurrent disease (Figeroa 2001). However, the long-term outcome of patients with subclinical carditis and RHD has not been established, and therefore, the persistence and development of permanent valvular lesions is not known.

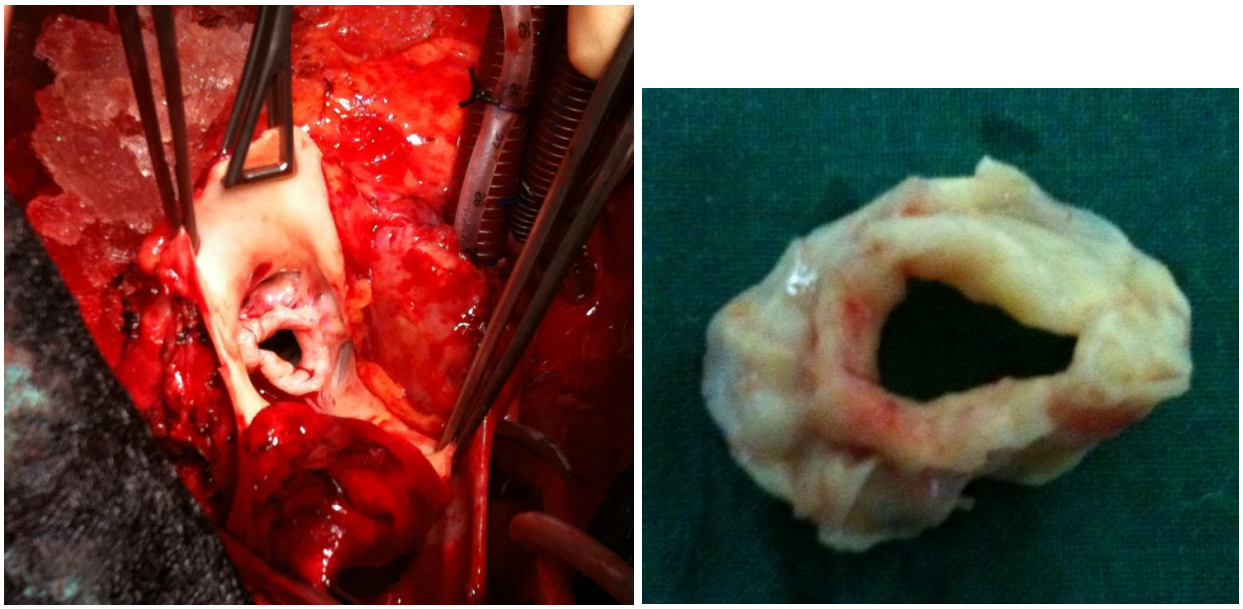


Figure 1.2 Thickened, fibrosed and fused leaflets of the aortic valve, leading to regurgitation

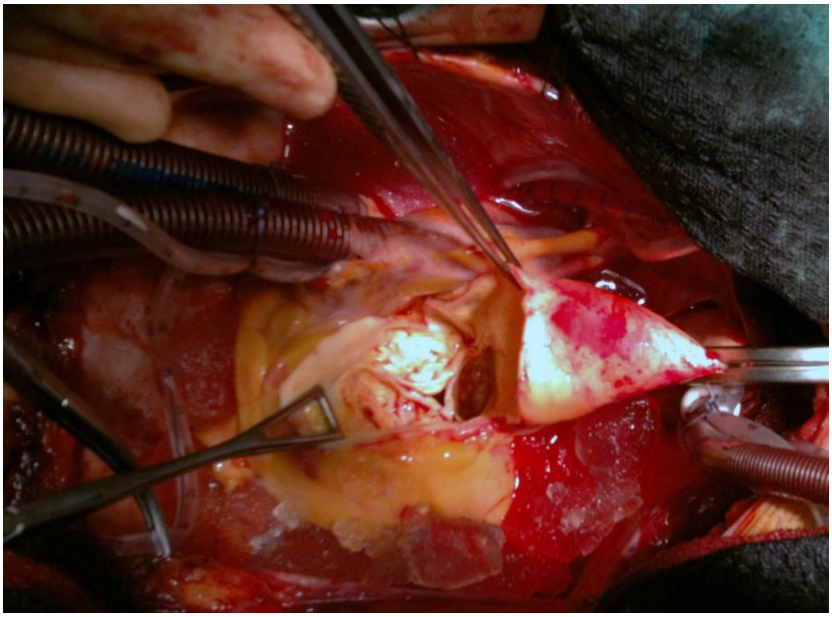


Figure 1.3 Calcific aortic valve

1.3.2 Risk factors

Variables that seem to correlate with severity of valve disease include the number of previous attacks of rheumatic fever, the length of time between the onset of disease and start of therapy, and gender. RHD has been reported to be more severe in females than in males as isolated mitral stenosis is more common in girls than in boys with a ratio of 1:2 (Cole 1976, Longo-Mbenza 1998) and carditis, valvulitis, and cardiomegaly are significantly more common in female patients (Mahmudi 2006).

Repeated episodes of RF are believed to lead to further inflammatory damage and subsequent scarring of cardiac valves (Veasy 1997, Majeed 1992). Mitral and aortic regurgitation are the most common events caused by repeated valvulitis (Guilherme 2009) while recurrence of RF leads to worsening of rheumatic valve lesions.

Meanwhile, reversibility has been reported and insufficiency from acute rheumatic valve disease has been shown to resolve in 60-80% of patients who adhere to antibiotic prophylaxis (Chin 2010). However, other, additional influencing factors have not yet been reported.

1.3.3 Prevention

The administration of penicillin has been the most extensively strategy used to prevent and treat RF (Markowitz 1993, Bisno 1997, Macris 1998, Gibofsky 1993, Bisno 2004, Bisno 2002), but the currently available methods for prevention of streptococcal infections and its sequelae are still insufficient and inadequate (Bisno 2005, Bisno 1997, Carapetis 2005[2], Jones Criteria 1992).

Primary prevention

Primary prevention of RF with prompt diagnosis and focus on antibiotic treatment of acute streptococcal pharyngitis (Tandon 2004, Bisno 2002) is beneficial in individual cases but has not been effective in controlling the disease, although the organism remains uniformly susceptible to penicillin (Bisno 2005, Bisno 1997, Betriu 1993). Therefore, penicillin is considered mandatory for the eradication of streptococcal infection of the upper respiratory tract (Bisno 2002) though it does not alter the cardiac outcome (Carapetis 2005[2], Meira 1993, Lennon 2000, Bisno 2002). However, patients receiving penicillin show a slight faster clinical improvement, and a modest

reduction in symptom duration with antibiotic therapy, with more rapid decrease in fever and symptoms.

A 10-day oral penicillin V regimen is required to prevent RF (Bisno 1997). This regimen can be replaced by an injection of benzathine penicillin G in regions where RF is still endemic. This preventive antibiotic treatment does not achieve more than 90 to 95% eradication of organisms from the throat, but is a reliable guideline for primary prevention of RF and the intrafamilial spread of virulent strains (Stollerman 2001, El Kholy 1980).

Secondary prevention

Repeated episodes of RF lead to further inflammatory damage and subsequent scarring of cardiac valves, therefore, the aim of secondary penicillin prophylaxis is to prevent development and worsening of RHD by reducing the incidence of recurrent RF (Veasy 1997, Majeed 1992, Massell 1988). Therefore, secondary prevention relies on accurate case detection for the appropriate use of prophylactic antibiotics and regular medical surveillance as it may be highly effective in preventing disease progression, even in children with subclinical disease. Penicillin prophylaxis is therefore a critical cost-effective intervention for preventing morbidity and mortality related to RF and the most practical strategy for prevention of recurrences of RF and the resultant progression to RHD (Michaud 1993, Carapetis 2005[2], Stewart 2007, Steer 2009).

Secondary prevention of RF consists of continuous long-term administration of intramuscular benzathine penicillin G (Manyemba 2002, Dajani 1995, Lennon 2002, Bisno 2005, Carapetis 2005[2], McDonald 2005, Manyemba 2003). The ideal dose, route, frequency, and duration of administration for penicillin as secondary prophylaxis have not been established specially for children and considerable variation is evident in practice and in guidelines around the world (WHO 2001, Gerber 2009, NHF Australia 2006, Saxena 2008). However, a systematic review of randomized controlled trials revealed that intramuscular injection of benzathine penicillin G is superior to daily oral penicillin (Manyemba 2002, Kassem 1992, Kassem 1996) and that a prophylaxis regimen of 2 or 3 weeks, rather than 4 weeks, reduces the number of recurrences of RF (Lue 1986, Lue 1994).

Most physicians believe that prophylaxis should continue at least until the patient is a young adult, 18 to 20 years, which usually is 10 years from an acute attack with no recurrence (Dajani

1995). The potential efficacy of prescription duration of prophylaxis five years or age 18 if no carditis during previous episodes, or ten years or age 25 in patients with only mild mitral regurgitation or healed carditis has been evaluated and shown to lead to a lower recurrence of RF attacks. Importantly, individuals with documented evidence of RHD should be on continuous prophylaxis indefinitely because RF can recur as late as the fifth or sixth decade of life. However, secondary prophylaxis with penicillin is expensive and has not been very effective in controlling RF, as the compliance to this regimen is low resulting in many recurrences of the attacks (Nordet 1992, Carapetis 1996). Nevertheless penicillin prophylaxis is the only proven cost-effective intervention (Carapetis 2005[2], McDonald 2005, Manyemba 2003), and should be promoted. This approach requires patient compliance for the treatment and a well-developed public health infrastructure to assure fidelity and maintenance of registries (McDonald 2005, Bisno 2005). Nevertheless, regardless of whether or not prophylaxis is continued, there should always be a low threshold to test and treat acute episodes of streptococcal pharyngitis.

1.3.4 Screening

The fact that subclinical carditis is relatively common in RF (Tubridy-Clark 2007) ranging up to 90% (Folger 1992, Abernethy 1994) and that detection at such an early stage is believed to limit the progression to and improve the prognosis of chronic valvular disease with adherence to secondary penicillin prophylaxis lending support to the fact that screening is essential. Therefore early detection of subclinical RHD is vital, as it presents an opportunity for case detection at a time prophylactic penicillin can prevent progression to important valve disease in young adult life. The fact that only screening can indicate the true disease burden and detect those in danger of developing or progressing into valvular disease highlights the importance of developing effective screening programmes to identify patients at risk or those who have asymptomatic disease in the community. Apart from its importance for applying secondary prevention, screening programs in different parts of the world where the disease is common, are essential for understanding the pathogenesis of the disease, developing new specific diagnostic methods and importantly helping the development of effective vaccines (Bisno 2005).

In populations with high prevalence, RHD satisfies all the Council of Europe's criteria for selecting diseases suitable for screening. The Council of Europe (1994) and the WHO

recommended school-based screening as a way to estimate disease burden for RHD and as a way to identify patients in an area with a high prevalence of RHD (Bisno 2001, WHO 2004, Council of Europe 1994). However, specific recommendations regarding screening methods were not mentioned. A study carried out by highly trained clinicians in New Zealand found that clinical diagnosis of heart valve lesions is often inaccurate (Jaffe 1988).

1.4 Echocardiography

Echocardiography is a highly accurate method for diagnosing and characterizing valve lesions (Jaffe 1988, Bisno 2001), giving excellent details of the structural and functional abnormalities in RF and RHD and increasing the case detection rate by 10-fold compared to clinical examination, identifying children at risk of developing RHD for whom secondary prevention with penicillin prophylaxis may be effective (Marijon 2001, Marijon 2007) and resulting in major potential public health benefits.

1.4.1 Echocardiographic criteria

Echocardiography is found to be the only approach with sufficient sensitivity and specificity for the detection of subclinical carditis and RHD in the absence of auscultatory findings and overt evidence of valvular or other cardiac involvement (Marijon 2007, Carapetis 2008), during both the acute and the quiescent phases of the disease (Veasy 1993, Veasy 1994, Wilson 1995) and is therefore the perfect screening tool. However, there are no universally agreed criteria for the echocardiographic diagnosis of RHD and there has been debate concerning the echocardiographic signs of early RHD and the criteria to be used as an echo definition of RHD based on the length, velocity, and persistence of the regurgitant jet may depend on the gain settings on the ultrasound equipment (Minich 1997, Wilson 1995, WHO 2004).

Based on the fact that progress and prognosis of RHD depends on case detection and adherence to penicillin prophylaxis shows the need for standard echocardiographic criteria to diagnose RHD. The evaluation of inclusion of echocardiographic assessment in the Jones criteria has led to the conduction several studies to define the role and value of echocardiography and screening in the diagnosis and assessment of subclinical RHD.

Criteria 1 (WHO 2004)

The currently utilized World Health Organization (WHO) criteria for the diagnosis of subclinical RHD refers to a set of consensus recommendations by a WHO expert panel, widely used by practitioners for the diagnosis of subclinical RHD, and are based only on Doppler characteristics of the valvular regurgitation, defined by the association of a regurgitant jet >1 cm in length, seen in at least 2 planes, a mosaic color jet with a peak velocity >2.5 m/s, persisting throughout systole or diastole (WHO 2004) (Table 1.3). This set of criteria emphasizes the presence of pathological valve regurgitation but does not include valve structural features of RHD presumably missing up to three quarters of cases of subclinically affected and therefore potentially treatable children with RHD.

Table 1.3 WHO criteria

Doppler criteria
A regurgitant jet >1 cm in length
A regurgitant jet in at least 2 planes
A mosaic colour jet with a peak velocity >2.5 m/s
The jet persists throughout systole or diastole

Criteria 2 (Carapetis 2008)

An stringent criteria that combined internationally accepted definitions of pathological valvular regurgitation (Bisno 2008) with evidence of structural changes to valve appearance that are typical of RHD has been developed defining regurgitation as pathological when meeting all four criteria defined by the WHO (Table 1.3) otherwise classifying it as physiological (Carapetis 2008, Carapetis 2008[2], Bisno 2008, Folger 1992, Wilson 1995) has also been used.

This criteria resulted from a study that developed an abbreviated protocol that took 5–10 min per child and focused on rheumatic pathology of the mitral and aortic valves with diagnostic criteria consistent with those cited in a number of reports (Bisno 2008, Wilson 1995, Carapetis 2008[2]). Because the WHO criteria do not provide guidelines for categorizing severity, the division between categories was subjectively based on the direction of the regurgitant jet and its width, length and size compared with the left atrium. Structural abnormalities of the mitral valve such as valvular thickening and/or an elbow deformity of the anterior mitral valve leaflet

characteristic of rheumatic carditis were required for the diagnosis of definite RHD. If the mitral valve appeared normal, a diagnosis of definite RHD was made only if regurgitation was of mild or greater severity. Very mild pathological mitral regurgitation with normal valve morphology and the available views were only suggestive of RHD leading to a diagnosis of borderline RHD. However, any degree of pathological aortic regurgitation, mitral or aortic stenoses were considered to indicate definite RHD as they represent a condition rare in healthy children (Folger 1992). Mitral stenosis was diagnosed if flow acceleration across the mitral valve and a mean pressure gradient greater than 4 mmHg were seen on echocardiography. Similarly, flow acceleration across the aortic valve with a peak velocity greater than 2 m/s was necessary for diagnosis of aortic stenosis. The severity of stenosis was also subjectively classified as very mild, mild, moderate or severe.

These criteria included stenotic lesions but ignored the leaflet mobility and the subvalvular apparatus. Furthermore, a combination of functional and structural characteristics was not always necessary for the diagnosis of RHD, which might lead to a lower sensitivity of the criteria for the diagnosis by neglecting structural features.

Criteria 3 (Marijon 2007)

A systematic echocardiographic school based screening achieved a case detection rate 10-fold that achieved by clinical examination only. The criteria used considered RHD by the presence of any definite evidence of mitral- or aortic-valve regurgitation seen in two planes by Doppler echocardiography, accompanied by at least two of the following three structural abnormalities of the regurgitant valve: (1) leaflet morphology (typical marked thickening of the margins), (2) leaflet mobility (abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures). Only left-sided valves were examined for features of RHD. Mild tricuspid regurgitation and pulmonary regurgitation were frequently noted but were not regarded as indicating RHD as these conditions are frequent and seldom rheumatic in origin. This criteria included valvular stenotic lesions in the evaluation but failed to specify the parameters used for the definition of relevant valvular disease and regurgitation on echocardiography (Marijon 2007).

Table 1.4 Criteria 3

Any degree of valvular regurgitation seen in at least 2 planes Associated with at least 2 structural signs:
Leaflet restriction Subvalvular thickening Valvular thickening

Recently, this criteria has been compared to the WHO criteria in the setting of systematic echocardiographic screening of randomly selected school children aged 6 to 17 years to assess the echocardiographic RHD prevalence, and evaluate both criteria showing a significant lower detection rate by the WHO criteria. This confirms that case detection may differ importantly according to the diagnostic criteria utilized and that emphasis of the pathological valvular function and exclusion of valvular structural features of RHD leads to underdiagnosis of up to three quarters of cases of subclinically affected individuals.

This data indicates that inclusion of structural valve features in addition to regurgitation has the potential to increase case detection rates by up to 3-fold, with major potential public health benefits. Nevertheless, the WHO criteria is more likely related to acute RHD, whereas structural changes as defined in the combined criteria is more likely to suggest chronic RHD lesions. The WHO criteria may be easier to use at screenings with a portable ultrasound system, however this set of criteria seems to suffer from a substantially lower sensitivity than the combined one (Marijon 2009).

1.5 Pathogenesis

RF is a delayed, non-suppurative consequence of a streptococcal pharyngeal infection with B-hemolytic streptococci of Lancefield group A (Group A Streptococcus, GAS), C (GCS) and G (GGS) resulting in an autoimmune response (Olivier 2000, Cunningham 2000, Guilherme 2009, Dinkla 2007). However, the pathogenesis of RF/RHD is complex and involves both microbial factors and host factors that predispose and influence the development of autoimmune reactions and sequelae following streptococcal infection (Guilherme 1995). Although the pathogenesis of RF and RHD remains somewhat elusive, RF is clearly the result of an exaggerated immune response related to molecular mimicry between streptococcal and human proteins triggering

factors leading to autoimmune humoral and cellular responses directed toward human tissues in the response to streptococci. However, the host genetic markers for the predisposition to develop RHD have not yet been identified (Carreno-Manjarrez 2000) although an increased frequency of specific HLA class II haplotypes has been observed among RHD patients (Guilherme 2009, Cunningham 2000, Bisno 2003).

1.5.1 Molecular mimicry

Sharing of antigenic determinants between epitopes on the pathogen, the streptococci and specific human tissues is implicated in RF and RHD (Bisno 2005, Zabriskie 2004) by triggering the autoimmune response that causes RF (Cunningham 2000, Guilherme 2001, Cunningham 2003). This molecular mimicry between streptococcal antigens and human host tissue is thought to be the basis of cross-reactivity (Guilherme 2006).

Streptococcal M proteins share epitopes with human heart tissue including cardiac myosin (Carapetis 2005[2]), troppomyosin, vimentin, laminin and sarcolemmal membrane proteins mediating B and/or T cell cross-reactions (Cunningham 2000). Therefore, antibodies against the M protein can cross-react with heart tissue and induce heart valve damage due to inflammatory reactions. This molecular mimicry between streptococcal and human proteins has been proposed as the triggering factor leading to autoimmunity in RF and RHD.

The structural and immunological similarities between streptococcal M protein and human myosin, both α-helical and coiled-coil molecules seems essential for the development of rheumatic carditis (Carapetis 2005[2]). However, valvular disease, rather than acute myocarditis, is responsible for most of the cardiac morbidity and mortality of RF (Williams 2002, Kamblock 2003, Gentles 2001, Essop 1993).

1.5.2 Cellular and humoral immune response

Humoral and cellular immune responses are involved in the development of RF autoimmune reactions (Guilherme 2005[2]) and both B- and T-cells play important role in the development of RF (Guilherme 2007). The role of the humoral immune response in the development of RHD lesions is to trigger endothelial inflammation, leading to subsequent T- cell infiltration (Guilherme 2005[2]).

Streptococcal and host antigen cross-reactive antibodies facilitate heart tissue infiltration by T-lymphocytes. Activation of $CD4^+$ T-cells which are believed to be the major effectors of heart tissue lesions is triggered by the presentation of streptococcal antigens, mainly, via HLA class II molecules leading to a degenerate pattern of antigen recognition between streptococcal antigens and autoantigens (Guilherme 2005[2], Guilherme 2007). T-cells are therefore able to recognize Streptococcal M5 protein peptides and produce various inflammatory cytokines such as TNF-α, IFN-γ, IL-10, IL-4 which could be responsible for progressive fibrotic valvular lesions (Chopra 2007).

The recognition of bacterial and/or human-antigens depends on antigen presentation by antigen-presenting cells (APCs), such as macrophages, dendritic cells, and B-lymphocytes, in the context of MHC class I or II to T-cells via T-cell receptor. Recognition of human-proteins by other cells is mediated by the mechanism of "epitope spreading," in which an initial immune response against a determined pathogen generates broad diversity recognition of human-antigens that triggers an amplification and diversification of the autoimmune response (Lehmann 1992).

The activation of $CD4^+$ T-cells is triggered by the presentation of streptococcal antigens, mainly, via HLA class II molecules. However, pathogen epitopes that present structural or sequential similarity to human-epitopes might activate autoreactive T-lymphocytes that have escaped immune tolerance by the molecular mimicry mechanism also activating B-cells that will produce pathogen and human-antigen-specific antibodies (Guilherme 2009).

Antigen-activated $CD4^+$ T-cells polarize to Th1 or Th2 or Th17 subsets, the three subsets of T-helper (Th) cytokines currently described, depending on the cytokine secreted. Once activated, T- cells secrete cytokines that activate autoreactive B-cells. Polarization of the immune response towards either B- or T- cells results in different manifestations of the disease, such as arthritis, Sydenham's chorea, and carditis. Th1 are involved with the cellular immune response and produce IL-2, IFNγ, and TNF-α. Th2 cells mediate humoral and allergic immune responses and produce IL-4, IL-5, and IL-13. Th17 cells have recently been identified as a new lineage of effector Th cells, with an important role in host defense against infections by recruiting neutrophils and macrophages to infected tissues (Stanevicha 2003) showing a type of proinflammatory response mediated by IL-17 with the differentiator factors being cytokines TGF-ß, IL-6, and IL-23 (Guilherme 2009).

It is suggested that cell-mediated immunity within the heart is responsible for the resulting cardiac damage (Stollerman 2001, Guilherme 2009) as there is presence of heart-reactive antibodies in the serum of patients with RHD and deposition of such antibodies in the myocardium, which emphasises the importance of humoral immunity. Therefore, it is believed that antigenic mimicry or cross-reactivity between streptococcal antigens and cardiac autoantigens may initiate the autoimmune response resulting in cardiovascular damage.

The description of intralesional T-cell reactivity demonstrates the role of $CD4^+$ T-cells as effectors of the autoimmune lesions and how these cells expand when driven by streptococcal cross-reactive heart tissue suggesting their role as major effectors of autoimmune reactions in the heart tissue in RHD patients. Therefore, the pattern of cytokine production in the heart lesions favors a Th1-mediated disease.

1.5.2.1 Cytokines

Cytokines are important secondary signals following an infection as they trigger effective immune responses and deleterious responses in autoimmune disease. Streptococcal throat infection triggers an inflammatory reaction that involves several proinflammatory cytokines, such as IL-1, IL-6, and TNF-α (Hackett 1992, Kotb 1993, Tomai 1990, Ramasawmy. unpublished observations). Cytokines appear to play a crucial role in triggering immunologic and inflammatory reactions in RF. Moreover, IL-6 and TNF-α are well-known inducers of acute phase reactants and show significant correlation with the CRP and ESR. (Yegin 1997) The presence of these cytokines shown in peripheral blood of acute RF and active RHD patients (Yegin 1997, Miller 1989, Morris 1993, Narin 1995, Samsonov 1995) as well as valvular tissue suggests a major role for inflammatory cytokines in mediating heart lesions in RHD, however, their definite role as major damage mediators have not been cleared yet (Guilherme 2009).

The cytokine pattern produced by Th cells in response to defined antigens is crucial to drive the humoral or cellular immune responses. In addition, the concept of pathological or physiological autoimmune reactions depends on the cytokine produced in response to the autoantigens that are being recognized by T-cells. The different manifestations of RF involve particular autoantigens as targets of pathological autoimmunity (Stollerman 1988). Arthritis, chorea and mild RHD are

in part due to a pathological autoimmune reaction probably mediated by Th2-type cytokines, leading to an exacerbated humoral response, as reported in several studies (Cunningham 2000). On the other hand, carditis, valvulitis and severe RHD are mediated mainly by T-lymphocytes (Guilherme 2001, Guilherme 1995, Guilherme 2007, Raizada 1983, Kemeny 1989).

Increased plasma levels of IL-1, IL-6, and TNF-α in patients with heart failure (Matsumori 1994, Mann 2001, Lane 1992, Smith 1992) and significant changes in serum values of IL-6 and TNF-α in the acute phase of RF have been reported (Miller 1989). Furthermore, the TNFa levels have also been shown to correlate with the IL-6 levels (Yegin 1997). It has therefore been suggested that estimation of IL-1α in carditis and IL-6 in arthritis may be helpful as minor criteria for diagnosis and follow-up of rheumatic activity, and advised therapy for rheumatic carditis with anticytokines such as anti-IL-1α and anti-TNF-α immediately after diagnosis to prevent or reduce valvular damage (Hafez 2001).

Interleukin-1 (IL-1)

IL-1β is predominantly expressed by monocytes while IL-1α is mainly expressed by keratinocytes. However, IL-1 is also produced by activated macrophages from different sources and by peripheral neutrophil granulocytes, T- and B-cells. The main biological activity of IL-1 is the stimulation of T-helper cells, which are induced to secrete IL-2 and to express IL-2 receptors. IL-1 acts directly on B-cells, promoting their proliferation and the synthesis of immunoglobulins and functions as one of the priming factors that makes B-cells responsive to IL-5 and by enhancing the expression of adhesion molecules IL-1 promotes the adhesion of neutrophils, monocytes, T-cells, and B-cells. In combination with other cytokines IL-1 is an important mediator of inflammatory reactions as it markedly enhances the metabolism of arachidonic acid (in particular of prostacyclin and PGE2) in inflammatory cells such as fibroblasts, synovial cells, chondrocytes, endothelial cells, hepatocytes, and osteoclasts. Furthermore, IL-1 is also a strong chemoattractant for leukocytes and activates oxidative metabolism in neutrophils. Apart from its clinical significance as a stimulator of T-cells, IL-1 has been shown to also promote wound healing, which is thought to involve effects on angiogenesis, the promotion of proliferation of fibroblasts, and the chemotactic activity on neutrophils.

IL-1 is reported to increase during active rheumatic carditis and significantly elevated amounts of IL-1α as well as IL-1b (Yegin 1997) have been found in ARF and active RHD patients (Morris 1993) persisting for up to 48 weeks (Miller 1989).

Interleukin-2 (IL-2)

IL-2 is produced mainly by T-cells expressing the surface antigen $CD4^+$. IL-2 causes and stimulates the proliferation of T-cells and is a growth factor for all subpopulations of T-lymphocytes being an antigen-unspecific proliferation factor for T-cells that induces cell cycle progression in resting cells and thus allowing clonal expansion of activated T-lymphocytes.

IL-2 also promotes the proliferation of activated B-cells in the presence of additional factors, for example, IL-4. Due to its effects on T-cells and B-cells IL-2 is a central regulator of immune responses and plays a role in anti-inflammatory reactions, hematopoiesis and tumor surveillance. IL-2 stimulates the synthesis of IFN-γ in peripheral leukocytes and also induces the secretion of IL-1, TNF-α and TNF-β. Increased production of IL-2 during ARF and in patients with active RHD correlating with the numbers of $CD4^+$ and $CD25^+$ cells on the periphery (Yegin 1997) has been reported (Morris 1993).

Interleukin-4 (IL-4)

IL-4 is produced mainly by a subpopulation of activated Th2 cells which are the biologically most active helper cells for B-cells and also secrete IL-5 and IL-6. However, a lesser extent of IL-4 is also produced by Th1 cells.

IL-4 promotes the proliferation and differentiation of activated B-cells, the expression of MHC class 2 antigens and low affinity IgE receptors in resting B-cells, also enhancing expression of MHC class 2 antigens on B-cells and promoting their capacity to respond to other B-cell stimuli and present antigens for T-cells. This promotes the clonal expansion of specific B-cells enabling the immune system to respond to very low concentrations of antigens. Furthermore, IL-4 stimulates the proliferation of thymocytes with the marker spectrum $CD4^-$ $CD8^-$, $CD4^+$, $CD8^+$. In $CD4^+$ cells IL-4 induces the expression of $CD8^+$.

IL-4 may be of clinical importance in the treatment of inflammatory diseases and autoimmune diseases since it inhibits the production of inflammatory cytokines such as IL-1, IL-6 and TNF-α

by monocytes and of TNF by T-cells. The lack of IL-4, a regulatory cytokine, in the valvular tissue is involved in the progression and permanence of RHD lesions. In addition, the lack of IL-4 is believed to perpetuate and/or exacerbate the production of the inflammatory cytokines TNF-α and IFN-γ (Guilherme 2005[3]).

Interleukin-6 (IL-6)

IL-6 is produced by many different cell types, mainly monocytes, fibroblasts, endothelial cells, macrophages, T-cells and B-lymphocytes. Physiological stimuli for the synthesis of IL-6 includes IL-1, bacterial endotoxins, TNF while IL-6 can also stimulate or inhibits its own synthesis, depending upon the cell type. IL-6 is a pleiotropic cytokine influencing antigen-specific immune responses and inflammatory reactions and is one of the major physiological mediators of acute phase reaction and also a B-cell differentiation factor and an activation factor for T-cells. In the presence of IL-2, IL-6 induces the differentiation of mature and immature T-cells into cytotoxic T-cells. IL-6 also induces the proliferation of thymocytes and probably plays a role in the development of thymic T-cells and is capable of inducing the final maturation of B-cells into immunoglobulin-secreting plasma cells if the cells have been pre-activated by IL-4 and stimulates the secretion of antibodies in B-cells.

Interleukin-8 (IL-8)

IL-8 is produced by stimulated monocytes, macrophages, fibroblasts and endothelial cells. In many cell types the synthesis of IL-8 is strongly stimulated by IL-1 and TNF-α while interferon IFN-γ can function as a costimulator. IL-8 differs from all other cytokines in its ability to specifically activate neutrophil granulocytes and causes a transient increase in cytosolic calcium levels in neutrophils and the release of enzymes from granules it also affects B-cells directly through a specific mechanism that is different from IFN-γ and IFN-α. IL-8 may be a marker of different inflammatory processes and is an important inflammatory cytokine in activation of chemotaxis and the accumulation of leucocytes into the inflammation site (Endo 1991, Seitz 1991, Whicher 1990).

Significant elevations of IL-8 have been observed in the plasma of RHD patients with cardiac failure which suggests that IL-8 might have a role in the pathogenesis of RF (Yegin 1997).

Interleukin-10 (IL-10)

IL-10 is produced by activated $CD8^+$ peripheral blood T-cells, by T-helper $CD4^+$ T-cell clones (resembling Th0, Th1, and Th2) after both antigen-specific and polyclonal activation, by B-cell lymphomas and monocytes following cell activation by bacterial lipopolysaccharides and mast cells. The synthesis of IL-10 by monocytes is inhibited by IL-4 and IL-10.

IL-10 inhibits the synthesis of a number of cytokines such as IFN-γ, IL-2 and TNF-β in Th1 T-helper subpopulations of T-cells but not of Th2 T-helper cells which can be antagonized by IL-4. The inhibitory effect on IFN-γ production is indirect and appears to be the result of a suppression of IL-12 synthesis by accessory cells. IL-10 is produced by, and downregulates the function of, Th1 and Th2 cells. In macrophages stimulated by bacterial lipopolysaccharides IL-10 inhibits the synthesis of IL-1, IL-6 and TNF-α. It also leads to an inhibition of antigen presentation. In human monocytes IFN-γ and IL-10 antagonize each other's production and function. IL-10 has also been shown to be a physiologic antagonist of IL-12 and reduce the production of IFN-γ and IL-2. IL-10 appears to be responsible for most or all of the ability of the conditioned medium of Th2 T-helper cells to inhibit cytokine synthesis by Th1 cells and acts as a costimulator of the proliferation of mast cells (in the presence of IL-3 and/or IL-4) and peripheral lymphocytes.

IL-10 is also a costimulator for the growth of mature and immature thymocytes (together with IL-2, IL-4 and IL7) and functions as a cytotoxic T-cell differentiation factor, promoting a higher number of IL-2 activated precursors of cytotoxic T-lymphocytes to proliferate and differentiate into cytotoxic effector cells. IL-10 sustains viability of B-cells and also stimulates B-cells and promotes their differentiation. It enhances the expression of MHC class 2 antigens on B-cells whereas it inhibits MHC class 2 expression on monocytes. In B-cells activated via their antigen receptors or via CD40, IL-10 induces the secretion of IgG, IgA and IgM. This effect is synergised by IL-4 while the synthesis of immunoglobulins induced by IL-10 is antagonized by TGF-β. Furthermore, the activation of macrophages can be prevented by IL-10.

Interleukin-12 (IL-12)

It is produced mainly by B-cells and to a lesser extent by T-cells and is secreted by peripheral lymphocytes after induction. The most powerful inducers of IL-12 are bacteria, bacterial products, and parasites. IL-12 stimulates the proliferation of human lymphoblasts following cell activation by phytohemagglutinin and activates $CD56^+$ NK-cells, being blocked by antibodies specific for TNF-α.

In peripheral lymphocytes of the Th1 T-helper cell type IL-12 induces the synthesis of IFN-γ and IL-2, and TNF. TNF-α also appears to be involved in mediating the effects of IL-12 on natural killer cells since the effects of IL-12 are inhibited by an antibody directed against TNF-α. IL-12 and TNF-α are costimulators for IFN-γ production with IL-12 maximizing the IFN-γ response and the production of IL-12, TNF, and IFN-γ is inhibited by IL-10. In Th2 T-helper cells IL-12 reduces the synthesis of IL-4, IL-5, and IL-10.

IL-12 synergises with suboptimal amounts of IL-2 in promoting the proliferation of mononuclear cells in the peripheral blood and in promoting the generation of LAK cells (lymphokine activated killer cells). However, picomolar concentrations of IL-12 are as effective as nanomolar concentrations of IL-2 in augmenting the cytolytic activity of natural killer cells expanded in vivo by IL-2.

TNF-α

TNF is secreted by macrophages, monocytes, neutrophils, T-cells, NK-cells following their stimulation by bacterial lipopolysaccharides. Cells expressing $CD4^+$ secrete TNF-α while $CD8^+$ cells secrete little or no TNF-α. The synthesis of TNF-α is induced by many different stimuli including interferons (IFN), IL-2, Immune complexes and PAF (platelet activating factor) and the production is inhibited by IL-6, TGF-β.

TNF-α enhances phagocytosis and cytotoxicity in neutrophilic granulocytes and also modulates the expression of many other proteins, including IL-1 and IL-6. In combination with IL-1, TNF-α is responsible for many alterations of the endothelium. TNF-α also inhibits anticoagulatory mechanisms and promotes thrombotic processes and therefore plays an important role in pathological processes such as venous thromboses, arteriosclerosis, vasculitis, and disseminated intravasal coagulation.

IL-6 suppresses the synthesis of IL-1 induced by bacterial endotoxins and TNF, and the synthesis of TNF induced by endotoxins. TNF mediates part of the cell mediated immunity against obligate and facultative bacteria and parasites. However, although TNF-α is required also for normal immune responses the overexpression has severe pathological consequences.

Elevation of TNFa levels may have important pathophysiological and clinical implications and the possible relation between cardiac contractility and circulating level of TNFa has been pointed. Furthermore, it has been shown that TNFa and other cytokines inhibit the contractility of cultured cardiac myocytes (Gulick 1989, Hasenpud 1989, Weisensee 1993) It has been suggested that TNF-α could be responsible for the development and progression of heart failure and that the production of TNF-α could be the result of immune response activation because of tissue injury, stress, or underperfusion of systemic circulation, but could also be locally produced by myocytes. It has also been shown that patients with chronic mitral valve regurgitation present high production of TNF-α in the plasma and increased expression in the myocardium, which suggests that TNF-α plays a role in the remodeling of left ventricular volume (Kevasan 1997, Kapadia 1998, Herrera-Garza 1999, Dibbs 1999).

RF and RHD patients present increased levels of TNF-α (Narin 1995, Samsonov 1995, Yegin 1997, Taneja 1989, Olmez 1993, Hernandez-Pacheco 2003, Miller 1989, Morris 1993) and TNF-α is apparently one of the cytokines with an active and prominent role in the pathogenesis of the rheumatic process the initiation of multisystemic inflammatory response in RF and pathogenesis of RHD. TNFa may therefore, be the major cytokine in RF leading to IL-6 and IL-8 production (Yegin 1997).

1.5.3 Cellular and humoral response in valvular tissue

Heart biopsies exhibit cellular infiltration with a predominance of $CD4^+$ T-cells (Guilherme 1995) with the highest number of both $CD4^+$ and $CD8^+$ T-cells in patients that present rheumatic activity (Guilherme 2001). It seems that during the acute episode there are expansions of auto reactive T-cells in the heart and after the acute episode these auto reactive T-cells decrease in numbers but remain important to maintain the heart lesions **(**Sampaio 2007). Inflammatory infiltrates may be sparse and patchy in the chronic lesions, but are usually dense in the acute

phase of the disease. In RHD the main finding is dense valvular inflammatory infiltrates characterized mainly as $CD4^+$ T-cells and macrophages at the site of heart lesions.

Intralesional $CD4^+$ T-cell clones from surgical fragments of severe RHD patients' crossreact with immunodominant M5 peptides and heart tissue proteins which indicates the existence of T-cell molecular mimicry between streptococci and heart tissues relevant in the pathogenesis of RHD. Apparently, adhesion molecules such as VCAM-1 facilitate the extravasation of $CD4^+$ and $CD8^+$ T-cells into the valves, after being up-regulated on the vascular endothelium of the mitral valve of ARF patients (Guilherme 2004, Roberts 2001, Guilherme 2004, Roberts 2001). The concomitant presence of fibrin deposits as verrucae on the endocardial surface implies that there is rheumatic activity **(**Sampaio 2007). The differential cytokine polarization in the heart in RHD is probably related to immigrant autoreactive T-cells, local chemokines produced by inflammatory cells and adhesion molecules (Guilherme 2004).

Mononuclear cells from heart lesions predominantly secrete inflammatory cytokines IFNγ and TNF-α, Th1-type cytokines such as the regulatory cytokine IL-10, in both acute RF and chronic RHD patients. This indicates that, even during the chronic phase, these mononuclear cells still produce inflammatory cytokines. Usually, there is a sparse production of IL-4 by valve-infiltrating cells and large numbers of IL-4 positive cells which suggests that low numbers of IL-4 producing cells in the valvular tissue and the lack of IL-4, a regulatory cytokine, may contribute to the induction of progression and maintenance of valvular RHD lesions. In addition, the lack of IL-4 probably perpetuates and/or exacerbates the production of inflammatory cytokines such as TNF-α and IFN-γ which are believed to be the mediators of valve lesions and the presence of few mononuclear cells (Guilherme 2007). Furthermore, the production of IL-1, IL-2 and TNF-α was correlated with the progression of the Aschoff nodules in the valve lesions of ARF patients (Kemeny 1989).

These observations show the involvement of different mononuclear and cytokine producing cells in the myocardium and valves reinforcing the putative role of regulatory and inflammatory cytokines in myocardium healing in RHD and in the induction of progressive and permanent valve damage (Guilherme 2004, Guilherme 2005[2], Guilherme 2007, Guilherme 2009). Whether the initial valvular insult is due to antibody or cell-mediated immunological damage is uncertain,

but the subsequent damage seems to be caused by T-cell and macrophage infiltration (Cunningham 2003, Roberts 2001, Guilherme 2004, Guilherme 2009).

1.5.4 Valvular architecture

The valve cusps are three-layered structures consisting of a ventricularis/atrialis, spongiosa, and fibrosa and composed of differing amounts of primarily collagen, elastin, and a glycosaminoglycan-rich ground substance, representing about 60, 10 and 20% of the dry weight of the valve, respectively (Kunzelman 1993). However, little quantitative information is available on the amount, location, orientation, and overall structure of these constituents in the three layers (Scott 1995).

RHD valves have shown altered architecture and histology, including fibrosis, calcification and neovascularization (Sampaio 2007) as well as changes in the collagen, elastin and glycosaminoglycans (GAGs) distribution and localization, which seem to lead to distortion of valve function and mechanics (Guilherme 2009[1], Sampaio 2007).

Valvulitis and endocarditis have been observed in excised RHD valves. The disease predominantly affects the valvular endocardium culminating in crippling valve deformities and the valves show various degrees of calcification, which is a sign of chronic valvular involvement. Interestingly, recent histopathological and clinical studies indicate that calcification is not merely an inactive, "dystrophic" process but involves a active and highly regulated inflammatory process associated with expression of osteoblast markers and neoangiogenesis (Messika-Zeitoun 2004). Furthermore, increased plasma osteopontin levels correlated with severity of mitral valve calcification (Chopra 2007). Importantly, deposition of calcium phosphate on the delicate valvular structures can lead to cumulative damage and result in valvular dysfunction and stenosis (Atar 2003). Features like fibrosis and neovascularization are observed in most of the valves, indicating that the valve has gone through previous episodes of rheumatic activity and usually, the degree of fibrosis is also proportional to the number of acute episodes (Sampaio 2007). Importantly, molecular mimicry between streptococcal and human antigens causes inflammation that leads to neo-vascularization, which enables further recruitment of T-cells, leading to granulomatous inflammation and the establishment of chronic RHD while repeated episodes of RF lead to further inflammatory damage and subsequent scarring of cardiac valves (Veasy 1997).

Elastin structures are found to contribute significantly to the mechanical function of the valve cusps (Vesely 1998) and damage to the elastin network of the cusps alters the valvular mechanics, subjects the tissue to abnormal loading conditions and accelerates mechanical degeneration. This is due the fact that damage to elastin in aortic valves leads to a passive elongation of the tissue, a reduction in extensibility, and an increase in stiffness.

Fragmentation of elastin and reconformation of collagen fibers may also have additional effects on this mechanism (Viidik 1982). It is also speculated that the permanent elongation of the valve cusps and their decreased extensibility results partly from the slippage of collagen fibrils and partly from the mechanical interaction between elastin and collagen. It is therefore believed that the initial response to mechanical loading is mainly due to elastin, while the final stiffness is defined by collagen.

Elastin structures impose tensile forces on collagen fibers during valve unloading and the dominant restorative force when the ventricularis is stretched in the radial direction is the tension produced by elastin as the collagen takes over the load and limits further extension as it relatively inextensible. Furthermore, the fibrosa passively relies on the ventricularis to pull it back, through the collagen mechanisms into its folded shape. Furthermore, valvular elastin also likely acts as a 'housekeeper' that restores the collagen fibre geometry back to its original conformation between successive loading cycles, and is therefore critical to proper valve function (Vesely 1998).

The function of elastin is highly dependent on its configuration and relative content in the given material, however elastin content alone does not dictate mechanics, important is its organization relative to collagen.

As the mechanics of the whole ventricularis is not identical in the radial and circumferential directions, it is suggested that the amount of collagen or the types of connections between the elastin and collagen structures are responsible for this anisotropy and not only the mechanics of the elastin itself. Importantly, increase in mature elastic fibers has been found with age (McDonald 2002, Bashey 1967), particularly between the second and fifth decades of human life (Sell 1965) and in pathological valves (Imayama 1989). Therefore, distortion of valve function and mechanics seems to be an interaction between elastin and collagen conditions and changes.

The collagen component is predominantly type I and III (74% and 24%, respectively) and collagen fibre bundles appear to be surrounded by elastin fibrils (Kunzelman 1993) which are

much thinner than collagen fibrils and arranged in a branching pattern (Stevens and Lowe 1997). Collagen I is found predominantly in the ventricularis while collagen III can normally be found in the fibrosa.

Glycosaminoglycans (GAGs) form a highly hydrated gel-like ground substance in which other matrix molecules are located (Kunzelman 1993) and are therefore critical to the function of numerous soft connective tissues, including heart valves, providing material properties such as viscoelasticity, resistance to compression and tension (Kinsella 2004, Rothenburger 2002). GAGs also play crucial roles in tissue differentiation, growth factor regulation and various pathologies (Rothenburger 2002) including many heart valve diseases. Furthermore, recent evidence also suggests that GAG's may act as a key component in the development of calcific AV disease (Grande-Allen 2007) and the loss of the GAG-rich spongiosa layer, which normally provides shear between the outer valve layers and thus enables complex valve leaflet movement (Vesely 2004), is known to be instrumental in the failure of porcine bioprosthetic aortic valves.

Valvular GAGs are complex in their composition and distribution and change throughout development and aging, when the heart valves experience significant changes in mechanical loading and tissue differentiation. However, the composition of GAGs is different between layers and varies regionally based on the type of mechanical loading experienced by these tissues, (Grande-Allen 2004) showing abundance in the spongiosa of the aortic valve. Importantly, regions experiencing tension containe fewer GAG overall, compared to regions experiencing compression. Meanwhile, human valves also demonstrate age-associated decrease in total GAGs, unique to specific histological layers as well as associated with differences in mechanical loading (Stephens 2008).

1.5.5 Structural and biochemical imaging techniques

Raman spectroscopy

It has been demonstrated that optical spectroscopy techniques, such as reflectance, fluorescence, infrared absorption, and Raman scattering can provide information about tissue composition at the molecular level (Lucas 1996, Buschman 2000, Hanlon 2000). MRI may allow the discrimination of large lipid pools, calcifications, and fibrous caps in atheromatous plaques but cannot quantify the chemical composition (Toussaint 1996). Therefore, of these

techniques, Raman spectroscopy shows great promise to provide valuable biochemical information for nondestructive diagnosis of cardiovascular disorders (Buschman 2000, Hanlon 2000). Raman spectroscopy of tissue yields more information about chemical composition because the Raman spectra of biological compounds are unique (Brennan 1994, 1995, 1995[2], 1997, 1997[2]).

Raman scattering is an inelastic process that occurs when a sample is illuminated with a monochromatic light source, such as a laser beam. In this process, energy from the incident photons is transferred to the sample's molecules, exciting them to high vibrational modes. Scattered photons have lower frequency than the incident ones due to the energy that is lost in the scattering process. This radiation frequency shift corresponds to the difference in molecular vibrational frequencies of the sample (Hanlon 2000). Raman spectra of tissues show narrow and well-resolved bands with line width of 10-20 cm^{-1}, revealing the presence of many biochemical molecules (Lucas 1996). The relative contribution of these biochemical molecules for the tissue Raman spectrum is proportional to their concentration in the tissue, which is the basis for the diagnostic data acquired by the Raman spectra (Buschman 2000). Among the many types of biomedical investigation made possible by Raman spectroscopy cardiac valves are among the most important diagnostic applications (Otero 2004).

Raman spectroscopy has shown to be a promising technique to characterize the chemical compounds found in biological tissues, allowing the identification of compounds in different states, and giving information about the molecular geometry from the analysis of the vibrational spectra (Elbagerma 2010). The use of Raman spectroscopy as a diagnostic tool for pathological alterations is based on the uniqueness of the Raman spectrum of a given molecule (Raman 1928, Manoharan 1996, Frank 1995, Lawson 1997). Using sensitive laboratory equipment, Raman spectra can be obtained in less than a second and most spectral features are visible in spectra collected in only a few seconds via optical fiber catheters (Brennan 1997).

Considerable advantages result from the fact that Raman spectroscopy is a fast and non-destructive technique and that spectra can be recorded directly from specimens even in an aqueous environment (De Beer 2004) providing quantitative information about the chemical composition (Baraga 1992). Furthermore, it has been shown that Raman spectroscopy can be used to quantify the relative amounts of protein, cholesterol, adventitial fat, and calcium salts

(Brennan 1997[2]). Spectroscopic methods for determination of stability constants have the advantages of sensitivity and reliability and are suitable for determination of stability constants in solution under different experimental conditions (Elbagerma 2010).

Atomic force microscopy (AFM) enables structural studies revealing nanometer-scale details of biological objects and when working in the contact mode in air is a useful tool for imaging surfaces of human heart valves at the supramolecular level. AFM images have revealed different local architecture of collagen fibrils on the valve surface showing areas with entirely different spatial organization of the collagen fibril bundles. This included zones with multidirectional, stacked collagen fibrils, as well as areas with regular dense packing of thin collagen fibrils. It was also found that different forms of fibril packing had an impact on the collagen D-spacing distance. The ventricularis layer, which is abundant in elastin, and fibrosa layer was recognized spectroscopically to contain mostly collagen. Moreover, carbohydrates, which are the main components of GAGs, and minerals deposits (hydroxyapatites) were also detected. Therefore, it has been shown that AFM imaging in the contact mode and FT-IR spectroscopy are suitable methods for structural characterization of heart valves at the molecular and supramolecular levels (Jastrzebska 2006).

1.6 Streptococcal pathogenesis

RF was believed to be caused by repeated infections with GAS (Figure 1.4) but recent studies suggest streptococci of group C and G are also linked to its pathogenesis (Dinkla 2007). Streptococci are able to adhere to and colonize (Neeman 1998) skin, oralnasal mucosa, and tonsils (McMillan 2004, Cunningham 2000) and are able to disseminate to other organs (Bisno 2005). Tissue invasion can lead to local suppurative complications or systemic infections (Cunningham 2000).

Streptococci manipulate innate immune defences at several levels and virulent streptococci down-regulate inflammation and restrict activation of the alternative complement system, which is critical to the phagocytosis and killing of GAS by the nonimmune host. Surface-associated C5a peptidase and M proteins inhibit detection and phagocytosis by polymorphonuclear

leukocytes (PMNs), by specific destruction of the early complement chemotaxin, C5a, and then by M protein–restricted deposition of C3b opsonin (Wang 2000).

Streptococcal strains express multiple adhesins, such as lipoteichoic acid, M protein, and fibronectin binding proteins (Cunningham 2000, Bisno 2003). These adhesins, of which M protein, fibronectin binding protein Sfb1/PrtF, and fibronectin binding protein 54 (FBP54) have been shown to promote efficient intracellular invasion of mammalian cells (Wang 2000). Meanwhile, immunity to GAS is mediated by antibodies to the M protein, which exists as a coiled-coil protein on the surface of the bacteria.

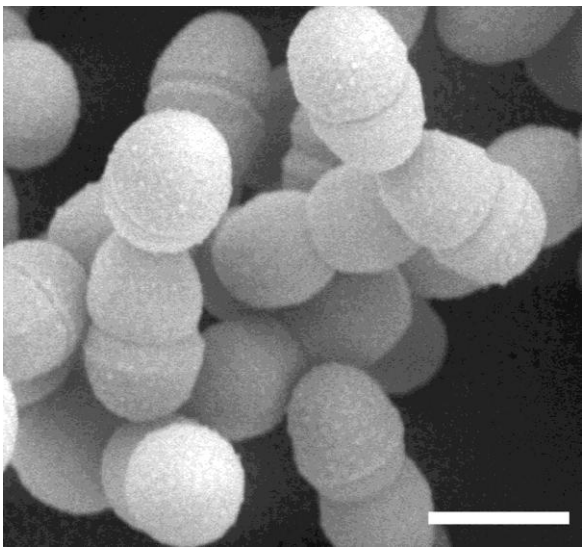


Figure 1.4 Group A streptococci

1.6.1 GAS

The classification of Group A streptococci (GAS) into specific types based on the type of M protein has been vital for the understanding of the epidemiology of streptococcal disease (Sheree 1994). The M protein was believed to be the major virulence determinant of the GAS organisms (Pruksakorn 1994) and also the major protective antigen (Lei 2004) as it enables streptococci to resist phagocytosis (Kotb 2002) by binding factor H, thus regulating complement deposition and preventing C3b-dependent phagocytosis.

The M protein is an α-helical coiled-coil protein (Carapetis 2005[2]) and consists of a variable nonrepeated amino terminal region, which contains determinants of type-specificity defining the

GAS serotype, and a highly conserved carboxy terminal C-repeat region, common in different serotypes. Antibodies directed against the type specific M protein epitopes neutralise the antiphagocytic property of M protein rendering cells susceptible to phagocytosis (Sheree 1994), by opsonizing streptococci in the presence of neutrophils. However, this antibody response is directed against the hyper-variable amino terminal region of the strepotococcal M protein, being thus type-specific (Robinson 1992, Malmanche 1994) and protecting only against GAS organisms of the same M serotype. Therefore, protective immunity to GAS has been associated with type specific opsonic antibodies against M protein (Sheree 1994). However, serum bactericidal antibodies targeting the streptococci M protein appear following natural infection of humans and are associated with protection against subsequent infection with the same serotype persisting for as long as 30 years following infection.

1.6.1.1 Variety of M-types

There are over 150 known different M protein types (Kehoe 1991, Beall 1995, Sheree 1994, Facklam 2002), and only a single type is expressed by each strain. The type specificity of each M protein is largely determined by the epitopes located in the amino-terminal 40 to 50 amino acid residues (Beall 1995) being the amino acid sequence of the amino terminus in the M protein responsible for the serotype of the organism (Kehoe 1991). Diversity in the *emm* sequence is generated by corrected frameshift mutations, point mutations, and small in-frame mutations. Therefore, variations in *emm* gene sequences occur and result in amino acid substitutions or insertion of short sequences within the N-terminal, the hypervariable segments of the M proteins. The high variability of the N-terminal region of the M protein between different streptococcal strains has lead to the presence of different streptococcal serotypes with a number of serotypes being much more common than others within a population, varying between distinct geographic locations leading to geographic variations among streptococcal strains (Carapetis 2000). Nevertheless, some strains are also believed to be more likely to cause RF than others (Carapetis 2005[2]) suggesting a particular rheumatogenic potential of certain strains of GAS.

Rheumatogenicity was considered a feature only of strains belonging to certain M serotypes, however results of several studies indicate no definitive association between group A

streptococcus emm sequence, class, or pattern group, and site of infection or ability to cause disease (Carapetis 2005[2]). Although amino-terminal M protein epitopes from common reference strains of GAS have been identified, protective epitopes from GAS strains prevalent within a GAS-endemic region remain unknown. Thus, the issue of potential and specific rheumatogenic strains remains unresolved (Stollerman 2001) as virulence and molecular differences between strains are not clear (Stollerman 2001).

M protein amino (N)-terminus

Phagocytic killing of GAS is promoted by antibodies targeting the amino (N)-terminus of the M protein, which also forms the basis for GAS serotyping. The amino terminus of the M protein is highly variable between the GAS serotypes and frequently undergoes genetic recombination of the amino sequence which gives rise to subtypes, and can lead to loss in opsonizing ability of type-specific antibodies. Antibodies to the amino terminus of the M protein are opsonic and provide protection against challenge from homologous organisms and are therefore type specific.

M protein carboxyl (C)-terminus

The M protein carboxyl (C)-terminus contains the so-called "C-repeats". These C-repeats are highly conserved epitopes among GAS serotypes, located at the proximal end of the M-protein (Pichichero 2004). It has been shown that epitopes within the C-repeat region of M proteins evoke antibodies that cross-opsonize many serotypes of streptococci and also opsonic antibodies specific to the C-region have been demonstrated in humans.

1.6.2 Group C streptococci (GCS) and group G streptococci (GGS)

Group C (GCS) and group G (GGS) are considered as important veterinary pathogens but are also involved in human infections and in geographical areas with a high incidence of RF there is a widespread carriage of these streptococcal groups (Haidan 2000, McDonald 2006, Ahmed 2003, Baracco, 2006). Their spectrum of diseases is very similar to the one of GAS, comprising pharyngitis, skin and soft tissue infections as well as bacteremia and streptococcal toxic shock syndrome (Baracco 2006) and some strains have the potential to induce autoimmunity against

cardiac myosin, suggesting contribution to the pathogenesis of RF/RHD (Haidan 2000). This might partially explain the high incidence of RF while pharyngeal isolation of GAS is rare.

1.6.3 Streptococcal PARF peptide and human collagen IV

It has been shown that rheumatogenic GAS strains are able to bind and aggregate human collagen. One mechanism applied by streptococcal strains capable of causing acute rheumatic fever is formation of an autoantigenic complex with human collagen IV (Dinkla 2003). The recent hypothesis is that human collagen IV, a major component of subendothelial basement membranes, acts as such an autoantigen after forming a complex with streptococcal strains, which have a potential of causing ARF. In sera of RF patients such a collagen autoimmune response was accompanied by specific reactivity against the collagen-binding proteins, linking the observed effect to clinical cases.

Binding to collagen occurs through M protein or M18 hyaluronic acid capsule. This aggregation of collagen leads to autoantigenicity, which in turn induces RF. The sera from ARF patients shows significantly increased titers of anticollagen antibodies as compared with healthy controls.

An M-like protein, FOG, which was described as an adhesin (Nitsche, 2006) was identified as the group G streptococcal surface protein that is capable of binding different members of the collagen family thereby inducing the collagen autoimmune response. The low selectivity of FOG within the collagen family suggests that the autoimmune response is not caused by collagen IV only and other collagen types that interact with FOG may therefore be involved. Considering the high similarity between collagens, it seems likely that the autoimmunity against collagen IV at least partially depends on cross-reactivity being part of a broader anti-collagen response.

Further analysis of streptococcal collagen interaction revealed that an octapeptide, AXYLZZLN, designated PARF (peptide associated with rheumatic fever) in M proteins of rheumatogenic strains is responsible for binding and aggregation of collagen. Direct binding of collagen to streptococcal surface components is, therefore, considered as one of the key mechanisms for induction of ARF (Figure 1.11). GGS and GCS, like GAS, have been shown to have a rheumatogenic potential and have been reported to express M protein with PARF sequences. Just like rheumatogenic GAS strains, they are capable of binding and aggregating collagen and generating anticollagen antibodies, through the identified octapeptide motif PARF. Taken

together, the data demonstrate that the identified octapeptide motif (PARF) plays a crucial role in the pathogenesis of RF through its action on collagen, which makes it a promising candidate for diagnosis of rheumatogenic streptococcal strains. Unfortunately, the mechanisms known today are still not sufficient to fully explain the pathogenesis of RF.

Re-evaluations of existing data or new epidemiological studies, which take these findings into account, may shed more light on regional characteristics of ARF pathogenesis and help to identify and eradicate the causative bacteria and the value of PARF as a diagnostic marker for the early detection of rheumatogenic strains (Dinkla 2007).

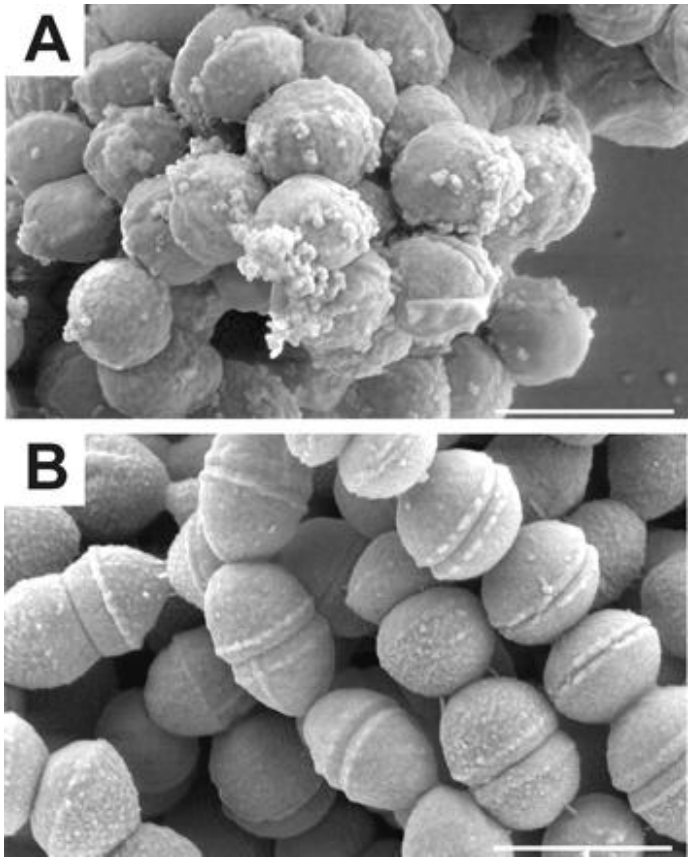


Figure 1.5 Binding and aggregation of collagen IV on the GGS surface. Field emission scanning electron micrograph of FOG-expressing strain G45 (*A*) and of a FOG-negative non-collagen binding strain G50 (*B*), which were incubated with soluble collagen IV. Only the FOG-positive strain shows massive aggregation of collagen on its surface. The *bars* correspond to 1 µm.

CHAPTER 2

AIMS AND PLAN OF INVESTIGATION

2.1 Aims

Many aspects of RF and RHD have still not been clarified. Data on epidemiology and geographical variety in clinical manifestation remain scarce with lack of systemic programs for screening and early detection and no consensus about diagnostic criteria and management while a high prevalence of the disease is suspected in different areas. The long-term outcome of patients with RHD has not been established, and therefore, the persistence and development of permanent valvular lesions is not known. Echocardiography is found to be the only approach with sufficient sensitivity and specificity for the detection of RHD, however, there are no universally agreed criteria and consensus of the echocardiographic signs of early RHD.

A major role for inflammatory cytokines has been suggested in mediating heart lesions in RHD with cellular infiltration of predominance of $CD4^+$ T-cells, showing geographic variety in immune response. However, the definite role of inflammatory cells as major damage mediators and signs of immune activation have not been cleared yet.

The key mechanism used by streptococcal strain and which defines rheumatogenicity seems to be the formation of a streptococcal autoantigenic complex with human collagen IV with binding and aggregation to collagen through PARF, which has been suggested as a diagnostic marker for the early detection of rheumatogenic strains and the diagnosis of RHD.

In addition, there are still gaps in the knowledge of the microbial, autoimmune and biochemical processes corresponding to the structural and functional features in RF and RHD. Importantly, better understanding of epidemiology, clinical characteristic and pathogenesis pathways of RF and RHD may facilitate improvement of the management of the disease, long term outcome and prognosis.

Therefore, we aim to:

-Determine the epidemiological and clinical characteristics of RF and RHD

-Perform in depth analysis of cellular and structural features in RF and RHD

-Atempt to define antigenic targets for evaluation and diagnosis of streptococcal infection

2.2 Plan of investigation

RF and RHD are endemic in Egypt where data on prevalence, characteristics, immune response and microbiology are lacking. Therefore, Egypt is a perfect setting for research and elucidation of the factors and mechanisms surrounding RF and RHD. This study was developed to unveil these factors in the Egyptian community.

DETERMINATION OF THE EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF RF AND RHD

In order to achieve this aim the following studies were performed:

School based screening for RHD

A cross-sectional screening was performed in a cohort of randomly selected school children in Aswan, in whom auscultation followed by echocardiography was used to identify the presence, characteristics and determinants of RHD. Aswan was chosen for this epidemiological study, as the area hosts a relatively stable population, with generally low income, living in a less humid or polluted environment, which has never been studied before regarding to this particular disease.

The assessment included a questionnaire containing questions regarding the patient's demographics, family environment, risk factors, history of RF and penicillin prophylaxis developed to detect the epidemiologic factors and determinants of RF and RHD. Physical examination included general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation and detailed transthoracic echocardiography evolving a newly developed protocol to define the characteristics of RHD and the role of echocardiography as a screening tool. This study evolves a set of rigid echocardiographic criteria, designed to enhance the specificity of the diagnosis of RHD to increase case detection rates and therefore the treatment of individuals at risk, preventing the progression of valvular lesions.

Characterization of RF in a hospital based study in Egypt

A cohort of patients with history of RF and RHD assessed at the penicillin clinic and cardiothoracic surgery department of the National Heart Institute (NHI), which is a major referral heart center, serving the population of Cairo and surrounding areas with a big influx of patients

from all over Egypt. A penicillin prophylaxis program of the Ministry of Health is based at the NHI for patients that have been previously diagnosed with RF and prescribed penicillin prophylaxis. This clinic is assessable to all patients in the area and receives patients from all over Egypt by self referral. Therefore, the population visiting the NHI is thought to represent the prevalence of the RF and RHD in the highly populated Northern part of Egypt.

The assessment included a questionnaire containing questions regarding the patient's demographics, family environment, risk factors, history of RF and penicillin prophylaxis developed to detect the epidemiologic factors and determinants of RF and RHD. Physical examination included general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation.

This study focused on the epidemiological profile and clinical manifestation of RF in Egypt as well as the use and compliance to secondary prevention and define the determinants of RHD.

Clinical characteristics and determinants of RHD in a hospital study in Egypt

The individuals screened at the NHI were assessed by detailed transthoracic echocardiography evolving a newly developed protocol.

Diagnosis of RHD: The characteristics of RHD and the role of echocardiography as a screening tool was defined. This study evolves a set of rigid echocardiographic criteria, designed to enhance the specificity of the diagnosis of RHD to increase case detection rates and therefore the treatment of individuals at risk, preventing the progression of valvular lesions.

Subclinical RHD in individuals with history of RF in Egypt: Subclinical RHD was determined in individuals with history of RF presenting at the penicillin clinic of the NHI by echocardiography.

Echocardiographic characteristics of RHD: Echocardiographic data of individuals diagnosed with RHD was analyzed for the determination of the echocardiographic characteristics.

Prognosis of RHD: The patients recruited in the penicillin clinic were reassessed 6 months after the first consultation for the evaluation of the lesions, progression and/ or resolution of the

cardiac condition to analyze the reversibility of subclinical valvular lesions with adherence to secondary prophylaxis regimens and determine the long-term outcome of patients with subclinical RHD.

Determinants of RHD: The factors influencing the presence and characteristics of RHD were analyzed in the cohort.

ANALYSIS OF CELLULAR AND STRUCTURAL FEATURES IN RF AND RHD

In order to achieve this aim the following studies were performed:

Immune mediators in RF and RHD

Blood samples and excised valve tissue material collected at the time of surgery from patients requiring valve replacement from the individuals recruited at the NHI collection of samples were analyzed for determination of the immune response in RF and RHD.

Haemal immune response in RF and RHD: Analysis of cellular features and the signs of immune activation included determination of haemal inflammatory parameters as CBC, ESR, ASOT and CRP and cytokine levels in the serum and valvular tissue. Serum and plasma were obtained and stored for further analysis of the acute phase proteins, pro- and anti-inflammatory cytokines and signs of immune activation to determine the molecular and cellular mechanisms

Valvular immune activation in RHD: The valves collected were analyzed by immunohistochemistry for the presence and distribution of pro- and anti-inflammatory markers to determine the cellular features of RHD and the role of regulatory cytokines in valvular damage.

Determinants of RF and RHD: The haemal and valvular immune activation and response were analyzed to determine the immune mediators in RF and RHD. The influence of inflammatory and immune parameters on valvular affection were analyzed to determine the mediators of valvular lesions in RHD.

Structural characteristics of RHD

Excised valve tissue material collected at the time of surgery from patients requiring valve replacement from the individuals recruited at the NHI collection of samples were analyzed by

histology and immunohostochmistry to determine the structural and architectural features of valvular damage related to RHD.

DEFINITION OF ANTIGENIC TARGETS FOR EVALUATION AND DIAGNOSIS OF STREPTOCOCCAL INFECTION

A throat swab and venous blood were collected from the individuals recruited at the NHI.

Streptococcal strain responsible for RF in Egypt: The throat swab were streaked across a blood agar plate for culturing of the organism and the hemolytic colonies identified were tested for antibiotic sensitivity for the analysis of the effectiveness of antibiotic treatment and prophylaxis in these cases. Cultures of throat swab material and serum samples were analyzed to identify the particular strain of Streptococci responsible for RF/RHD in Egypt and its rheumatogenicity through serotyping for the particular type of M protein as well as DNA studies of the genes which encode these virulence factors (emm).

Atempt to determine the potential of PARF as a diagnostic marker: Collagen-binding assays were performed for the evaluation and analysis of the binding between the octapeptide PARF and collagen IV and the potential of PARF as a diagnostic marker for the early detection of rheumatogenic strains and the diagnosis of RHD.

2.2.1 Design of the investigations

Our research project included three main branches, namely community-based studies, hospital-based research and laboratory work (Diagram 2.1).

(1) Community Work 1 (National Heart Institute – Cairo, Egypt)

Epidemiology studies

Prevalence study

Follow-up of participants in epidemiology and clinical studies

Establishment of a follow-up facility for patients with RF and RHD

(1) Community Work 2 (Aswan Heart Centre – Aswan, Egypt)

Training of local staff

Design and Installation of Research Facilities

Contribution to establishment and development of Aswan Heart Centre

Epidemiology studies

Prevalence study

Establishing cohort of school children

Follow-up of participants in epidemiology and clinical studies

Establishment of a follow-up facility for patients with RF and RHD

(2) Clinical Research 1 (National Heart Institute – Cairo, Egypt)

Establishment of a Penicillin Prophylaxis Registry

Data collection

Clinical follow-up of patients

Blood and tissue collection and storage

(2) Clinical Research 2 (Aswan Heart Centre – Aswan, Egypt)

Organization and participation in surgical missions

Establishment of surgical registry

Pre- and post-operative management

Establishment of a RF and RHD registry

Data collection

Blood and tissue collection and storage

Clinical follow-up of patients

(3) Laboratory Studies (United Kingdom and Germany)

Performing the laboratory work

Diagram 2.1. Work undertaken by the investigator in the four main sites of research

2.2.2 Ethical considerations

Individuals were notified about the results of the tests and eventual need of any further treatment or follow-up and were informed that they are free to withdraw from the study at any time without giving any specific reasons.



Figure 2.1 Cardiac surgery at the Aswan Heart Centre

2.3 Layout

The work presented in this thesis has been divided in 11 chapters, starting with an introductory chapter, followed by the current chapter describing the aims and plan of investigation.

Chapter 3 details of the design of research and the procedures used to implement the research program, including the methods used in the different studies.

The school based screening for RHD is described in chapter 4, which involves large-scale echocardiographic screening and determination of prevalence of RHD in Aswan including description of the sampling method, issues related to preparation and implementation and the results found.

Chapter 5 presents a study in a cohort of patients with history of RF and RHD assessed at the penicillin clinic and cardiothoracic surgery department of the National Heart Institute (NHI) to unveil the epidemiology of RF in Egypt focusing on the epidemiological profile and clinical manifestation of RF as well as the use and compliance to secondary prevention and define the determinants of RHD.

Chapter 6 describes the clinical characteristics and determinants of RHD patients with history of RF in Egypt assessed by detailed transthoracic echocardiography evolving evaluation of a newly developed protocol. The different parts of the chapter present the determination of subclinical RHD as well as the echocardiographic characteristics of RHD, the prognosis of valvular lesions and determinants of the disease.

Chapter 7 describes the pattern of immune response in RF and RHD with analysis of cellular features and the signs of immune activation including haemal inflammatory parameters and cytokine levels. The valvular activation in RHD is described in the second part of the chapter, analyzing the inflammatory markers and the role of regulatory cytokines in valvular damage determining the immune mediators of RHD.

The structural features seen in valvular damage related to RHD are presented in chapter 8 as analyzed by histology and immunohostochmistry to determine the structural and architectural characteristics of the disease.

Determination of the antibiotic sensitivity and resistance rates of the found hemolytic colonies is described in chapter 9.

Chapter 10 presents the studies performed for definition of the Streptococcal strain responsible for RF in Egypt and its rheumatogenicity through serotyping for the particular type of M protein as well as DNA studies of the genes which encode these virulence factors (emm). Part 2 of the chapter describes the collagen-binding assays performed for the evaluation and analysis of the binding between the octapeptide PARF and collagen IV for the potential of PARF as a diagnostic marker for the early detection of rheumatogenic strains and the diagnosis of RHD.

The last chapter summarizes the conclusions of our work and proposes new research lines into the mechanisms of RF and RHD.

CHAPTER 3

METHODS

3.1 Epidemiology study

Egypt

Egypt is located in North Africa, with the Sinai Peninsula forming a land bridge in Western Asia. Covering an area of about 1,010,000 square kilometers (390,000 sq mi) (Figure 3.1).



Figure 3.1 Map of Africa, with Egypt marked in red.



Figure 3.2 Detailed map of Egypt

At 1,001,450 square kilometers (386,660 sq mi), Egypt is the world's 38th-largest country. Egypt is divided into 29 governorates, which are further divided into regions. Egypt is one of the most populous countries in Africa and the Middle East with its 83,082,869 (July 2009 est.) citizens. For population characteristics see (Appendix I).

The great majority of the population lives near the banks of the Nile River, in an area of about 40,000 square kilometers (15,000 sq mi), where the only arable agricultural land is found. The large areas of the Sahara Desert are sparsely inhabited. The population density is estimated 81.5 per sq km (Figure 3.3).

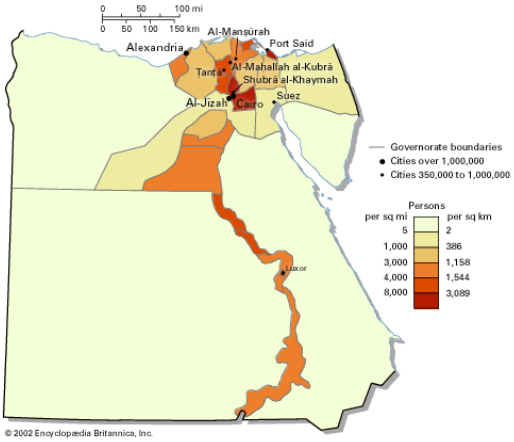


Figure 3.3 Population density

3.1.1 Epidemiological set-up

Study area

Cairo

The Egyptian capital has a surface of 214 km2 and 18,3 million inhabitants (2008 estimate) with a density exceeding 37,136 inhabitants/km^2 (Figure 3.4).



Figure 3.4 Site of Cairo (top centre)

National Heart and Lung Institute

The National Heart Institute (NHI), in Imbaba, Cairo, is one of the major heart centres in Cairo and surrounding areas and has a big influx of patients from all over Egypt. The NHI, founded in 1965 as a public institute, is a part of the General Organization of the Teaching Hospitals and Institutes "GOTHI" and has a total of 350 beds. A penicillin prophylaxis program of the Ministry of Health is based at the NHI and is meant for patients that have been previously diagnosed with RF and prescribed penicillin prophylaxis. This clinic is assessable to all patients in the area and receives patients from all over Egypt who choose to be treated at the NHI.

Aswan

In order to perform the screening of school children in Aswan an epidemiological study was performed.

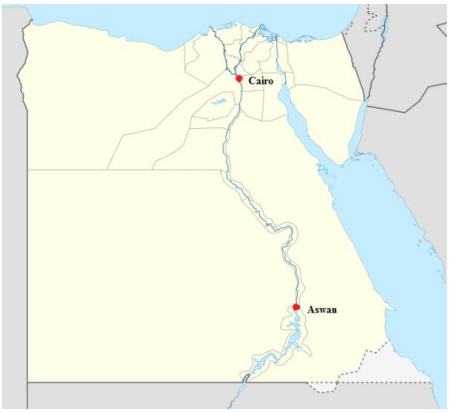


Figure 3.5 Site of Aswan

Aswan Governorate is the south most governorate in Upper Egypt (Figure 3.5). The distance between Cairo and Aswan is 879 km (549 miles). The population of the governorate is around 1,484,433 and its area is 34,608 km². Primary school attendance is over 95%. The schools are distributed among 7 areas. The distribution of the schools and school children in each area is shown in Appendix II. There are in total 51 402 school children distributed among 95 schools (Table 3.1).

Table 3.1 Amount of school children per age group

Age group	number
5 years	1 249
6-12 years	14 457
13-15 years	35 696
Total	51 402

Hospital-based study

Individuals were recruited at the National Heart Institute (NHI), in Imbaba, Cairo, one of the major heart centers in Cairo and surrounding areas with a big influx of patients from all over Egypt, where a penicillin prophylaxis program of the Ministry of Health is based for patients that have been previously diagnosed with RF and prescribed penicillin prophylaxis. This clinic is assessable to all patients in the area and receives patients from all over Egypt who choose to be treated at the NHI.

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI were assessed for the investigation of the epidemiological and clinical characteristics, molecular and cellular mechanisms and microbiology of RF and RHD.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

The individuals recruited in the penicillin clinic were reassessed 6 months after the first consultation for evaluation of the development, progression and/ or resolution of the cardiac manifestation to analyze the reversibility of valvular lesions with adherence to secondary prophylaxis regimens and determine the long-term outcome of patients with subclinical RHD.

We think that it is legitimate to compare both patient groups, as they form part of the same patient population that visits the NHI and group II represents group I in a later stage of the disease. Therefore, this allowed us to compare different stages and presentations of the disease: individuals on penicillin prophylaxis with and without RF and/or cardiac involvement, individuals with evidence of RHD with and without history of RF and/or penicillin prophylaxis.

School-based screening

A cross-sectional screening of schoolchildren from 5 to 15 years of age was performed in Aswan, Egypt.



Figure 3.6 School children awaiting assessment in Aswan during the screening

Sample size calculation

Previous studies in Mozambique and Cambodia reveal a prevalence of RHD as detected by echocardiography of 3.0% (95% CI 2.3-3.8) and 2.2% (95% CI 1.7-2.6) respectively in schoolchildren aged 6-17 years. Assuming that the clinical prevalence of RHD in school children in Egypt is comparable to the previous studies we hypothesize a prevalence of RHD in school children in Aswan around 2.5 to 3.0%. This means that 25 to 30 RHD cases would be detected per 1,000 screened children.

In epidemiological studies and studies that investigate factors associated with RHD, it is required to have a substantial number of RHD cases, approximately 50 cases, in order to be able to do multivariable analyses. Therefore it was decided to screen 3,000 children which is expected to detect 75 children showing characteristics of RHD following the earlier provided prevalence forecast.

Sample selection

The Governorate of Aswan provided us with the map of Aswan, distributed in 7 geographical areas and created a list of all schools in the area. By this mean we were able to obtain the number of schools and school children for each age group in the different areas.



Figure 3.7 Map of Aswan City divided in 7 geographical areas

Sample size

The number of children needed to screen from each area was calculated as following:

$$\frac{Children_{area}}{Children_{total}} = Representation_{area} \quad (1)$$

$Children_{area}$ = total school children in the area
$Children_{total}$ = total of school children in Aswan
$Represenation_{area}$ = representation of the area in the total cohort

$$Representation_{area}\ x\ Screen_{total} = Screen_{area} \quad (2)$$

$Representation_{area}$ = representation of the area in the total cohort
$Screen_{total}$ = total number to screen (in this case= 3000)
$Screen_{area}$ = number needed to screen in the area

The schools are distributed in 3 age groups: 5 years, 6 to 12 years and 13 to 15 years. The number of children screened in each age group was dependent on their representation in the age groups in the area so that the screened population was representative for the area as well as the age group. The number of children needed to screen from each age group was calculated as following:

(3)

$$\frac{Children_{agegroup}}{Children_{area}} = Representation_{agegroup}$$

$Children_{agegroup}$ = total of children in a certain age group
$Children_{area}$ = total school children in the area
$Represenation_{agegroup}$ = representation of the age group in the area

(4)

$$Representation_{agegroup}\ x\ Screen_{area} = Screen_{agegroup}$$

$Represenation_{agegroup}$ = representation of the age group in the area
$Screen_{area}$ = number needed to screen in the area
$Screen_{agegroup}$ = number needed to screen in the age group

The distribution of the children by area, age group, representation and the number needed to screen per area and age group can be found in appendix III.

Sampling

Selection of the sample was done by randomization in three stages stratified by area, school and age group and weighed by distribution of school children across the 7 areas and 3 age groups.
The schools were stratified by area and age group and randomly selected using computer-generated random-number sampling.
On the day of screening, the children were invited to participate in the study and attributed numbers for random-number sampling, according to the number needed to screen, weighed by the representation of the age group in the area. There was no exclusion criteria and all children had the same probability chances of being chosen. The list of schools visited, selected by age group as well as the number of children screened can be found in Appendix IV.

The number of children screened in each school and area exceeds slightly the number needed to screen due to logistical matters. Area 7 did not have schools in the category of 5 year olds and the number needed to screen in area 1 for this category was 1 which was not feasible due to logistics.

3.2 Clinical study

Examination protocol

The screened individuals underwent a standardized examination protocol consisting of detailed medical history recorded by the investigator, physical examination including general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation with the patient in the supine and left lateral decubitus positions. From individuals assessed at the NHI blood samples and throat swabs were obtained. A unique identification number was attributed to all participants. The patient flow chart for clinical investigation in RF and RHD patients at the NHI penicillin clinic are shown in diagram 2.2 and diagram 2.3. The flowchart for clinical investigation in a cohort of school children in Aswan is shown in diagram 2.4.

Disease manifestations and auscultatory findings were evaluated according to internationally approved criteria, including the published data from World Health Organization (WHO 1988, Figueroa 1992). Detailed transthoracic echocardiography was performed with the use of a hand-carried, battery operated echocardiographic system (Vivid *i*, General Electric) with M-mode, two-dimensional, and Doppler (pulsed, continuous, and color) imaging. The following were obtained: apical two-, four-, and five-chamber views, parasternal long-axis and short-axis views at the level of the papillary muscles and the aortic valve and subcostal views.

This study developed an abbreviated protocol that took 5–10 min per child and focused on rheumatic pathology of the mitral and aortic valves. Particular attention was paid to valve morphology on cross-sectional, two dimensional imaging and the degree and extent of mitral and aortic regurgitation, assessed by color flow Doppler imaging. The apical imaging included anterior angulation to evaluate the left ventricular outflow tract and the aortic valve. Transvalvular flow was assessed by measuring the peak velocity with continuous wave Doppler

imaging. Hard copies with detailed location and type of lesions (Appendix V, page 3) and electronic records of relevant data were kept for offline re-evaluation.

The echocardiographic study was performed by the candidate. Two blinded experienced cardiologists reviewed the records. The diagnosis of RHD was accepted when there was agreement of at least two cardiologists, including the researcher. All children suspected of cardiac involvement or lesions were reassessed by echocardiography at the Aswan Heart Centre.

Visit to Penicillin clinic at the NHI

(Referred by health unit, referred by the community or self-referral)

↓

Registration (after informed consent)

↓

Clinical history and examination

Echocardiography

↓

Throat swab

Storage of B-hemolytic strains in THB tubes for bacteriology studies (Germany)

↓

Blood collection

Full Blood count

ESR, CRP, ASOT

Storage of plasma, serum and blood cells at -80˚C for studies on pathogenesis (UK)

↓

Follow-up: medical appointment after at least 6 months

Diagram 2.2 Flowchart for clinical investigation in RF and RHD patients at the NHI penicillin clinic

Admission to cardiothoracic surgery department at the NHI

(Referred by health unit, referred by the community or self-referral)

↓

Registration (after informed consent)

↓

Clinical history and examination

Echocardiography

↓

Throat swab

Storage of B-hemolytic strains in THB tubes for bacteriology studies (Germany)

↓

Blood collection

Full Blood count

ESR, CRP, ASOT

Storage of plasma, serum and blood cells at -80˚C for studies on pathogenesis (UK)

↓

Surgery

Collection of valvular tissue during procedure and storage of samples

Diagram 2.3 Flowchart for clinical investigation in RHD patients scheduled for surgery at the NHI

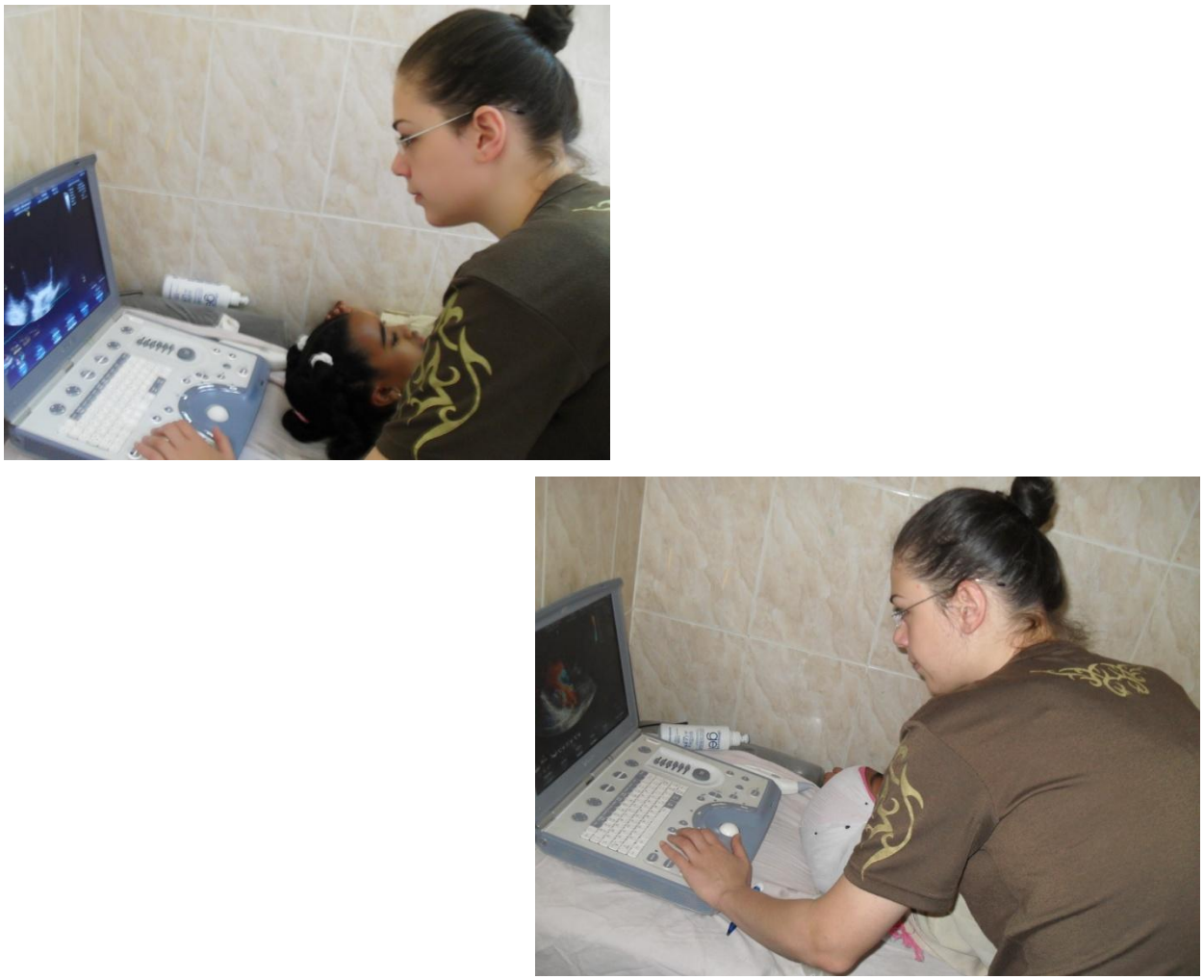


Figure 3.8 Echocardiographic screening of school children

Randomization of the schools by area, age and school

↓

Visit to schools in Aswan

Randomization of the children in the school

↓

Registration

↓

Clinical history and examination

Echocardiography

↓

Revision of retrieved echocardiographic data

↓

When required: Follow-up and reassessment appointment at the AHC

Diagram 2.4 Flowchart for clinical investigation in a cohort of school children in Aswan

Sample collection

A throat swab was taken and blood was collected by venipuncture into 1 siliconised EDTA, 1 non-additive and 1 citrate ESR tube. The throat swab was streaked immediately across a blood agar plate for culturing of the organism. The hemolytic colonies identified were tested for antibiotic sensitivity, stored into THB agar tubes.

A complete CBC, ESR analysis, qualitative determination and semi-quantitative determination of the ASOT and CRP was done. Serum and plasma was obtained and stored.

From the patients scheduled for valve replacement excised valve tissue material was collected at the time of surgery. The collected valves were immersed in formalin upon dissection followed by embedding of parts of the tissue sample in paraffin.

Demographic data

Demographic data from every subject selected were collected in the form of a standardized questionnaire (Appendix V). The questionnaire contains questions regarding the patient's demographics, family environment, risk factors, history of RF, its severity, treatment and prophylaxis.

Issues regarding the onset age of RF, symptoms, severity, presence of recurrences of RF, medication and penicillin prophylaxis were questioned on the history of RF and the presence of symptoms to confirm the presence of RF and clinical presentation. Penicillin prophylaxis schedule was divided into 3 categories, following the most used and prescribed schedules for penicillin prophylaxis, every 15 days, 3 weeks or monthly. Compliance rate for the penicillin prophylaxis was evaluated by asking the patient about the schedule, how often he ignored or postponed the injection date and if he was strict to the schedule. Compliance was then also be evaluated in relation to the schedule. A schedule of less than 9 injections a year was considered poor. A schedule of 9 to 12 injections a year was considered fair, while an excellent compliance rate was more than 12 injections a year. Many individuals were receiving prophylaxis for the first time therefore questions on schedule and compliance was not applicable.

The disease awareness as classified in 3 categories evaluated on the patients' knowledge of the RF history, symptoms and penicillin prophylaxis. The disease awareness was also be evaluated by the amount and type of questions answered on the knowledge of RF and RF history. The disease awareness was evaluated as poor when the patient had no knowledge of the reason for penicillin prophylaxis. When the patient was aware of RF or the valvular damage, and knew that penicillin is a way of prophylactic prevention but did not know the link between RF and the valvular damage the awareness was evaluated as fair. The patient's awareness was evaluated as good when he was also aware of the correlation between RF and cardiac disease (RHD). The same scale was used for the parents' awareness. Dental care was evaluated during collection of throat swabs by the general condition of the mouth and teeth.

Echocardiographic criteria

Based on recent studies on the design and development of diagnostic criteria for RHD (WHO 2004, Marijon 2007, Carapetis 2008, Marijon 2009) and our own clinical experience a new comprehensive set of criteria to determine the presence and extent of cardiac involvement was developed (Criteria 4, Aswan Heart Centre (AHC) criteria).

Our suggested criteria for the diagnosis of RHD consists of the presence of any Doppler-detected valvular affection of the right-sided valves in association with at least one structural rheumatic valvular feature. Regurgitation should be defined as pathological when meeting all four criteria defined by the WHO (Table 1.3). However, including trivial affection. Mitral stenosis should diagnosed in case of flow acceleration across the mitral valve and a mean pressure gradient greater than 4 mmHg. Similarly, flow acceleration across the aortic valve with a peak velocity greater than 2 m/s is necessary for diagnosis of aortic stenosis.

The structural criteria should be based on the study of (1) quantitative leaflet morphology (typical marked thickening of the margins, greater than 4mm), (2) leaflet shortening with abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures) (Table 3.2).

Table 3.2 AHC criteria: Echocardiographic criteria for the diagnosis of RHD (Criteria 4)

Functional criteria
Mitral or Aortic Regurgitation (at least mild)
Mitral or Aortic Stenosis
Structural criteria
Thickening of valve cusps
Doming of valve cusps
Fused subvalvular apparatus
Leaflet shortening and restriction

After completion of the hospital based study and evaluation of the data we have decided to adjust the echocardiographic criteria for the diagnosis of RHD in the school based screening (Criteria 5). We believe that in a young population subclinical RHD can present as single and isolated lesions rather than combinations of lesions which are expected to appear in a more chronic phase.

In this criteria any degree of regurgitation together with valvular thickening of leaflet restriction lead to the diagnosis of RHD, as well as the finding of isolated fusion of the subvalvular apparatus and/or commisure or aortic regurgitation, as these features are uncommon in healthy children. Isolated valvular thickening, leaflet restriction or at least mild mitral regurgitation indicate possible RHD (Table 3.3). We believe that this criteria is more sensitive for screening purposes in young children, avoiding over- and underdiagnosis of RHD.

Table 3.3 Modified AHC criteria: Echocardiographic criteria for the diagnosis of RHD (Criteria 5)

Definite RHD
Valvular thickening & any degree of regurgitation
Leaflet restriction & any degree of regurgitation
Fusion of subvalvular apparatus or/and commisures
Aortic regurgitation caused by cusp retraction
Possible RHD
Isolated valvular thickening
Isolated leaflet restriction
Isolated ≥ mild mitral regurgitation

3.3 Laboratory study

3.3.1 Haemal assays

Sample collection: The blood was collected following international venipuncture protocols.

Detailed steps of venipuncture:

- The median cubital and cephalic veins of the arm were palpated and traced with the index finger
- The patient as positioned so he or she was comfortable and safe in case of fainting and falling
- The venipuncture site was prepared with an alcohol swab in a circular fashion.
- The tourniquet was applied 3-4 inches above the selected puncture site.
- The venipuncture was performed with the patient's arm in a downward position.
- The tourniquet was removed as soon as blood appeared in the tube
- Succeeding tube was placed in the holder puncturing stopper to initiate flow
- While each successive tube was filling the previous tube was inverted gently 5 times
- When all tubes of blood had been collected, the last tube was removed from the vacutainer holder, and a cotton ball or gauze was placed over the site and the needle was withdrawn in a smooth and cautious manner
- After withdrawing the needle fully, pressure was applied to the cotton ball over the puncture site and hold pressure. The patient was asked to apply pressure for 3 to 5 minutes until the bleeding stops.

Blood was collected into:

-1 siliconised glass tubes containing EDTA

-1 non-additive tube

-1 citrate ESR tube

Preparation of samples

EDTA tube: The tube containing blood on EDTA was run through the Sysmex KX-21N for CBC count and the centrifuged to obtain plasma.

Non-additive tube: The blood collected in the non-additive tube was centrifuged to obtain serum. This serum was used for the CRP and ASOT analysis. 1 ml of whole blood was aliquoted in an Eppendorf tube for storage.

ESR citrate tube: 1 ml of blood was collected in an ESR tube to determine the rate with the Wintrobe method.

Preparation of serum

- blood samples collected in non-additive blood collection tubes were centrifuged at 4000 rpm for 4 min
- the supernatant (serum) was aliquoted into 2 Eppendorf tubes (approximately 1ml of serum each)
- the eppendorf tubes were stored in a freezer at -40°C
- the samples were later transported to the Helmholtz Centre in an icebox

Preparation of plasma

- blood samples collected in EDTA collection tubes were centrifuged at 4000 rpm for 4 min
- the plasma was aliquoted into 2 Eppendorf tubes (approximately 1ml of plasma each)
- the Eppendorf tubes were stored in a freezer at -40°C

3.3.2 Valvular tissue assays

Sample collection: Excised valves of patients with a history of RF/RHD were collected during routine valve replacement procedures. The valvular tissue was stored in formalin directly after collection. Parts of the tissue was later embedded in paraffin.

Paraffin embedding protocol

- The tissue was fixed in 70% Ethanol
- The tissue was then placed in the tissue processor.
- The specimens were embedded in paraffin.

- The tissue block was attached to a hardwood block by melting the back of the tissue block with a warm spatula and firmly pressing the two together.
- 8 μm thick sections were cut with a standard microtome
- The sections and paraffin blocks were stored at room temperature.

2.3.3 Bacteriology assays

Sample collection: The specimen for throat culture were obtained by the following protocol:

- The patient was asked to tilt the head back and open the mouth wide.
- With the tongue depressed and the patient saying "ah," the back of the throat and the tonsils were wiped with a sterile swab.
- The swab is removed gently without touching the teeth, gums, or tongue.
- The swab was streaked immediately across a blood agar plate

Culturing

- The swab was streaked immediately after collection across a blood agar plate
- The blood agar plate was allowed to incubate at (35°–37°C) for 24–48 hours to allow the growth of bacteria
- After incubation the colonies on the agar plates were analyzed
- Hemolytic colonies were identified

Sensitivity testing: Susceptibility testing is used to determine which antimicrobial inhibits the growth of the bacteria causing the infection. If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition). The size of the zone and the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. The results from this test helped determine which antibiotic was the most effective in treating the infection.

- Antibiotics tested:
  - Levofloxacin 5 mcg (BioAnalyse)
  - Erythromycin 15 mcg (BioAnalyse)
  - Vancomycin 30 mcg (BioAnalyse)
  - Ciprofloxacin 5 mcg (BioAnalyse)
  - Gentamycin 10 mcg (BioAnalyse)
  - Doxycicline 30 mcg (BioAnalyse)
  - Penicillin 10U (BioAnalyse)
  - Bacitracin 10U (BioAnalyse)
  - Taxo- differentiation discs (Benex Limited)

Detailed steps:

- Samples of the identified colonies were re-streaked on antibiotic agar plates
- Antibiotic discs were placed on the agar plate
- After incubation the colonies on the agar plates were analyzed for the growth pattern
- The antibiotic sensitivity was analyzed

Storage

- Samples of the identified colonies were collected with a sterile loop
- Sterile loop were stabbed in THB agar tubes
- The tubes was allowed to incubate at (35°–37°C) for 24–48 hours
- The agar tubes were stored at 4°C for a maximum of 3 weeks
- The tubes were transported to the Helmholtz Centre at room temperature

3.4 Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences, version 16 (SPSS 16). Firstly all variables were tested for normality using Kolmogrov-Smirnov test. If the test was significant, non-normality was accepted otherwise double-check using graphs, skewness and kurtosis were required to confirm normality (Chan 2003).

Most of the quantitative variables in this research were not normally distributed and accordingly are presented as median (range). Quantitative data are presented as mean ± SD when normality assumptions were satisfied. Qualitative data as frequencies are presented as number (percentage).

Categorical variables were compared using Chi-square analysis (x^2) (Chan 2003[2]). A P-value of <0.05 was considered statistically significant (Chan 2003). Because most of quantitative data were not normally distributed, variables were compared between two related samples using the Spearman test and two unrelated samples using the Mann Whitney U test. When normal distribution was confirmed, data were compared using paired and unpaired T-test, respectively (Chan 2003).

3.5 Ethical considerations

Hospital-based study

Ethical approval was granted by the Imperial College London (appendix VI) and the National Heart Institute. Patient information sheet and informed consent were designed (Appendix VII). All volunteers were given written and oral detailed information, interviewed through the RF and RHD characteristic questionnaire and asked to sign an informed consent form which was translated into Arabic.

All subjects were asked to undertake physical and echocardiographic examination and collection of blood and throat swab samples. The physical examination included general analysis of the patients' health, recognition of Jones criteria, cardiac auscultation and echocardiography. From the patients scheduled for valve replacement excised valve tissue material was collected at the time of surgery. Individuals were notified about the results of the tests and eventual need of any

further treatment or follow-up and were free to withdraw from the study at any time without giving any specific reasons.

Cohort school children

The research project was approved by the appropriate authorities at the Governorate of Aswan and the Egyptian Ministry of Health and Education, as representatives of the population and education system (Appendix VIII).

The local authorities and school personnel were given written and oral detailed information. The physical examination included general analysis of the patients' health, recognition of Jones criteria, cardiac auscultation and echocardiography. The local health authorities and caretakers were notified about the results of the tests and eventual need of any further treatment or follow-up. Individuals were free to withdraw from the study at any time without giving any specific reasons. Children with cardiac affection were offered free treatment, follow-up and surgery if indicated at the Aswan Heart Centre in Aswan.

CHAPTER 4

SCHOOL BASED SCREENING FOR RHEUMATIC HEART DISEASE

4.1 Background

In Egypt, a high percentage of schoolchildren have been reported to have clinical evidence of cardiac valvular damage due to RHD, with a severe and aggressive couse of the disease (Kassem 1982, Abdin 1968, El Sherif 1975). Although these studies have been helpful in forming an idea on RHD in the Egyptian youth, they have not been systematic and did not use echocarediographic evaluation and diagnosis of the valvular lesions. Echocardiography is found to be the only approach with sufficient sensitivity and specificity for the detection of subclinical carditis and RHD (Marijon 2007, Carapetis 2008) and has resulted in high prevalence rates of RHD in different areas confirming that the prevalence of RHD in school-aged children is far greater than earlier estimates.

Due to the lack of data on prevalence, characteristics and determinants we designed a cross-sectional screening using systemic sampling in a cohort of randomly selected school children in Aswan, in whom auscultation followed by echocardiography was used to identify the presence, characteristics and determinants of RHD.

The aim of this study was to evaluate the valvular affection and diagnosis of RHD and determine the epidemiological and clinical characteristics as well as risk factors and determinants of RHD in an area where data on regard the disease is completely lacking.

4.2 Participants and methods

Screening

A cross-sectional screening of schoolchildren from 5 to 15 years of age was performed in Aswan as described in chapter 3, pg. 76.

Sample size calculation

A total of 3,062 children were screened for the characteristics of RHD following the earlier provided prevalence forecast.

Sample selection

The number of schools and school children for each age group in the different areas was obtained.

Sample size

The number of children needed to screen from each area was calculated as following:

$$\frac{Children_{area}}{Children_{total}} = Representation_{area} \quad (1)$$

Children$_{area}$ = total school children in the area
Children$_{total}$ = total of school children in Aswan
Represenation$_{area}$ = representation of the area in the total cohort

$$Representation_{area}\ x\ Screen_{total} = Screen_{area} \quad (2)$$

Representation$_{area}$ = representation of the area in the total cohort
Screen$_{total}$ = total number to screen (in this case= 3000)
Screen$_{area}$ = number needed to screen in the area

The schools are distributed in 3 age groups: 5 years, 6 to 12 years and 13 to 15 years. The number of children screened in each age group was dependent on their representation in the age groups in the area so that the screened population was representative for the area as well as the age group. The number of children needed to screen from each age group was calculated as following:

$$\frac{Children_{agegroup}}{Children_{area}} = Representation_{agegroup} \quad (3)$$

Children$_{agegroup}$ = total of children in a certain age group
Children$_{area}$ = total school children in the area
Represenation$_{agegroup}$ = representation of the age group in the area

$$Representation_{agegroup}\ x\ Screen_{area} = Screen_{agegroup} \quad (4)$$

Represenation$_{agegroup}$ = representation of the age group in the area
Screen$_{area}$ = number needed to screen in the area
Screen$_{agegroup}$ = number needed to screen in the age group

The distribution of the children by area, age group, representation and the number needed to screen per area and age group can be found in appendix III.

Sampling

Selection of the sample was done by randomization in three stages stratified by area, school and age group and weighed by distribution of school children across the 7 areas and 3 age groups.

The schools were stratified by area and age group and randomly selected using computer-generated random-number sampling.

On the day of screening, the children were invited to participate in the study and attributed numbers for random-number sampling, according to the number needed to screen, weighed by the representation of the age group in the area. There was no exclusion criteria and all children had the same probability chances of being chosen. The list of schools visited, selected by age group as well as the number of children screened can be found in Appendix IV.

The number of children screened in each school and area exceeds slightly the number needed to screen due to logistical matters. Area 7 did not have schools in the category of 5 year olds and the number needed to screen in area 1 for this category was 1 which was not feasible due to logistics.

Examination protocol

The screened individuals underwent a standardized examination protocol consisting of detailed medical history recorded by the investigator, physical examination including general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation with the patient in the supine and left lateral decubitus positions.

Disease manifestations and auscultatory findings were evaluated according to internationally approved criteria, including the published data from World Health Organization (WHO 1988, Figueroa 1992). Detailed transthoracic echocardiography was performed with the use of a hand-carried, battery operated echocardiographic system (Vivid *i*, General Electric) with M-mode, two-dimensional, and Doppler (pulsed, continuous, and color) imaging. The following were obtained: apical two-, four-, and five-chamber views, parasternal long-axis and short-axis views at the level of the papillary muscles and the aortic valve and subcostal views.

Particular attention was paid to valve morphology on cross-sectional, two dimensional imaging and the degree and extent of mitral and aortic regurgitation, assessed by color flow Doppler imaging. The apical imaging included anterior angulation to evaluate the left ventricular outflow

tract and the aortic valve. Transvalvular flow was assessed by measuring the peak velocity with continuous wave Doppler imaging. Hard copies with detailed location and type of lesions (Appendix V, page 3) and electronic records of relevant data were kept for offline re-evaluation. The echocardiographic study was performed by the candidate and two blinded experienced cardiologists reviewed the records. The diagnosis of RHD was accepted when there was agreement of at least two cardiologists, including the researcher. All children suspected of cardiac involvement or lesions were reassessed by echocardiography at the Aswan Heart Centre.

Demographic data

Demographic data from every subject selected were collected in the form of a standardized questionnaire (Appendix V). The questionnaire contains questions regarding the patient's demographics, family environment, risk factors, history of RF, its severity, treatment and prophylaxis.

Echocardiographic criteria

Only left-sided valves were evaluated for features of RHD as affection of the right sided valves is fairly common and not related to the condition (Lembo 1988, Kostucki 1986, Lembo 1988, Yoshida 1988). RHD was diagnosed by the different echocardiographic diagnostic criteria as following:

Criteria 1 (WHO 2004): (1) a regurgitant jet >1 cm in length, (3) seen in at least 2 planes, (3) a mosaic color jet with a peak velocity >2.5 m/s, (4) persisting throughout systole or diastole

Criteria 2 (Carapetis 2008)*:* (1) valvular regurgitation (meeting all four criteria defined by the WHO) with evidence of structural changes to valve appearance: (a) valvular thickening (b) elbow deformity of the anterior mitral valve leaflet (2) any degree of pathological aortic regurgitation (3) mitral stenosis (flow acceleration and a mean pressure gradient > 4 mmHg) (4) aortic stenoses (flow acceleration with peak velocity > 2 m/s)

Criteria 3 (Marijon 2007)*:* any degree of valvular regurgitation seen in at least 2 planes accompanied by at least two of the following three structural abnormalities of the regurgitant valve: (1) leaflet morphology (typical marked thickening of the margins), (2) leaflet mobility (abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures)

*Criteria 4 (*AHC criteria): The suggested criteria for the diagnosis of RHD consists of the presence of any Doppler-detected valvular affection of the right-sided valves in association with at least one structural rheumatic valvular feature (as extensively described in chapter 3, pg. 90). Regurgitation should be defined as pathological when meeting all four criteria defined by the WHO (Table 1.3), however, including trivial affection. Mitral stenosis should diagnosed in case of flow acceleration across the mitral valve and a mean pressure gradient greater than 4 mmHg. Similarly, flow acceleration across the aortic valve with a peak velocity greater than 2 m/s is necessary for diagnosis of aortic stenosis.
The structural criteria should be based on the study of (1) quantitative leaflet morphology (typical marked thickening of the margins, greater than 4mm), (2) leaflet shortening with abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures) (Table 3.2).

Criteria 5 (Modified AHC criteria): During the clinical work and evaluation of the data, criteria 4 was adjusted and modified (Criteria 5). In this criteria any degree of regurgitation together with valvular thickening or leaflet restriction lead to the diagnosis of RHD, as well as the finding of isolated fusion of the subvalvular apparatus and/or commisure or aortic regurgitation, as these features are uncommon in healthy children. Isolated valvular thickening, leaflet restriction or at least mild mitral regurgitation indicate possible RHD (Table 3.3).

4.3 Results

4.3.1 Demographics

A total of 3062 pupils between the ages 5 and 15 were screened in Aswan, representing 6% of the schools population in the area.

Age: The mean and median age was 10 years ± 2.6 years. The distribution of the age groups was representative for each group in the population of schoolchildren (Figure 6.1).

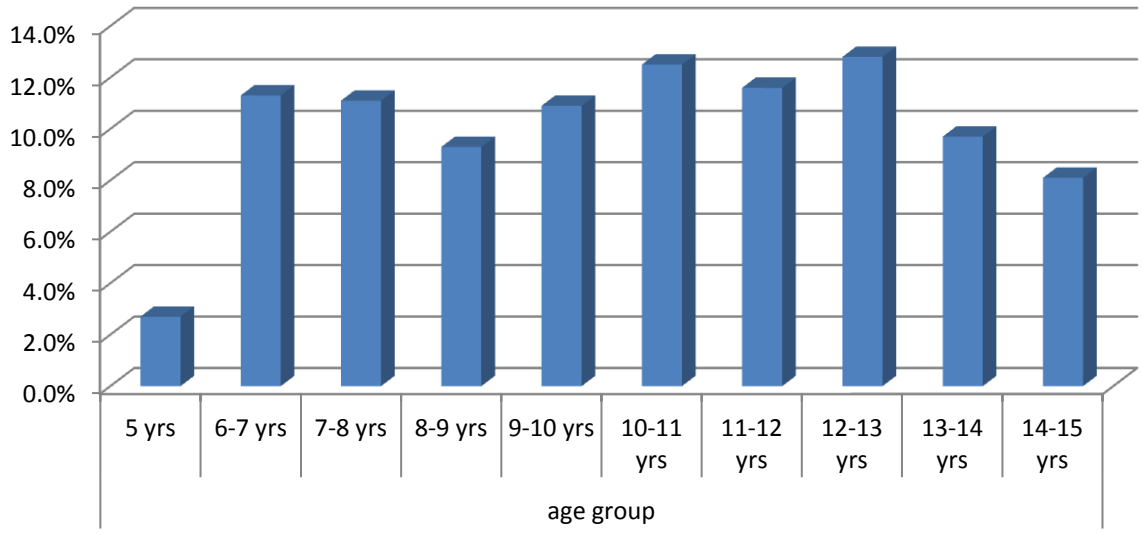


Figure 4.1 Distribution of age groups

Gender: There was a slight male predominance (58.7%, n=1796) in the group screened.

Social factors: The number of siblings ranged from 0 to 11 (3.14 ± 1.54) in the total of 1515 (49.47%) children that answered the question.

The majority of the children (90.2%, n=1776) reported that the father had an active job, while literacy of the father was reported in 74.7% (n=1085). A part of the children (20.4%, n=372) reported that the mother had an active job, with 63.6% (n=885) reporting literacy.

Medical history: The medical history remained unknown as the caretakers were not present during the screening and the lecturers and education staff did not have any information on the medical history.

4.3.2 Clinical findings

No major general health issues were detected and none of the children showed characteristics of the Jones Criteria for the diagnosis of RF.

Cardiac auscultation revealed murmurs in 0.4% (n=12) children, of which 0.23% (n=7) were free of cardiac affection as analyzed by echocardiography. Moderate aortic regurgitation was the cause of a cardiac murmur in 0.1% (n=3) of the children, while the remaining children (0.07%, n=2) had moderate or severe mitral regurgitation.

4.3.3 Echocardiographic findings

Structural features

In our series 3.3% (n=101) of the children screened had structural abnormality of the heart valves, namely thickening of the valve leaflets or cusps (≥4 mm). Isolated fusion or thickening of the subvalvular apparatus was seen in 0.03% (n=1) of the children. A combination of thickening of the valve leaflets or cusps and fusion or thickening of the subvalvular apparatus was seen in 0.1% (n=3) of the children. Prolapse of the mitral valve was seen in 0.16% (n=5) of the children.

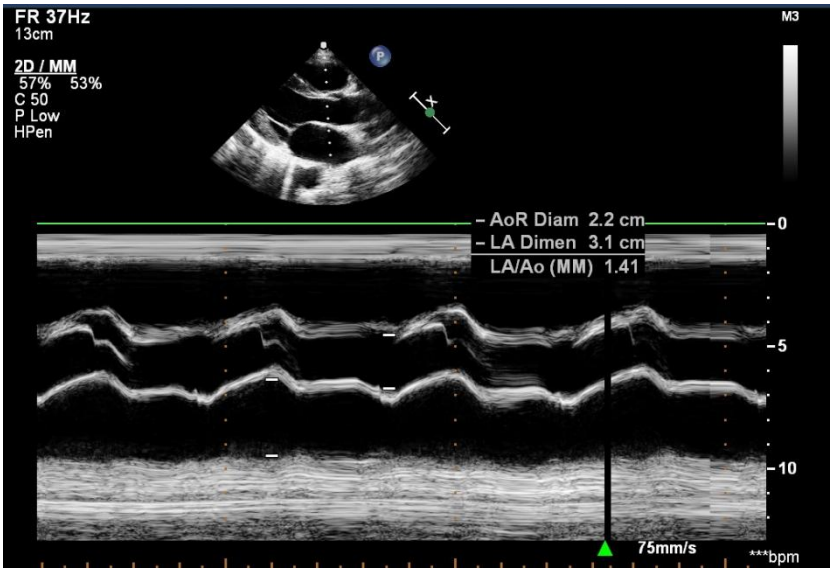


Figure 4.2 Measurement of the aorta and left atrium in a parasternal long-axis

Functional affection

Functional affection of the right sided heart valves was found in 16.3% (n=501) of the children assessed (Figure 4.2). Trivial mitral valve regurgitation was found in 14.3% (n=440) of the children while mild mitral regurgitation was seen in 1.9% (n=57) of the children. Moderate and severe mitral regurgitation was seen in 1 child (0.03%), respectively. Isolated mitral valve affection was seen in 16.2% (n=498) of the children.

Mild aortic regurgitation was seen in 0.1% (n=3) of the children. Isolated affection of the aortic valves was seen in 2 (0.07%) and a combination of mild mitral and aortic regurgitation was found in 1 (0.03%) child. Trivial and mild tricuspid regurgitation was seen in 0.4% (n=12) and 0.1% (n=4), respectively. No stenotic lesions were found in our study.

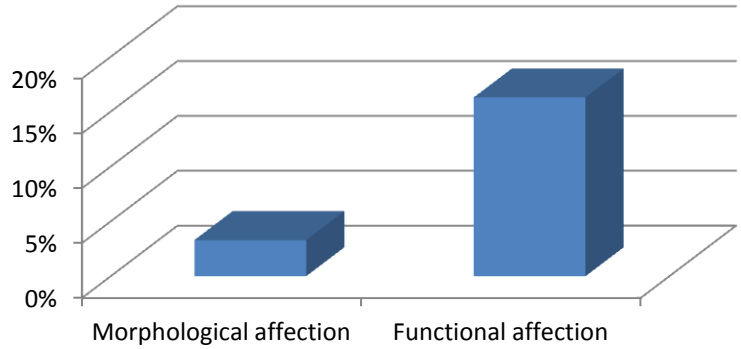


Figure 4.3 Valvular affection

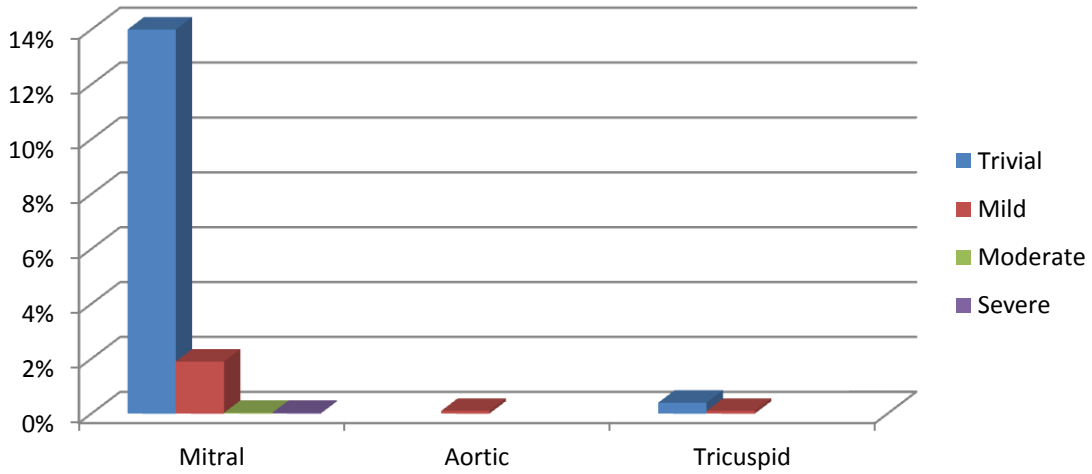


Figure 4.4 Distribution of regurgitant valvular affection

Features of RHD

Structural features: Thickening of the valve leaflets or cusps was seen in 94.3% (n=66) of the children diagnosed with RHD. Isolated fusion or thickening of the subvalvular apparatus was seen in 1.4% (n=1) of the RHD cases. A combination of thickening of the valve leaflets or cusps and fusion or thickening of the subvalvular apparatus was seen in 4.3% (n=3) of the children.

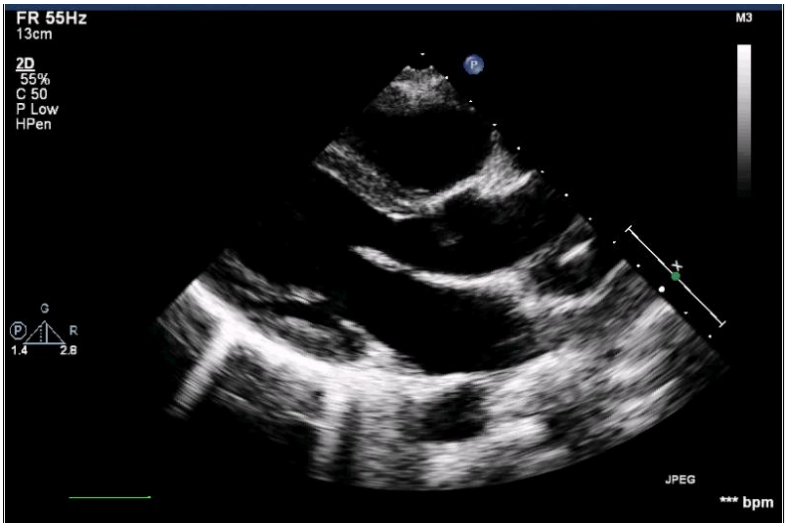


Figure 4.5 Thickening of the mitral leaflets in a parasternal long-axis

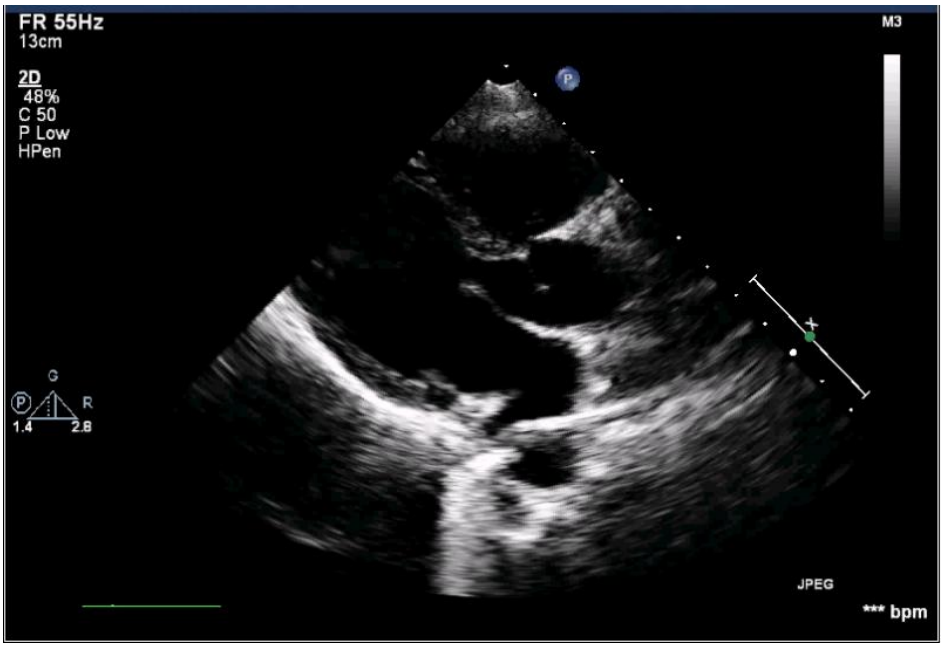


Figure 4.6 Thickening of the tip of the mitral leaflet in parasternal long-axis

Functional features: Isolated mitral regurgitation was seen in 96% (n=67), isolated aortic regurgitation in 2.9% (n=2) and both in 1.4% (n=1) of the RHD cases. No cases of mitral or aortic stenosis were seen. Trivial or mild mitral regurgitation accounted for 44.2% (n=31) and 51.4% (n=36) of the RHD cases, respectively. The regurgitation was mild in all cases of aortic affection (4.2%, n=3).

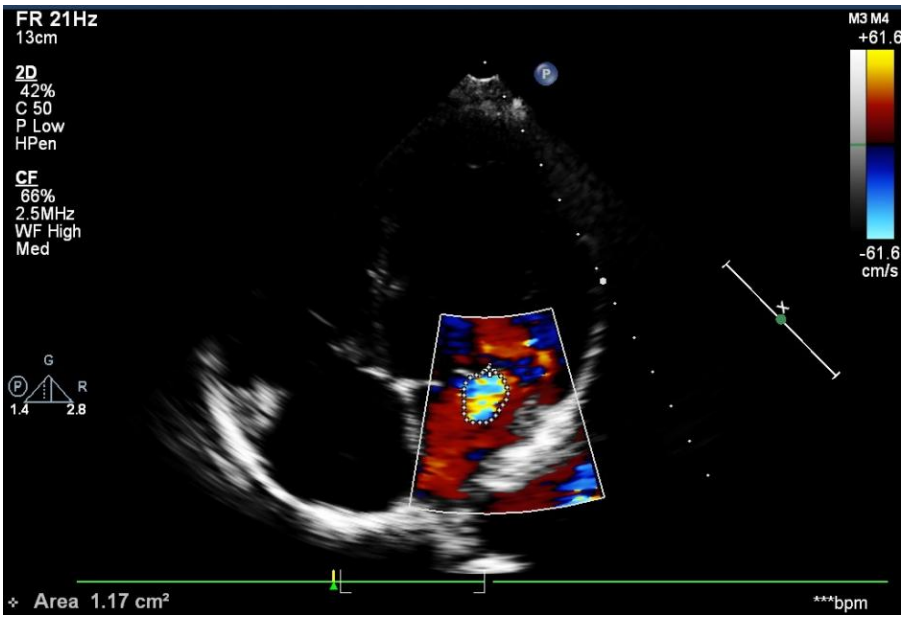
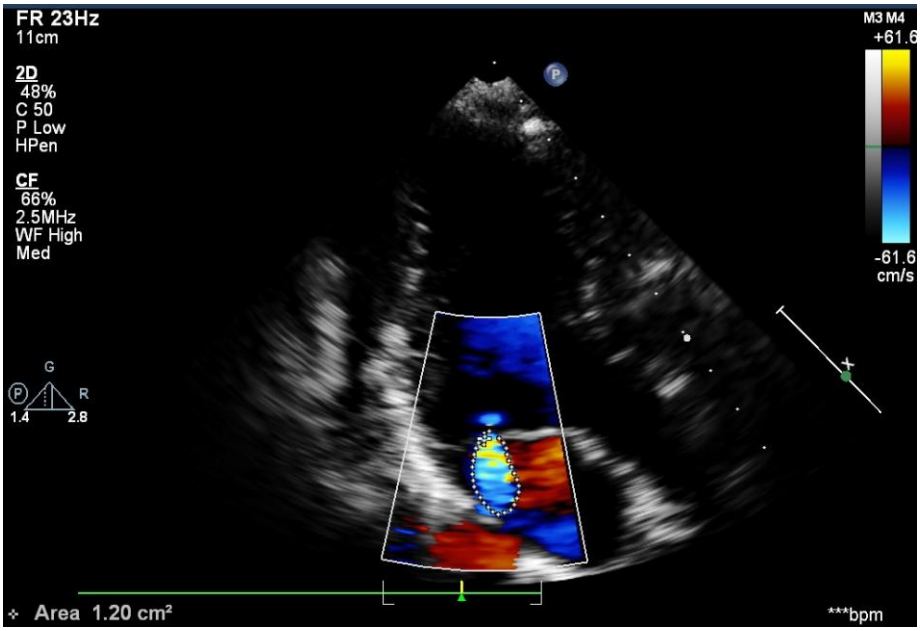


Figure 4.7 Mitral regurgitation in apical four-chamber view



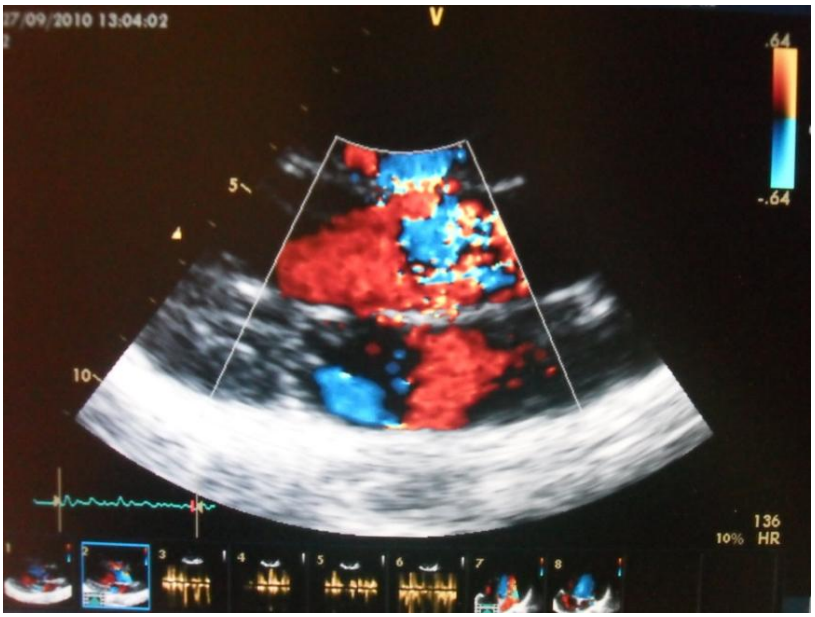


Figure 4.8 Mitral regurgitation in parasternal long-axis

Congenital Heart Disease

We identified 3 children with congenital heart disease (prevalence 1 per 1000) in the form of PDA (Patent Ductus Arteriosus), PAVC (Partial Atrioventricular Canal) and TGA (Transposition of the Great Arteries).

4.3.4 Evaluation of newly developed echocardiographic diagnostic criteria

Prevalence of subclinical RHD following the different diagnostic criteria

Following criteria 1 (WHO 2004), which is based on the finding of at least mild mitral and/or aortic regurgitation 2% (n=61) would be diagnosed with RHD.

Following criteria 2 (Carapetis 2008) a total of 1.76% (n=54) would be diagnosed with RHD of which 0.1% (n=3) would have been diagnosed by the presence of pathological aortic regurgitation and 0.5% (n=16) by the presence of mild mitral regurgitation. A combination of mild mitral regurgitation and structural characteristics was seen in 1.1% (n=35) of the children. A small number (0.4%, n=12) of the individuals would be diagnosed with borderline RHD in by the presence of trivial mitral regurgitation. Following criteria 3 (Marijon 2007) which combines valvular regurgitation with at least 2 structural signs a total of 0.09% (n=3) individuals would be

diagnosed with RHD as a combination of at least 2 of the structural findings was found in only 0.1% (n=4) of the individuals. Following our earlier suggested criteria (criteria 4, AHC criteria) which combines 1 functional and 1 structural feature (as described in chapter 3, pg. 89), 2.1% (n=66) of the children would be diagnosed with RHD.

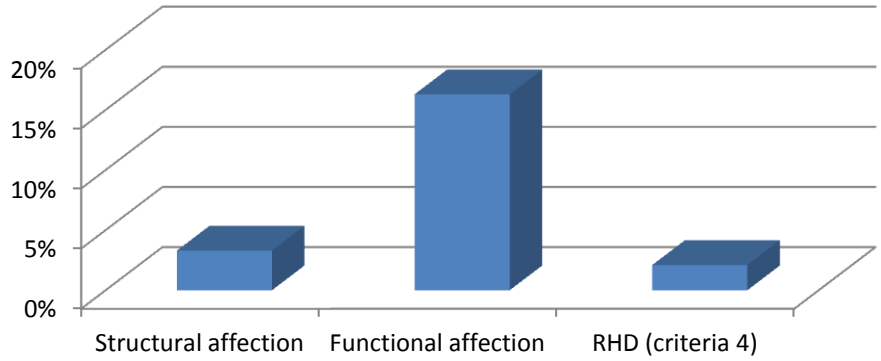


Figure 4.9 Valvular affection and diagnosis of RHD by criteria 4 (AHC criteria)

By the newly suggested criteria (Criteria 5, modified AHC criteria) (as described in chapter 3, pg. 90) 2.3% (n=70) of the children would be diagnosed with RHD, corresponding to a prevalence of 23 cases per 1000 children. Isolated thickness of the leaflets or cusps was seen in 0.6% (n=19) and isolated at least mild mitral regurgitation was found in 0.7% (n=22) of the children, leading to a total of 1.3% (n=41) of possible RHD cases.

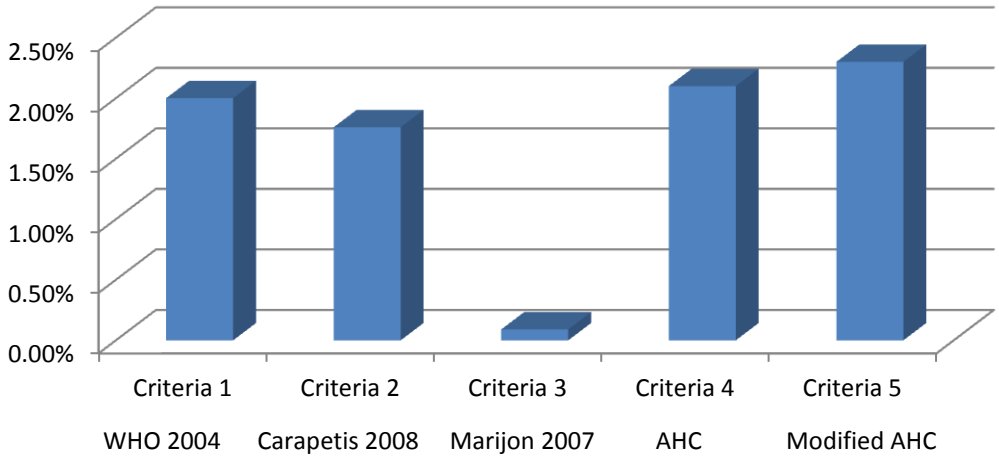


Figure 4.10 Diagnosis of RHD following the different criteria

4.3.5 Clinical versus echocardiographic diagnosis of subclinical RHD

Clinical assessment by cardiac auscultation revealed cardiac affection in 7.1% (n=5) of the children diagnosed with RHD by echocardiography, showing a 14-fold increase of the case detection rate by echocardiography.

4.3.6 Determinants of RHD

There was no significant relation between the age, the gender, or the amount of siblings and the presence of structural or functional cardiac affection or the diagnosis of RHD. However, there was a slight peak of RHD diagnosis in the group aged 11-12 years (Figure 4.6). Furthermore, there was also a slight statistically not significant male predominance in the diagnosis of RHD (Figure 4.7).

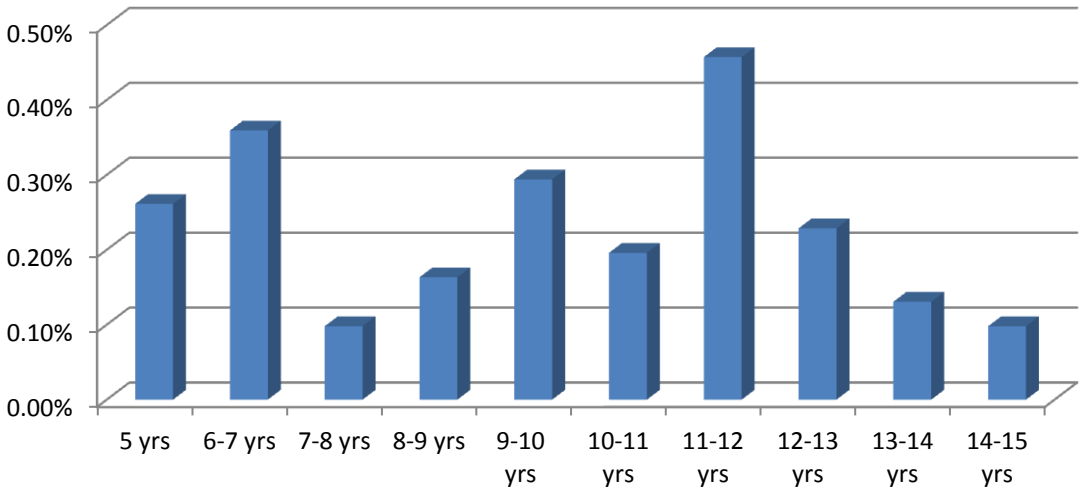


Figure 4.11 RHD affection by age

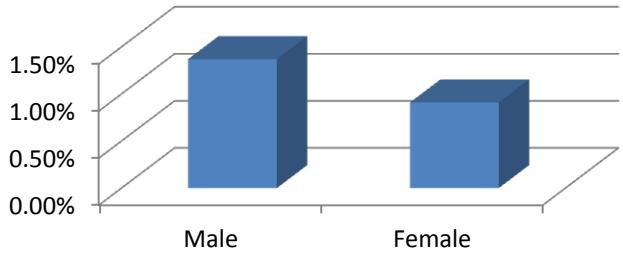


Figure 4.12 RHD affection by gender

4.4 Discussion

Our study shows a prevalence of 23 cases of RHD in 1,000 screened school children in Aswan, Egypt, which is comparable to earlier reports of systemic echocardiographic screenings from Cambodia (21.5 per 1,000) and Mozambique (30.4 per 1,000) (Marijon 2007) and exceeds the previously estimated prevalence based on clinical examination of 5.1 per 1000 schoolchildren in Egypt (Alwan 1993, WHO 1992). However, previous echocardiographic screenings have resulted in prevalence rates for RHD up to 62 in 1,000 (Anabwani 1996, Paar 2010, Marijon 2007), providing an average point prevalence of 40 in 1,000 in school-aged children (Paar 2010). Importantly, the difference in the reported prevalences may rely on geographical differences as well as the used criteria.

The age of the cohort studied was in the range with the previous school based screenings (Marijon 2007, Carapetis 2008, Paar 2010) enhancing the comparison of the results. Our series did not confirm the age differences for the cardiac affection, although RHD is believed to be more common in the 11-16 years age group than in younger children (Thakur 1996, Longo-Mbenza 1998) peaking in those aged 10-12 year (Carapetis 2008). However, there was a slight peak in the diagnosis of RHD in the group aged 11-12 years.

It has been reported that the prevalence of RHD increases significantly with age (Marijon 2007, Carapetis 2008, Marijon 2009), peaking in adults aged 20–40 years, with the number of cases of RHD in school-aged children representing less than 20% of the cases in the whole population (Carapetis 2005[1], Steer 2009). Therefore, in order to reflect the true number of prevalent cases in the general population the prevalence of RHD in children is increased five- to seven-fold (Carapetis 2005[1]). In our case this would lead to a horrifying prevalence of 115 to 161 RHD cases per 1000 Egyptians.

Our series does not confirm the earlier suggested gender predisposition for RHD with female predominance in the rheumatic affection (Marijon 2007, Marijon 2009), in fact, we have seen a statistically not significant male predominance in the diagnosis of RHD.

Structural features were found in 3.3% of the children screened and in 94.3% of the RHD cases, particularly in the form of thickening of the valve leaflets or cusps, which has been reported to be

the commonest feature of RHD, found in up to 83.8% of the cases (Vijayalakshmi 2008). Fusion or thickening of the subvalvular apparatus was less common and only seen in 1.5% of the children diagnosed with RHD and a combination of structural features was the second most common structural characteristic of RHD.

Functional affection of the left sided heart valves was found in 16.3% of the children assessed with predominance of trivial mitral valve regurgitation, which is in concordance with earlier reports (Thakur 1996, Longo-Mbenza 1998). The distribution of the valvular affection in the RHD cases found in our series was similar to the study performed in Mozambique (Marijon 2007) which reports mitral valve affection in up to 95.5%, aortic affection in 1.5% and affection of both valves in 3% of the cases. However, we found a slightly higher affection of the aortic valve (2.9%) and lower combined affection of the aortic and mitral valves (1.4%) (Marijon 2007). Nevertheless, studies in Cambodia and Tonga report lower representation of mitral valve affection, ranging from 77.2% to 85% and more affection of the aortic valve (12,7% and 9%, respectively) and combinations of mitral and aortic pathology (10.1% and 6%, respectively) (Marijon 2007, Carapetis 2008).

Importantly, although the prevalence of aortic regurgitation was lower, the severity of the affection was higher than earlier reported (Carapetis 2008). However, moderate or severe valvular affection was seen in only a small number of children, suggesting that in this age group the majority of the lesions are mild. No stenotic lesions were found in this age group as expected (Marijon 2007, Carapetis 2008). This series suggests a slight geographical variety of clinical presentation of RHD with a higher affection of the mitral valve and less combined lesions or affection of the aortic valve.

None of the screened children had physical signs of RF as defined by the Jones criteria and only a small number of children had a cardiac murmur. Our series confirms that cardiac auscultation detects an insufficient number of RHD cases, and particularly in those who have trivial or mild affection of the valves, showing an increase in case detection rate by echocardiography up to 14-fold compared to clinical examination, identifying children at risk of developing RHD for whom secondary prevention with penicillin prophylaxis may be effective (Marijon 2007). Comparable results have been reported earlier, as murmurs are known to be missed and subclinical and even

moderate cardiac affection may not be clinically audible, underdiagnosing milder lesions (Marijon 2007, Carapetis 2008), leading therefore to a higher sensitivity and accurate diagnosis of valvular involvement by echocardiography (Ramakrishnan 2009).

Screening based only on auscultation misses therefore many cases of RHD, however, the increased detection rate of valvular affection by echocardiography has generated debate (Carapetis 2007, Carapetis 2008[3], Marijon 2008). Detection of mild lesions is important because children with mild disease benefit most from secondary prophylaxis, however, echocardographic evaluation by Doppler and two-dimensional imaging is believed to be subtle and subjective. This would suggest a potential of over-interpreting normal physiological features, leading to incorrect diagnoses and overestimation of disease burden (Steer 2009). However, in areas with high prevalence of RF, the consequences of underdiagnosis are significantly greater than overdiagnosis (Ramakrishnan 2009) as secondary prevention of RHD can dramatically reduce morbidity and mortality, however, relying upon effective screening and identification of the children at risk.

There has been debate concerning the echocardiographic signs of early RHD to define the criteria to be used for the diagnosis of subclinical RHD and for the fact that echo definition of RHD based only on the length, velocity, and persistence of the regurgitant jet may depend on the gain settings on the ultrasound equipment (Minich 1997, Wilson 1995, WHO 2004).

The detection rate of the newly developed echocardiographic criteria (Criteria 4 (AHC criteria) and 5 (modified AHC criteria) is similar, however, the adjusted criteria seems to be more sensitive and specific for the screening and diagnosis of RHD in a younger population as it detects milder lesions and indicates them as rheumatic. Importantly, these criteria also had a similar detection rate as criteria 1 (WHO criteria), however, this was based on different echocardiographic criteria which only considers mild mitral or aortic regurgitation to indicate RHD, excluding all milder lesions and not evaluating the valvular structure. Furthermore, this criteria may also cause overdiagnosis due to the presence of physiological regurgitation. Criteria 2 (Carapetis 2008) showed a lower detection rate as it excludes trivial regurgitation and misses therefore all trivial and milder functional affection of the valves compared to our criteria. Criteria 3 (Marijon 2007) showed the lowest detection rate due the fact that it requires 2 structural

features for the diagnosis of RHD. We believe that this criteria underdiagnoses RHD especially in a young population, which still has mild and subclinical vlvular affection and who might have not developed combinations of structural rheumatic features as these are more prone to be present in chronic and long standing phases of the disease.
Furthermore, we believe that restriction of leaflet mobility should be only diagnosed in combination with shortening of the leaflet, forming together a structural feature of the condition. Criteria 3 (Marijon 2007) considers isolated abnormal motion of the leaflet a sign of rheumatic affection, leading to a higher diagnostic rate of this finding in their series, while in our study this feature was less commonly diagnosed by the requirement of leaflet shortening in combination with the mobility restriction. This might explain the discrepancy between the detection rates reported by the criteria suggested in this series and criteria 3 (Marijon 2007).

Inclusion of structural characteristics in the diagnostic criteria is believed to prevent overdiagnosis of the condition by overcoming the boundary between physiological valve regurgitation and authentic but minimal rheumatic lesions. Furthermore, the similar structural and functional rate of affection seen in RHD suggests that a combined criteria is more sensitive and accurate, avoiding misdiagnosis of the condition and seems to be better suited for screening of subclinical RHD. Based on the presented results we would recommend the newly developed criteria to be taken in consideration for the diagnosis of subclinical RHD, especially in a young population, as it seems to recognize early signs of the condition. We also believe that the most common structural feature found in early RHD is thickening of the valvular leaflets and cusps. Therefore we think that it is logic to regard isolated thickening as a sign of possible RHD as this feature might develop before the related valvular regurgitation. Furthermore, isolated aortic regurgitation is considered to indicate RHD as represents a condition rare in healthy children (Folger 1992). Importantly, standardization of the diagnostic criteria will allow comparison of prevalence in different areas and improve case detection.

It is now recognized that RHD can develop in individuals who did not develop manifest streptococcal infection or RF which can sometimes be asymptomatic. Moreover, the fact that over 93% of the found RHD cases in our series were subclinical, occurring in asymptomatic

children without audible murmurs shows the need for echocardiographic screening to optimize case identification and targeted prevention measures. Especially as many of the children diagnosed with subclinical cardiac affection will progress to clinically manifested disease in young adulthood (Carapetis 2005[2]). Importantly, detection at an early stage is believed to limit the progression to and improve the prognosis of chronic valvular disease with adherence to secondary penicillin prophylaxis lending support to the fact that screening is essential, and therefore, RHD satisfies all standard criteria for a screening program (Council of Europe 1994). The use of register-based disease-control programs is recommended in populations with a high prevalence of RHD (WHO 2004) to ensure coordinated, ongoing care with a particular focus on adherence to secondary prophylaxis regimens trough long-term, regular delivery of penicillin to patients with RHD or a history of RF to prevent recurrences and the worsening of valve lesions. However, the clinical importance of subclinical rheumatic valves lesions is still unknown (Carapetis 2008), which shows the need for follow-up of patients with subclinical lesions to determine whether their disease evolves in a similar way to clinically apparent RHD, and whether secondary prophylaxis improves disease outcome.

This study aimed to optimize case identification and targeted (secondary) prevention measures by identifying children at risk of developing rheumatic valve disease for whom secondary prevention with penicillin prophylaxis may be effective (Marijon 2001, Marijon 2007). We used stringent criteria that combines internationally accepted definitions of pathological valvular functional affection (Bisno 2001) with evidence of structural changes to valve appearance that are typical for RHD, expectedly raising case detection rates with major potential public health benefits. This study has led to the establishment of a registry for all the RHD cases found during the screening for regular follow-up and monitoring of the compliance to the penicillin prophylaxis. We hope hereby to enhance the disease awareness and increase the compliance to the penicillin regimen. This will also allow us to monitor the development or progression of the valvular affection and lesions for early recognition and treatment of RHD leading to better knowledge on long-term prognosis of subclinical rheumatic lesions. We will offer and provide free treatment, follow-up and surgery if indicated at the Aswan Heart Centre in Aswan.

Due to practical and ethical considerations no blood samples were taken, which forms a limitation of the study, as it would allow us to analyze the activity of the process. However, earlier similar studies have also not included this aspect in the screening (Anabwani 1996, Marijon 2007, Carapetis 2008, Paar 2010). Improtanlty, the significance of the findings and analysis of progression with determination of long-term prognosis should be addressed by follow-up of this cohort, which is uppermost fundamental.

4.5 Conclusion

This series shows that the prevalence of rheumatic valvular abnormalities in school-age children in Egypt is far greater than earlier estimates and extend the observations to show a high prevalence of subclinical disease in children in Aswan, Egypt, who may be at great risk for developing chronic progressive RHD. We have also confirmed that comprehensive echocardiographic screening identifies up to 14 times as many children with RHD as were identified by the traditional strategy of clinical cardiac auscultation.

The presented results reinforce the opinion that screening of asymptomatic school-age children is a useful strategy to identify new cases of RHD and subclinical rheumatic valvular affection in whom secondary prevention with penicillin prophylaxis may be effective in preventing development and worsening of RHD and might encourage a strategy of echocardiography-based screening programs. This study has contributed in the insights of the prevalence and characteristics of RHD in Aswan, Egypt and confirmed the importance of a combined criteria based on structural and functional valvular features. The screening protocol designed in this study can easily be used in other regions with high RHD prevalence, improving case finding, delivery of effective primary and secondary prevention, and adequate planning of health services as RHD remains a major cause of morbidity and mortality in different areas.

CHAPTER 5

CHARACTERIZATION OF RHEUMATIC FEVER IN A HOSPITAL BASED STUDY IN EGYPT

5.1 Background

The high worldwide incidence of RF with geographical variety in the epidemiology (Bisno 1997, Dinkla 2003) raises great concern as the disease remains endemic in different areas leading to considerable morbidity and mortality through RHD (Olivier, 2000). RF presents with a variety of symptoms outlined in the Jones Criteria (Stollerman 2001, Guilherme 2009[1]), which has shown decreased diagnostic sensitivity mainly due to the considerable variations in the prevalence of the symptoms and clinical manifestation of RF in different areas.

The aim of this study was to determine the epidemiological and clinical characteristics of RF in an area where data on regard the disease is completely lacking. The elucidation of determinants influencing the development of RF and RHD is of importance for early recognition of individuals at risk of developing RF and subsequently RHD and for the future development of preventive methods. Furthermore, we believe that the clinical presentation of RF is different in Egypt, with a different pattern of prevalence and incidence of the variety of symptoms. Therefore, it is of importance to evaluate and validate the Jones Criteria for a new classification of the diagnostic criteria RF for early recognition, diagnosis, follow-up and clinical management of RF.

5.2 Patients and methods

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI, in Imbaba, Cairo were assessed for the investigation of the epidemiological and clinical characteristics of RF as described in chapter 3, pg. 75.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

5.2.1 Data collection

Demographic data from every subject selected were collected in the form of a standardized questionnaire (Appendix V). The questionnaire contains questions regarding the patient's demographics, family environment, risk factors, history of RF, its severity, treatment and prophylaxis.

Issues regarding the onset age of RF, symptoms, severity, presence of recurrences of RF, medication and penicillin prophylaxis were questioned on the history of RF and the presence of symptoms to confirm the presence of RF and clinical presentation. Penicillin prophylaxis schedule was divided into 3 categories, following the most used and prescribed schedules for penicillin prophylaxis, every 15 days, 3 weeks or monthly. Compliance rate for the penicillin prophylaxis was evaluated by asking the patient about the schedule, how often he ignored or postponed the injection date and if he was strict to the schedule. Compliance was then also be evaluated in relation to the schedule. A schedule of less than 9 injections a year was considered poor. A schedule of 9 to 12 injections a year was considered fair, while an excellent compliance rate was more than 12 injections a year. Many individuals were receiving prophylaxis for the first time therefore questions on schedule and compliance was not applicable.

The disease awareness as classified in 3 categories evaluated on the patients' knowledge of the RF history, symptoms and penicillin prophylaxis. The disease awareness was also be evaluated by the amount and type of questions answered on the knowledge of RF and RF history. The disease awareness was evaluated as poor when the patient had no knowledge of the reason for penicillin prophylaxis. When the patient was aware of RF or the valvular damage, and knew that penicillin is a way of prophylactic prevention but did not know the link between RF and the valvular damage the awareness was evaluated as fair. The patient's awareness was evaluated as good when he was also aware of the correlation between RF and cardiac disease (RHD). The same scale was used for the parents' awareness. Dental care was evaluated during collection of throat swabs by the general condition of the mouth and teeth.

5.2.2 Statistical analysis

The statistical analysis was performed as described in chapter 3, pg 100.

5.3 Results

5.3.1 Epidemiological profile in RF

A total of 639 individuals were recruited at the NHI of which 498 seen at the penicillin clinic and 141 seen at the cardiothoracic surgery department. The patient demographics is shown in Table 5.1.

Age: The mean age was 23.78 years and the median was 21 years (Figure 5.1). One third (36%, n=230) was in the age group 3 to 17 years. Another third (37.40%, n= 239) were young adults between 20 and 35 years of age (Figure 5.1). One child (0.2%) was 3 years old and another child (0.2%) was 4 years old at the time of presentation at the penicillin clinic. A small number (1.1%, n=7) of the individuals screened were 5 years old.

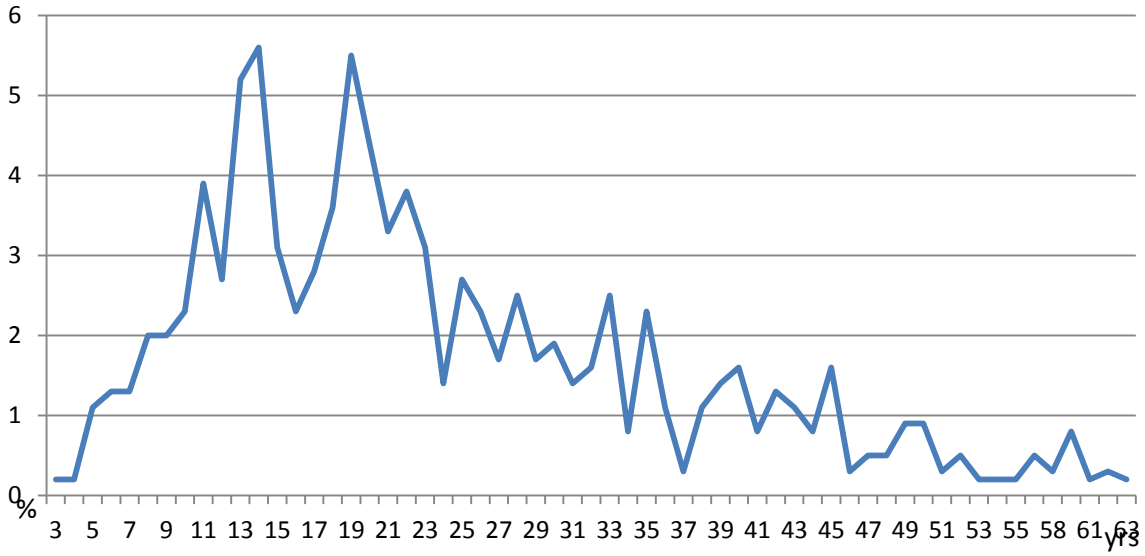


Figure 5.1 Age at the time of screening

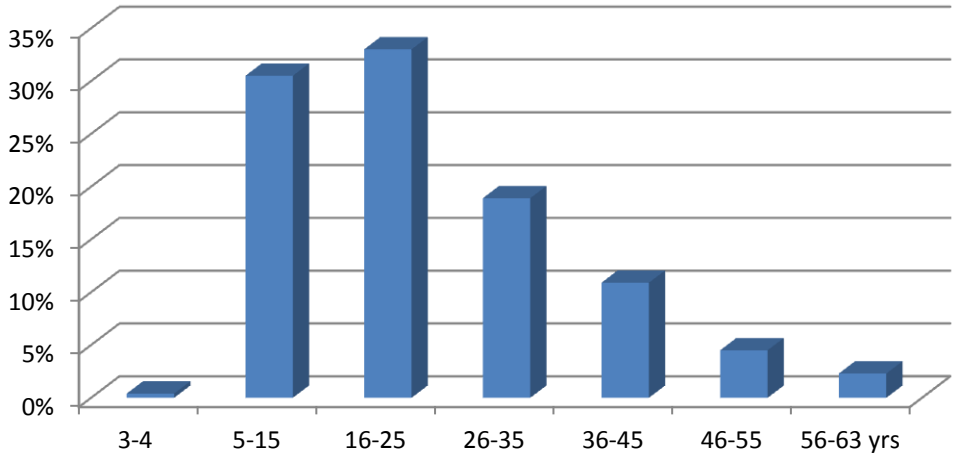


Figure 5.2 Distribution of age groups

Gender: There was a slight female predominance (52.6%, n=336) with a male to female ratio of 1:1,1.

Social factors: The number of siblings ranged up to 14, but most individuals came from families with a mean of 4 siblings and had good dental care.

Medical history: The majority had no cardiac risk factors and only a small fraction (2.5%, n=16) reported other risk factors like diabetes, atrial fibrillation or hypertension.

Family history: The majority of the individuals had a negative family history for cardiac diseases and RF. A small fraction of patients (5.4%, n=59) did not know if there was RF in the family.

Disease awareness: Most individuals (43.4%, n=265) had poor disease awareness. A small fraction of the individuals (5.28%, n=34) were too young to answer the questions on the disease awareness. A total of 182 parents were questioned about their child's health condition and the majority had a fair awareness of the disease.

Table 5.1 Patient demographics

	% (nr)	Mean ± SD	range
Age		23.78 ± 12.50	3-63
Sex			
Male	47.4% (n=303)		
Female	52.6% (n=336)		
Nr of siblings		4.0 ± 2.21	0-14
Dental care			
Poor	4.3% (n=27)		
Fair	47.8% (n=300)		
Good	47.8% (n=300)		
Family history for cardiac diseases			
Positive	7.2% (n=42)		
Negative	92.8% (n=543)		
Family history for RF			
Positive	19.8% (n=116)		
Negative	80.2% (n=469)		
Other risk factors			
Yes	2.5% (n=16)		
No	97.5% (n=620)		
Disease awareness			
Poor	43.4% (n=265)		
Fair	33.8% (n=206)		
Excellent	22.8% (n=139)		
Parents disease awareness			
Poor	26.8% (n=49)		
Fair	41.5% (n=76)		
Excellent	31.7% (n=58)		

Previous interventions: A small amount of the individuals had a history of previous interventions (5.9%, n=38), including balloon valvuloplasty, mitral valve repair and valve replacement (Table 5.2). The intervention date from 3 months to 20 year (6.38 ± 5.43).

Table 5.2 Previous interventions

	Nr (%)
Type of intervention	
Balloon	38.2% (n=13)
Valve repair	29.4% (n=10)
Valve replacement	32.4% (n=11)
Mv	54.54% (n=6)
Av	27.27% (n=3)
Double	18.9% (n=2)

5.3.2 Clinical manifestation of RF in Egypt

History of RF: The majority of the individuals had a positive history of RF. A fraction of the individuals (5.7%, n=37) did not recall the clinical manifestations (Table 5.3).

Age of RF: The age of onset of RF ranged from 2 months to 44 years (10.69 ± 6.24). A noticeable amount of patients (17.92%, n=95) was 5 years or younger at the time of the first RF attack, with 5.85% (n=31) patients being 3 years or younger at the time of the attack. The majority (73.96%, n=392) of individuals was between the 5 and 15 years of age with a peak at the age of 10 (14.9%, n=79). A fraction of the patients (7.92%, n=42) were between 20 and 35 years of age and 0.38% (n=2) were 38 and 44 years old. The median age at the time of the first RF attack was 10 years (Figure 5.3).

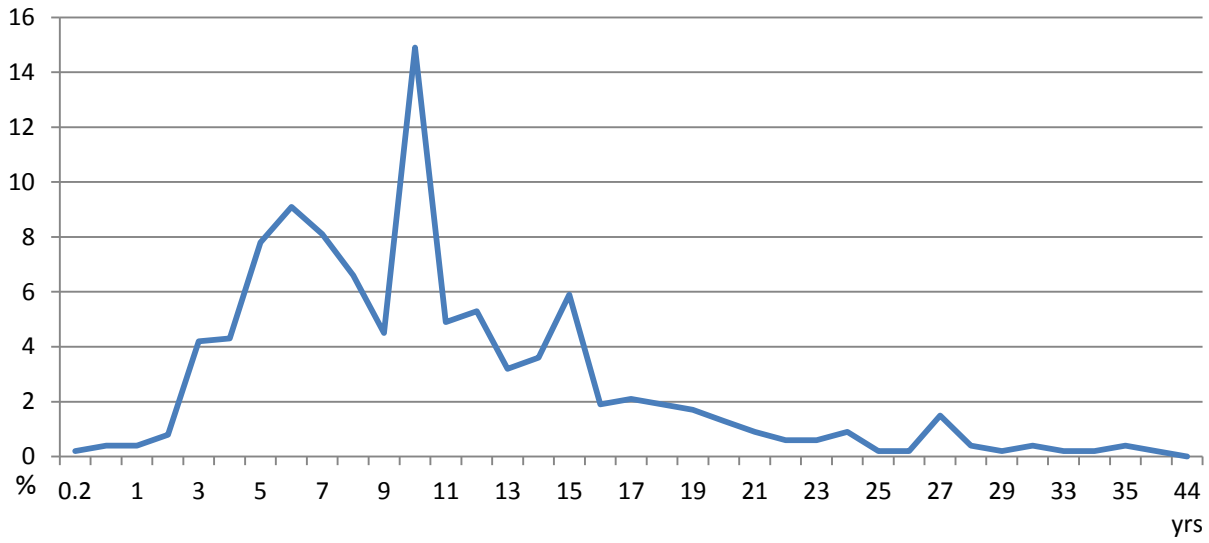


Figure 5.3 Age at the time of the first RF attack

Sixty individuals (11.3%) reported the attacks being in the last 2 months. However, most of the attacks were in the last 12 months (48.6%, n=257) and 24 months (68.5%, n=363), respectively. A fraction of the patients reported the RF attacks 5 (14.2%, n=75), 10 (6.22%, n=33) and over 20 years (2.45%, n=13) ago (Figure 5.4).

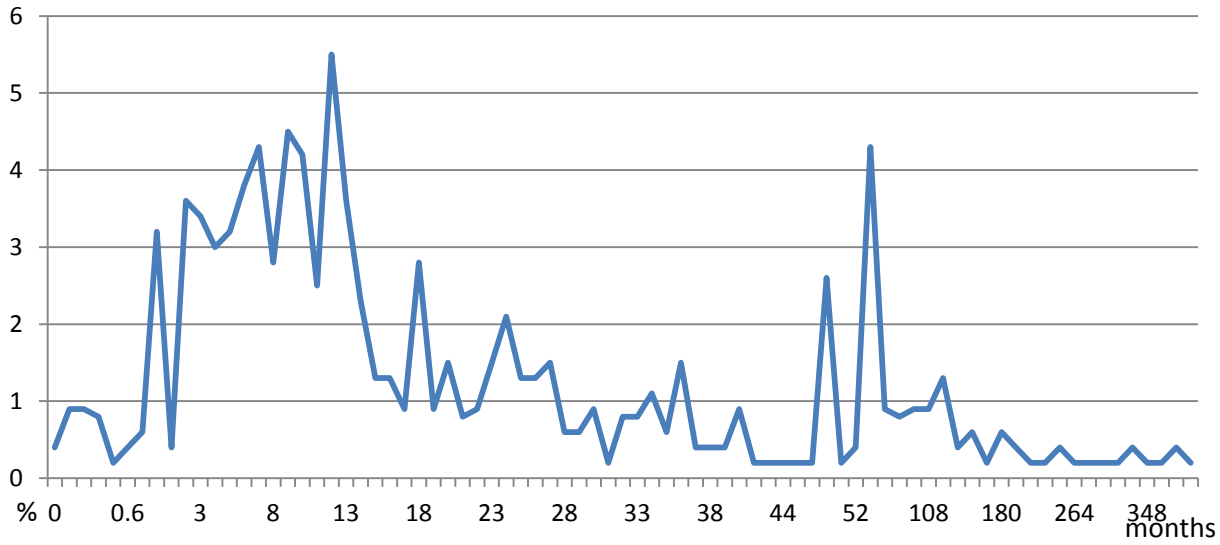


Figure 5.4 Date of first RF attack

Clinical manifestation: Arthritis was the most prominent symptom reported followed by a history of fever and pharyngitis and only a small fraction reported carditis and chorea. None of the individuals reported nodules.

Table 5.3 RF history

	Nr (%)
History of RF	
Yes	87.8% (n=560)
No	12.2% (n=78)
Symptoms	
Major manifestations	
Polyarthritis	87.9% (n=495)
Carditis	3.7% (n=21)
Chorea	0.4% (n=2)
Nodules	0% (n=0)
Erythema marginatum	0% (n=0)
Minor manifestations	
Fever and pharyngitis	65.5% (n=369)
Tonsillectomy	
Yes	21.0% (n=122)
Diagnosis	
RF symptoms	72.6% (n=464)
Cardiac symptoms/ affection	27.4% (n=175)

Recurrences: Most individuals reported recurrences of the RF attacks with the majority of the attacks reported in the last 3 to 4 weeks (98.2%, n=321), while 25.7% (n=84) reported recurrence in the last week (Table 5.4). Five (23.8%) of the individuals that reported carditis also reported recurrences of the carditis attacks.

Table 5.4 Recurrences

	Nr (%)	Mean +/- SD	range
RF recurrences			
Yes	59.6% (n=333)		
No	40.4% (n=226)		
Date recurrences		8.49 ± 0.76	1 week-6 months
Carditis recurrences			
Yes	0.9 % (n=5)		
No	99.1% (n=16)		
Date of recurrences		11.0 ± 9.59	2- 24 months

5.3.3 Diagnostic criteria

The difference in incidence between our series and the expected prevalence of the various manifestations of RF is shown in Figure 5.5 (CAVE: in the figure the minimum expected prevalence is shown).

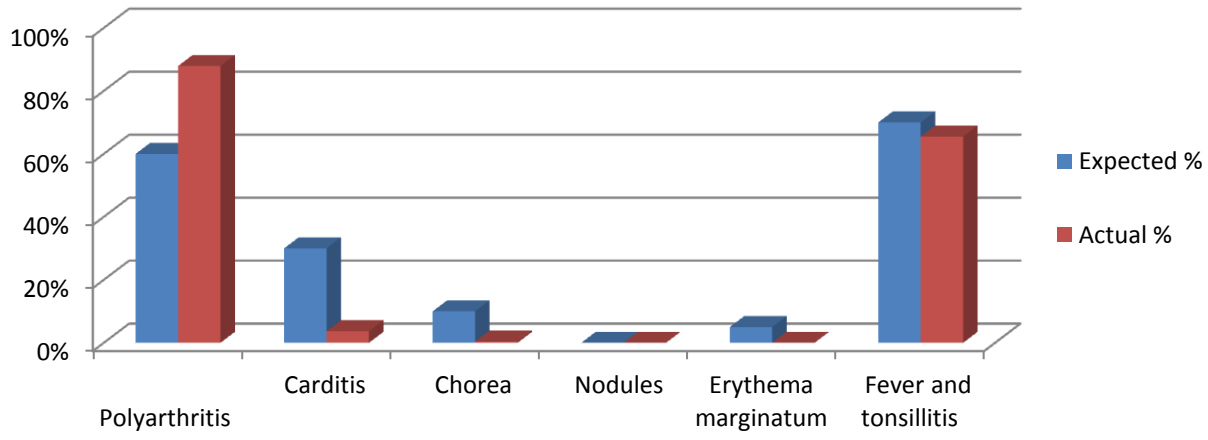


Figure 5.5 Distribution of symptoms

When using the Jones Criteria purely on the presence of two major, or one major and two minor manifestations, 51.64% (n=330) of the individuals screened would be diagnosed with RF. The prevalence of the combinations of symptoms leading to the diagnosis of RF is shown in Figure 5.6.

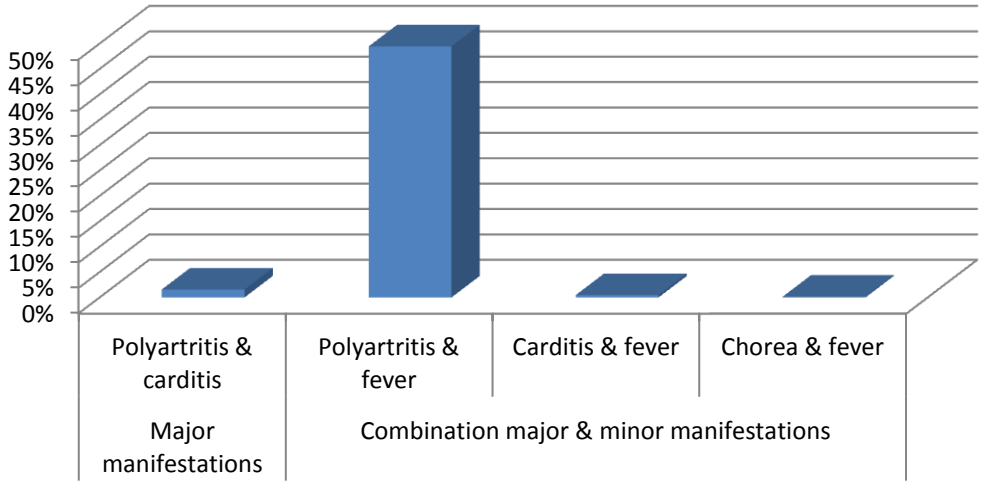


Figure 5.6 Diagnosis of RF based on the Jones Criteria

5.3.4 Secondary prevention

Penicillin prophylaxis: The majority of individuals were on penicillin prophylaxis, and only a small number of individuals had ceased the prophylaxis. The most common prophylaxis schedule was reported to be every 15-day, followed by the monthly prophylaxis routine (Table 5.5).

The start date of the prophylaxis ranged up to 432 months (36 years), with a mean of 21.82 months and standard deviation of 48.36 months (Figure 4.6). The period since prophylaxis cessation ranged from 1 month up to 84 months (7 years), with a mean of 1.21 months and a standard deviation of 6.10 months. Most individuals reported a fair or excellent compliance to the penicillin prophylaxis while a fraction of the individuals (9.78%, n=63) were receiving the penicillin prophylaxis for the first time.

Table 5.5 Penicillin prophylaxis

	Nr (%)	Mean +/- SD	range
Penicillin prophylaxis			
Yes	84.5% (n=522)		
No	15.5% (n=96)		
Stopped	3.3% (n=21)		
Prophylaxis schedule			
15-day	64.6% (n=318)		
21-day/ 3 weeks	12.6% (n=62)		
1-month	22.8% (n=112)		
Start date prophylaxis		21.82 ± 48.36	0 – 432 months
Period of cessation		1.21 ± 6.10	1 – 84 months
Compliance			
Poor	16.0% (n=78)		
Fair	33.4% (n=163)		
Excellent	50.6% (n=247)		

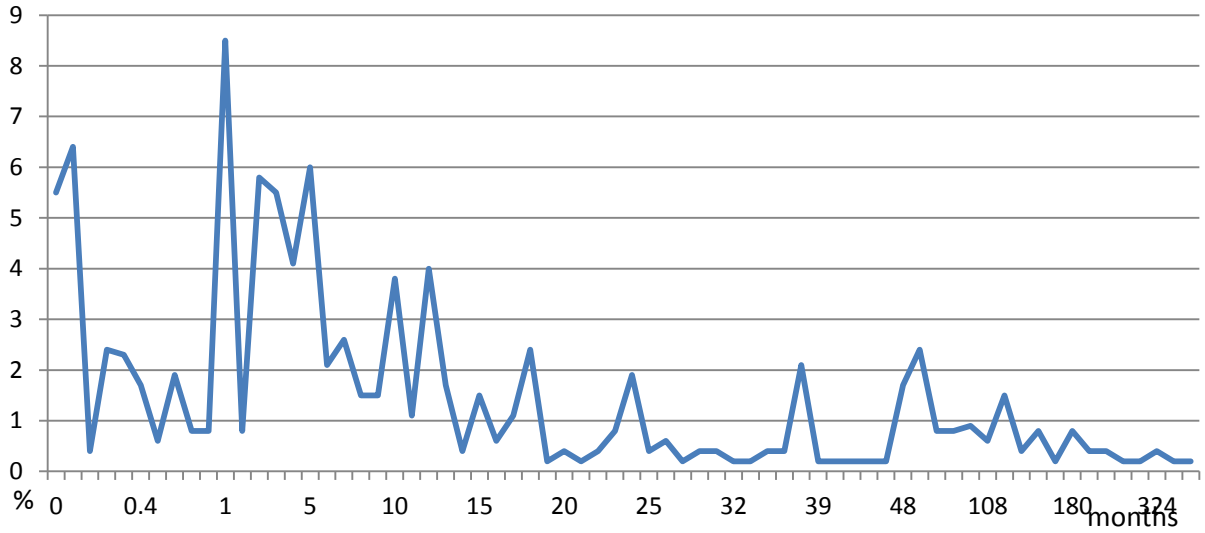


Figure 5.7 Date of start of penicillin prophylaxis

5.3.5 Determinants of RF

Age: The older patients at the time of the screening tended to have a negative history of RF (p=0.00) and more often a cardiac diagnosis instead of a RF symptom based diagnosis (p=0.00). Recurrences seemed to be less common among the older patients as the younger the patients the more recurrences reported (p=0.001).

The older individuals failed more often to be on prophylaxis (p=0.00) and had a higher tendency for the 21-day and monthly penicillin schedule (p=0.001). Furthermore, older individuals showed a lower compliance to the prophylaxis regimen (p=0.002) and had more often a history of previous interventions (p=0.00).

Gender: Of the individuals with a positive history of RF 45.7% (n=256) were male and 54.3% (n=304) were female (p=0.016) showing that females had more often a positive history of RF (90.7%, n=304) than males (84.5%, n=256) (Figure 5.8). Females tended to be older at the time of the first RF attack (p=0.011) but there was no gender difference in how long ago the first RF attack had been. Most individuals (56%, n=277) that reported arthritis were female, while 44.0% (n=218) were male (p=0.051).

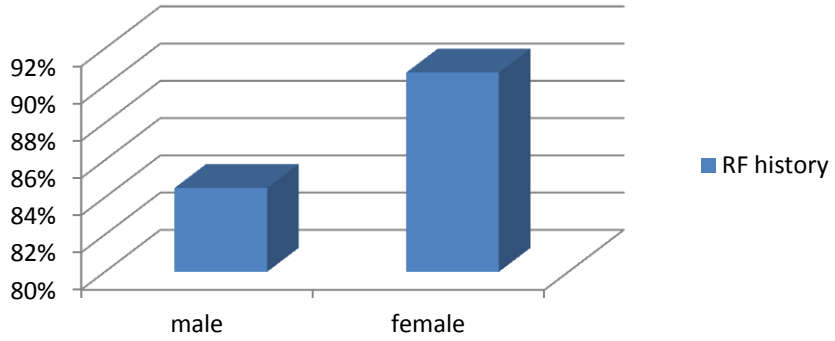


Figure 5. 8 RF history among males and females

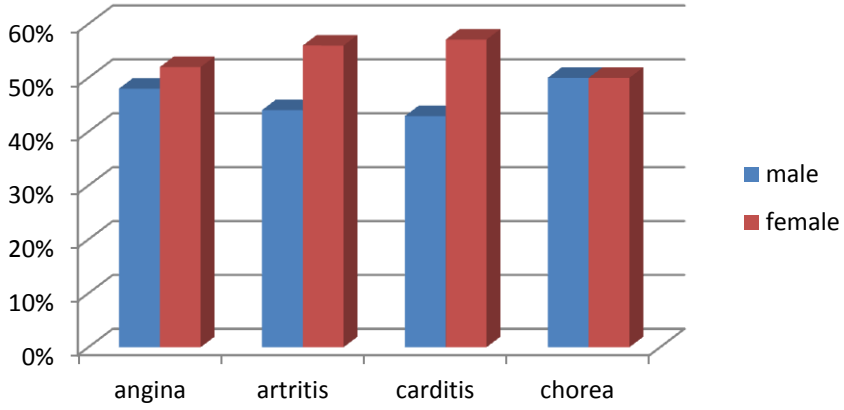


Figure 5.9 Distribution of symptoms by gender

Females reported more recurrences (66.3%) than males (53.4%) and 58.9% of the recurrences were found in females (p=0.002). There was a positive correlation between the gender and the type of diagnosis (p=0.019) with females being more often diagnosed trough the RF characteristics than males (76.5%, n=257 and 68.3%, n=207, respectively) and males being more often diagnosed by cardiac affection (31.7%, n=96) than females (23.5%, n=79) (Table 5.9). Females tended to have started later with the penicillin prophylaxis (p= 0.002) but seemed to have stopped the prophylaxis use later than males (p=0.027). However, no correlation was found between a positive history for previous interventions and the patients gender (p=0.614).

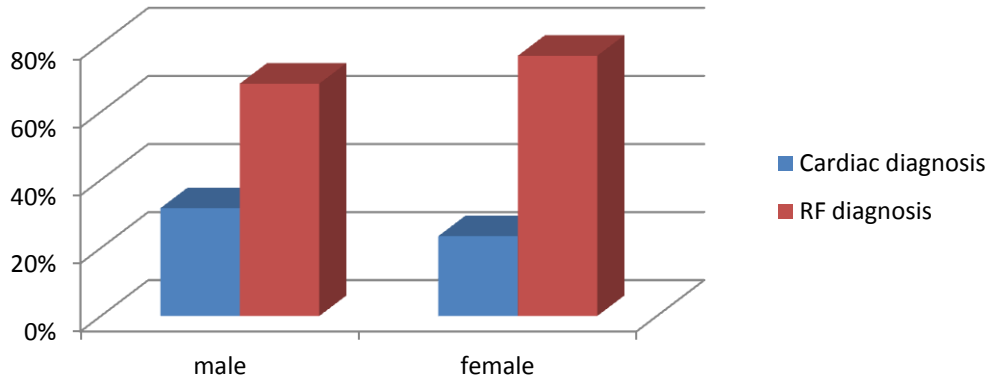


Figure 5.10 Type of diagnosis by gender

Familial predisposition: No correlation was found between a positive family history and history and the age during the first RF attack. However, positive family histories for RF lead to more diagnosis made by RF symptoms than by cardiac affection (81.9% and 18.1% respectively).

Tonsillectomy: The majority (95.1%, n=116) of the individuals who underwent tonsillectomy had a positive history of RF, while 4.9% (n=6) did not (p=0.03). The older the patient during the first RF attack the less often he underwent tonsillectomy (p=0.00).

RF history: Of the individuals with a negative history of RF the majority was diagnosed trough cardiac symptoms (82.1%, n=64) while only 17.9% (n=14) were diagnosed by the RF symptoms (p=0.00) (Figure 5.11).

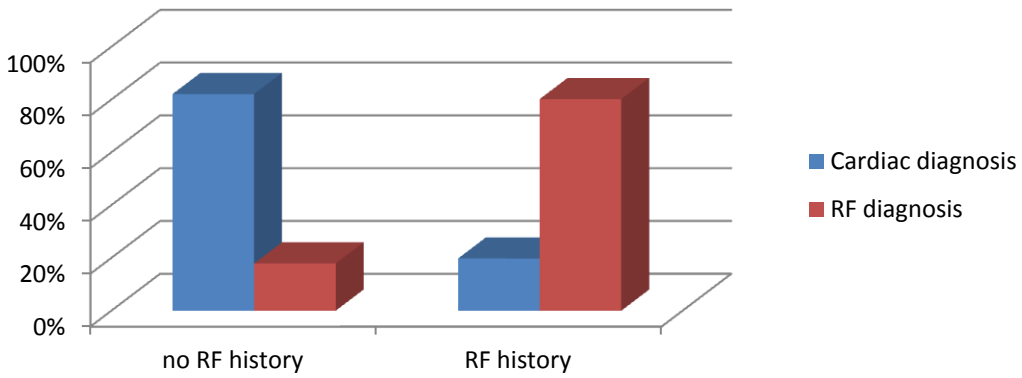


Figure 5.11 Type of diagnosis by history

The majority of the individuals on penicillin (91.0%, n=474) had history of RF while 87.4% (n=474) of the individuals with history of RF were on penicillin and 12.6% (n=68) were not (p=0.000). However, 9.0% (n=47) of the individuals on penicillin prophylaxis did not recall a history of RF (Table 5.12).

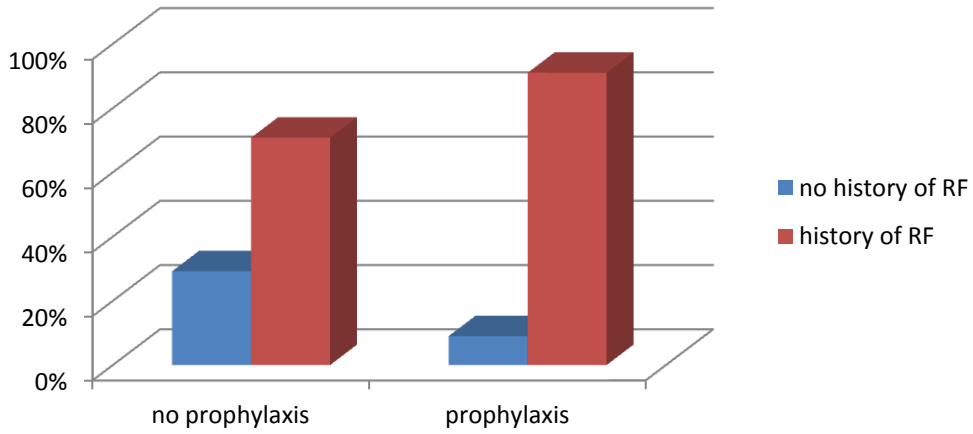


Figure 5.12 The use of penicillin prophylaxis and history of RF

Age of RF: The older the patient during the first RF attack, the less often fever and pharyngitis was reported as one of the initial symptoms (p=0.00). No correlation was found between the age during the first RF attack and the presence of arthritis symptoms. However, the older the patient

during the first RF attack the more often carditis was found (p=0.00). Furthermore, the longer ago the first RF attack had been, the less recurrences were reported (p=0.001).
No correlation was found between the age of the first RF attack and the presence of a history of RF, age of the first RF attack, type of diagnosis and the use of penicillin prophylaxis. However, the longer ago the first RF attack had been, the lower the reported compliance to the penicillin prophylaxis (p=0.002 respectively). The start date of the penicillin prophylaxis correlated to the date of the first RF attack (p=0.00) meaning that most of the patients started the penicillin prophylaxis shortly after the first RF attack. No correlation was found between the age of the first RF attack and the presence of a history of previous interventions (p=0.447). However, the longer the interval since the first RF attack the more often a history of previous interventions was found (p=0.00).

Diagnosis: The majority (77.4%, n=404) of the individuals on penicillin were diagnosed by RF symptoms while 22.6% (n=118) were diagnosed by valvular affection (p=0.000). Importantly, patients who were firstly diagnosed by the cardiac affection and symptoms had more often a history of previous interventions (p=0.003).

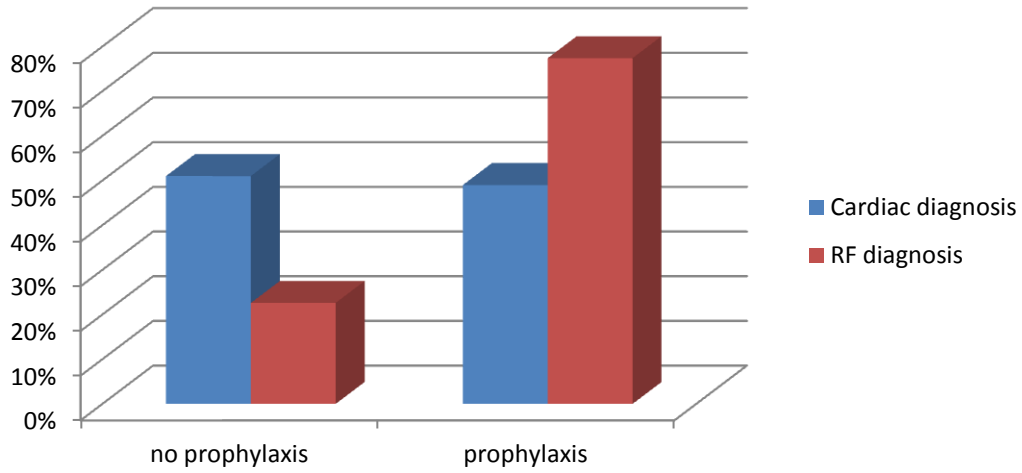


Figure 5.13 Type of diagnosis and the use of prophylaxis

Recurrences: Diagnosis on basis of valvular affection was more often made in patients who did not report recurrences of RF (59.8%, n=67) than in those who had RF recurrences (40.2%, n=45), showing that the amount of individuals first diagnosed with RF trough cardiac findings is higher in the population that does not have recurrences of the attack (p=0.00). Therefore, diagnosis through RF symptoms were more common in individuals with RF recurrences (64.4%, n=288) than in patients without recurrences (35.6%, n=159) (Figure 5.14). Most of the individuals (62.1%, n=293) of the individuals on penicillin prophylaxis reported recurrences of RF while 37.9% (n=179) of the individuals on prophylaxis did not report recurrences.

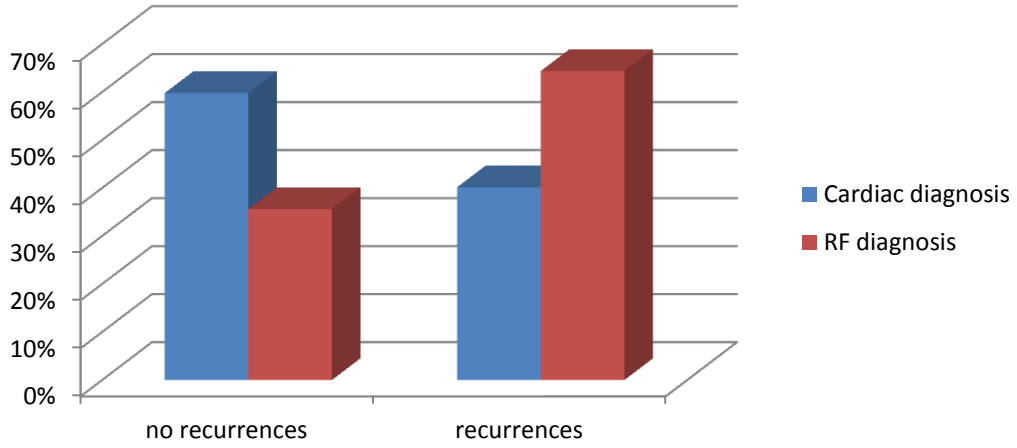


Figure 5.14 Type of diagnosis depending on recurrences

Disease awareness: The higher the disease awareness the more often history of RF was reported (p=0.00). Furthermore, symptoms of fever and pharyngitis seemed to raise the patient disease awareness (p=0.018) and patients with a positive family history for RF had a higher disease awareness than patients who did not (p=0.007). There was also a significant correlation between the patients dental care and the disease awareness (p=0.00) as the better the dental care, the higher the awareness.

No correlation was found between the patient's age and the disease awareness and the age during the first RF attack did not influence the disease awareness. However, the longer ago the RF attack had been, the higher the disease awareness (p=0.001). Importantly, a correlation was found between the disease awareness and the type of diagnosis (p=0.000) as a lower disease

awareness resulted in a higher number of individuals diagnosed by cardiac symptoms (41.7%, 23.3% and 12.2% respectively) while higher disease awareness resulted in a higher amount of diagnoses made trough the RF symptoms (58.3%, 76.7% and 87.8% respectively). However, no correlation was found between the disease awareness and history for previous interventions (p=0.606).

A correlation was also found between the disease awareness and the pattern of secondary prevention (p=0.000) as the higher the disease awareness, the higher the adherence to the prophylaxis (76.3%, 91.0% and 93.1% respectively) and the better the compliance to the prophylaxis (36.1%, 49.4% and 60.5% respectively).

Penicillin prophylaxis: Patients that had recently stopped the penicillin prophylaxis had less recurrences than those that had ceased the prophylaxis longer ago (p=0.031). No correlation was found between the schedule of the penicillin prophylaxis and the presence of recurrences.

5.4 Discussion

The age of the population screened in our series ranged from 3 to 63 years, with a median of 21 years, including therefore the three major age groups of children, adolescents and adults. Although no gender predilection is believed to exist and both sexes are believed to be equally predisposed to developing RF (Stollerman 1975), female as well as male predominance has been reported in the incidence of RF showing male/female ratios of 1:1.3 (Woo 1983) and 2.1:1 (Vinker 2010, Miyake 2007, Dajani 1993, Mahmudi 2006). Nevertheless, the found female predominance in our series is slightly lower than the male to female ratio reported earlier (1:1.25) (Bharani 2010).

The female predominance could have been explained by the treatment seeking profile or attitude but most females also tended to have more often a positive history for RF and arthritis symptoms than males. Therefore, females tended to be diagnosed trough the RF symptoms while males were more often diagnosed later by cardiac affection. Interestingly, females tended to be older at the time of the first RF attack.

The majority of the individuals was in good general health and did not have any other cardiac risk factors. The dental hygiene was evaluated to analyze the general hygiene and physical condition as the dental care and oral hygiene is believed to be a general indicator of personal hygiene and health care practices, including access to and use of health care services (DeStefano 1993). In concordance, our series shows that the better the dental care, the higher the disease awareness and vice versa.

Most individuals were from average sized families, with a mean of 4 siblings and from an equivalent social and financial status. Heritability for RF among Egyptians has been reported trough a positive family history in around one-third of the cases, suggesting a possible genetic contribution (Settin 2006). However, other reports show a positive family history ranging up to 14% (Mahmudi 2006, Dajani 1992), which is more in concordance with our own results.

Our data confirms that the burden imposed by RF is present throughout the life course, with a peak in the age group 5 to 15 with a median of 10 years and cases presenting up to the age of 44. Although all age groups may be affected, an elevated number of cases is known to occur among school-age children between the ages of 5 and 15 (Smith 1989, Falck 1992, Steer 2009, Thakur 1996) with a median age of 10 years and only 20% of the cases occuring in adults. Surprisingly, RF was also reported under the age of 3, and 18% of our patients were younger than 5 years at the time of the first RF attack (Chin 2010).

The majority of the RF attacks were reported in the last 12 and 24 months, showing that the population on prophylaxis had a recent diagnosis of RF and were shortly on penicillin prophylaxis, with a mean of 21 months. Only a small fraction of the individuals reported RF a longer time ago, suggesting therefore that the longer ago RF was diagnosed the more probable it was that patients had withdrawn from the prophylaxis.

The most prominent symptom of RF found in our series was arthritis which is not surprising as it is believed that up to 88% of the affected children display this symptom (Khriesat 2003), which tends to occur early in the disease course and is frequently the presenting complaint (Feinstein 1962). Importantly, in adolescents and adults, arthritis is often the sole major manifestation on

which the diagnosis is based since carditis and chorea are less common in first attacks occurring in these age groups. In our study population only 3.7% of the individuals reported episodes of carditis while 30 to 80% of the individuals affected are believed to develop carditis which causes heart damage with pericardial, myocardial and endocardial involvement followed by progressive and permanent valvular lesions leading to RHD (Mahmudi 2006, Dajani 1992, Carapetis 2007). Sydenham chorea and erythema marginatum (Vijayalakshmi 2005) were scarcely represented and subcutaneous nodules which are currently an infrequent manifestation of RF were totally absent in our series (Massell 1958, Khriesat 2003, Rosenthal 1968, Tani 2003, Chockalingam 2004, Canter 2004). Erythema marginatum is believed to have become irrelevant as a major criterion as it has shown a very low incidence in different studies and has therefore, been suggested to be replaced by abnormal echocardiographic findings in the Jones Criteria. This sounds encouraging as we believe that early and precise diagnosis of carditis in acute rheumatic fever, though difficult, is very important in preventing the development of RHD and the following morbidity and mortality.

Importantly, the age at the time of the first RF attack seems to influence the clinical presentation, as older patients reported less fever and pharyingitis as the initial symptoms. Nevertheless, studies from other endemic areas show a different distribution of the RF symptoms, with carditis being the most common clinical manifestation seen in up to 81% of the cases (Khriesat 2003, Mahmudi 2006), followed by arthritis (69%) (Mahmudi 2006) and a more abundant incidence of chorea, seen in up to 42% and nodules in 15% of the individuals (Commerford 2006).
In our series only 51.64% of the individuals screened would have been diagnosed with RF following the current Jones Criteria while 87.8% had a positive history of RF. Most of them would be diagnosed by the presence of polyarthritis, fever and history of pharyngitis while the majority of the individuals were diagnosed by the presence of fever in combination with one major criterion. Therefore, it seems that in Egypt, the most sensitive criteria is the presence of polyarthritis in combination with history of fever and pharyngitis and that strict adherence to the Jones criteria may result in underdiagnosis and lack of treatment of patients with RF and at risk of developing RHD.

We have used pharyngitis rather than arthralgia as a diagnostic criterion as we were assessing the history of RF retrospectively and symptoms of arthralgia would have been missed as they are easier to forget than the pharyngitis. Furthermore, history of an antecedent sore throat 1-5 weeks prior to onset of RF is present in up to 78% of older children and young adults (Mahmudi 2006, Guilherme 2009[1]).

Our series shows a history of an antecedent sore throat in 65.5% and polyarthritis in 87.9% of the individuals screened suggesting a high incidence of individuals that do not recall the initial pharyngitis. The morbidity caused by polyarthritis seems to draw attention and allows detection of most of the initial episodes of RF as well as recurrences in the community setting. It is also believed that when carditis is the only clinical manifestation, patients typically seek health care much later in the course of the disease (Narula 1999). It has also been noted that patients with severe arthritis or carditis are the ones who most often seek medical attention (Loeffler 1995) and that patients with mild rheumatic carditis alone are less likely to be symptomatic or to seek medical attention.

We also ignored the requirement of evidence of previous streptococcal infection as this was a retrospective study and most of the individuals screened did not have a medical record or reports on the medical history or laboratory findings.

Females and younger patients reported more often recurrences, while patients that reported a longer interval since the first RF attack tended to have less recurrence. Importantly, diagnosis on basis of valvular affection was more often made in patients who did not report recurrences of RF than in those who had RF recurrences. Therefore, recurrences seem to lead to awareness and recognition of the disease before affection of the heart. Nevertheless, a positive family history of RF seems to raise awareness as it leads to more diagnosis made based on the RF symptoms. Importantly, one quarter of the population screened had been diagnosed with RF trough cardiac symptoms and affection of the heart which seemed to lead to an increased presence of history of previous interventions. One prominent risk factor therefore was a low disease awareness, which resulted in more diagnosis based on cardiac symptoms rather than RF symptoms. Furthermore,

individuals that reported the first RF attack a longer time ago had more often a history of previous cardiac interventions, showing progression of the disease.

Although the compliance to the penicillin prophylaxis is known to be insufficient varying from 40 to 86% in previous reports (Kumar 1997, Saran 1985, Ravisha 2003) our study population reported regular penicillin prophylaxis regimens and a good compliance. However, we lack the methods of monitoring the penicillin prophylaxis regimen used and the compliance as there are no registries or data on the patients on prophylaxis or follow-up. Adherence to penicillin prophylaxis was found to be dependent on the history of RF and the disease awareness, as individuals with history of RF and better disease awareness are more often on regular regimens. The results presented on a compliance rate to the prophylactic regimen in two-thirds of the patients (Bassili 2000) is comparable to earlier studies in Alexandria, Egypt, bearing in mind that we assessed a population visiting the hospital for their prophylaxis and we lack data on the total of patients frequenting the clinic and the amount of patients that withdraws from or neglects their prophylactic regimen. Nevertheless, it is estimated that more than seven-tenths of patients with established RHD do not receive secondary prophylaxis (Strasser 1981). However, older age at the time of screening, lower disease awareness and a longer time since the RF attack were found to be risk factors for a lower compliance to the prophylaxis. Furthermore, older patients also tended to be more often on 21-day penicillin regimen than younger individuals.

Regular prophylaxis with 3 or 4 weekly benzathine penicillin injections is perhaps more realistic but for high-prevalence regions 3- or even 2-weekly penicillin is recommended (WHO 2004, Kassem 1992, Kassem 1996). It has been stated that penicillin has clearly failed to eradicate this disease process (Kaplan 2005) but in fact, this may be caused by the lack of precise diagnosis and use and adherence to penicillin prophylaxis, rather than the failure of penicillin (Vijayalakshmi 2005).

We found that more than half of the individuals reported recent recurrences of the RF attack, while on penicillin prophylaxis with the advised schedule of injections every 15-days (Kassem 1992, Kassem 1996). In Alexandria recurrences were seen in one-third of the patients with non-compliance to the prophylaxis being the significant risk factor. However, in our series,

compliance did not seem to affect the presence of recurrences, although cessation of the penicillin prophylaxis was found to be a risk factor for recurrences (Kassem 1992). Treatment of streptococcal pharyngitis with penicillin has been shown to markedly reduce the incidence of initial attacks of RF and to dramatically reduce recurrences of RF (Stollerman 1955, Wannamaker 1951), however, the main questions regarding secondary prevention remain on which regimen to use, how long to give it, and whether all patients required the same vigor and duration of prophylaxis (Narula 1999).

We have succeeded in building a registry of the patients visiting the NHI penicillin clinic for regular follow-up and monitoring of the compliance to the penicillin prophylaxis. We hope hereby to enhance the disease awareness and increase the compliance to the penicillin regimen. This will also allow us to monitor the development or progression of the valvular affection and lesions for early recognition and treatment of RHD. However, one of the study limitations is the fact that this study cohort only reflects those who seek secondary prophylaxis at the NHI, which undoubtedly under-represent the number of individuals affected, receiving prophylaxis elsewhere and specially those neglecting prophylaxis.

5.5 Conclusions

This series confirms that there are considerable variations in the prevalence of the symptoms and clinical manifestation of RF in different areas and age groups which shows the need for a validation of the criteria in different regions where the disease is common and still endemic. The diagnosis of RF based on symptomatology remains difficult as there is no gold standard diagnostic feature and the clinical manifestations tend to vary. This shows the need for revision of the Jones Criteria, which might be misdiagnosing disease in many countries, and better diagnostic methods, based on more specific laboratory tests and objective findings.

Nevertheless, in Egypt the initial episode of RF is often unnoticed in a substantial proportion of children, and arthritis tends to be reported less often than expected. Consequently, secondary prophylaxis is often not initiated. Furthermore, there is only limited enforcement of secondary prophylaxis and the penicillin injections are frequently missed. Therefore, recurrences are very

common, and may contribute to the rapid progression to advanced valvular RHD. The majority of the individuals screened had a poor awareness of the disease and although most of them had a positive history of RF they did not know the risks, complications, possibility of sequelae and treatment and prophylaxis requirement and need. It is believed that the disease awareness affects the treatment seeking profile and attitude and the use and compliance to the penicillin prophylaxis and that the success of secondary prophylaxis is critically dependent on patient education. In our series, adherence to penicillin prophylaxis was found to be dependent on the history of RF and the disease awareness, as individuals with history of RF and better disease awareness are more often on regular regimens, showing that lower disease awareness is a risk factor for non-compliance to the penicillin prophylaxis.

Overall, the volume and pattern of cases challenge the common assumption that RF presents predominantly in children, however our study shows onset of RF in young adults and adults in Egypt RF, being also characterized by considerable variations in the prevalence of the symptoms and a high tendency to recur even in patients following regular prophylactic programs.

In conclusion, our study confirms that RF is a notifiable condition, and highlights the persistent burden of newly diagnosed cases within the Egyptian population showing that RF remains one of the major public health problems in Egypt. However, no measure has been taken till date to ensure that cases are enrolled in registers and are offered secondary prophylaxis with regular penicillin injections. It remains to the patient and patient's responsibles to comply to the penicillin regimen while secondary prophylaxis is a cost-effective and proven intervention for the prevention of progression of RHD. This is worrying as the majority of individuals has a poor awareness and knowledge of the disease and are not aware of the importance and need for secondary prophylaxis, leading therefore to insufficient compliance to the penicillin regimen. The presented results show the urgent need for the registration of RF and RHD cases in high burden countries, as a measure to ensure control of the disease through the implementation of registry based secondary prophylaxis programs to improve the compliance to the penicillin prophylaxis.

This data has important clinical and public health implications for Sub-Saharan Africa, the wider African continent, and other parts of the world where RF and RHD are endemic. In conclusion,

this study provides important insights into the still large and complex burden of RF within the Egyptian community, supporting the statements that RF and RHD are of sufficient importance to warrant urgent attention of the national and international public health and research community. Nevertheless, the found results are irrefutable proof of the need for more laboratory, epidemiological, and clinical research.

CHAPTER 6

CLINICAL CHARACTERISTICS AND DETERMINANTS OF RHD IN HOSPITAL BASED STUDY IN EGYPT

6.1 Background

The prevalence and distribution of the different manifestations of RHD show geographical variation with some affections being more reported in some areas. In Egypt, a high percentage of schoolchildren have clinical evidence of cardiac valvular damage due to RHD, nevertheless, the prevalence of subclinical RHD in a population with history of RF is still unknown. Since there is a high incidence of RF and RHD in the Egyptian population, there is likely a high prevalence of subclinical and undiagnosed RHD with a different pattern of manifestation including higher prevalence of mitral stenosis, which might be a sign of a fast progression from mitral regurgitation to stenosis showing a severe course of the disease. A high rate of progression of subclinical valvular lesions has been shown in different series (Meira 2005) while also reversibility of the valvular lesions has been reported in patients who adhere to antibiotic prophylaxis (Chin 2010, Marijon 2007). However, the determining and influencing factors as well as the long-term outcome of subclinical RHD are still unknown.

The aim of this study was to analyze the manifestation and pattern of RHD in a population with history of RF in an area where data on regard RF and RHD is completely lacking and determine the early echocardiographic signs and features of subclinical valvular damage. This will lead to the development of a screening protocol and comprehensive criteria for the detection of subclinical RHD and early management of patients at risk of developing permanent, progressive and chronic valvular lesions. We also aimed to evaluate the progress and reversibility of subclinical valvular lesions in a population with history of RF to determine the long-term outcome of RHD as well as the determinant factors in Egypt.

6.2 Diagnosis of RHD

6.2.1 Introduction

Cardiac involvement in RF and RHD is often established by finding of a new murmur of mitral or aortic insufficiency. Nevertheless, doppler echocardiography improves the detection of carditis without overt evidence of valvular or other cardiac involvement (Marijon 2007, Carapetis 2008), detecting clinically silent RHD 10 times more than clinical examination.

Echocardiography is therefore the golden standard tool for diagnosing and characterizing valve lesions and detection of rheumatic carditis and subclinical RHD (Veasy 1993, Veasy 1994, Wilson 1995). However, there are no universally agreed criteria for the echocardiographic diagnosis of RHD and definition of cardiac involvement based on functional characteristics may depend on the gain settings on the ultrasound equipment, therefore, we hypothesized that the use of Doppler criteria for abnormalities associated with structural valve changes will improve the specific diagnosis of RHD (Marijon 2007) and is better suited for screening for subclinical RHD.

This study aims to analyze the characteristics and pattern of rheumatic cardiac affection and features in a population with history of RF. We hypothesized that echocardiographic diagnosis of subclinical RHD is superior and more specific than clinical diagnosis and detection of a murmur. We therefore aimed to evaluate the diagnostic rate, sensitivity and specificity of both methods for early diagnosis of subclinical RHD to be used for screening purposes for the diagnosis and better management of individuals with subclinical valvular damage which might progress to permanent and chronic valvular lesions (RHD).

The different diagnostic criteria in use were compared and analyzed with the aim to evaluate and validate the development of useful and comprehensive echocardiographic criteria for the early detection of subclinical RHD and purpose a new classification of characteristics of valvular affection and lesions in RHD that could be used in screening purposes as well as clinical diagnosis and management of patients.

6.2.2 Participants and methods

The study population consisted of individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI, Imbaba, Cairo as described in chapter 3, pg 75:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

Examination protocol

The screened individuals underwent a standardized examination protocol consisting of detailed medical history recorded by the investigator, physical examination including general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation with the patient in the supine and left lateral decubitus positions.

Disease manifestations and auscultatory findings were evaluated according to internationally approved criteria, including the published data from World Health Organization (WHO 1988, Figueroa 1992). Detailed transthoracic echocardiography was performed with the use of a hand-carried, battery operated echocardiographic system (Vivid *i*, General Electric) with M-mode, two-dimensional, and Doppler (pulsed, continuous, and color) imaging. The following were obtained: apical two-, four-, and five-chamber views, parasternal long-axis and short-axis views at the level of the papillary muscles and the aortic valve and subcostal views.

Particular attention was paid to valve morphology on cross-sectional, two dimensional imaging and the degree and extent of mitral and aortic regurgitation, assessed by color flow Doppler imaging. The apical imaging included anterior angulation to evaluate the left ventricular outflow tract and the aortic valve. Transvalvular flow was assessed by measuring the peak velocity with continuous wave Doppler imaging. Hard copies with detailed location and type of lesions (Appendix V, page 3) and electronic records of relevant data were kept for offline re-evaluation.

The echocardiographic study was performed by the candidate and two blinded experienced cardiologists reviewed the records. The diagnosis of RHD was accepted when there was agreement of at least two cardiologists, including the researcher.

Echocardiographic criteria

Only left-sided valves were evaluated for features of RHD as affection of the right sided valves is fairly common and not related to the condition (Lembo 1988, Kostucki 1986, Lembo 1988, Yoshida 1988). RHD was diagnosed by the different echocardiographic diagnostic criteria as following:

Criteria 1 (WHO 2004): (1) a regurgitant jet >1 cm in length, (3) seen in at least 2 planes, (3) a mosaic color jet with a peak velocity >2.5 m/s, (4) persisting throughout systole or diastole

Criteria 2 (Carapetis 2008)*:* (1) valvular regurgitation (meeting all four criteria defined by the WHO) with evidence of structural changes to valve appearance: (a) valvular thickening (b) elbow deformity of the anterior mitral valve leaflet (2) any degree of pathological aortic regurgitation (3) mitral stenosis (flow acceleration and a mean pressure gradient > 4 mmHg) (4) aortic stenoses (flow acceleration with peak velocity > 2 m/s)

Criteria 3 (Marijon 2007)*:* any degree of valvular regurgitation seen in at least 2 planes accompanied by at least two of the following three structural abnormalities of the regurgitant valve: (1) leaflet morphology (typical marked thickening of the margins), (2) leaflet mobility (abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures)

*Criteria 4 (*AHC criteria)*:* The suggested criteria for the diagnosis of RHD consists of the presence of any Doppler-detected valvular affection of the right-sided valves in association with at least one structural rheumatic valvular feature (as extensively described in chapter 3, pg. 90). Regurgitation should be defined as pathological when meeting all four criteria defined by the WHO (Table 1.3), however, including trivial affection. Mitral stenosis should diagnosed in case of flow acceleration across the mitral valve and a mean pressure gradient greater than 4 mmHg. Similarly, flow acceleration across the aortic valve with a peak velocity greater than 2 m/s is necessary for diagnosis of aortic stenosis.

The structural criteria should be based on the study of (1) quantitative leaflet morphology (typical marked thickening of the margins, greater than 4mm), (2) leaflet shortening with abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures).

Statistical analysis

The statistical analysis was performed as descrived in chapter 3, pg 95.

6.2.3 Results

A total of 639 individuals were screened, excluding individuals with a history of valve replacement.

6.2.3.1 Echocardiographic screening

Structural findings

Our series showed that 85.6% (n=534) of the individuals assessed had structural affection of the heart valves. 28.4% (n=177) had 1 abnormality of the heart valves and 24.0% (n=150) had a combination of 2 different abnormalities of the valves.

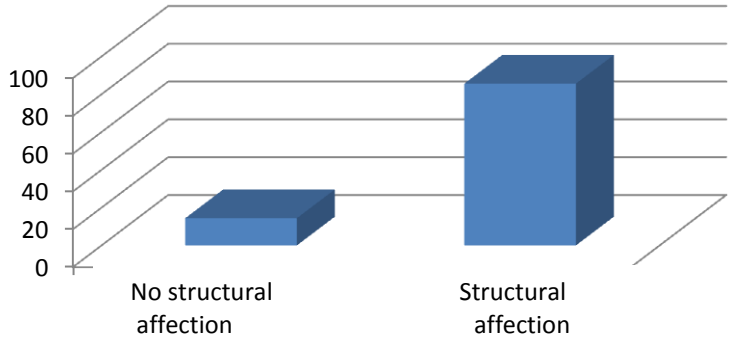


Figure 6.1 Structural affection

Thickening of the heart valves and subvalvular thickening was found in 79.6% (n=497) and 27.8% (n=173) of the individuals, respectively. Doming was found in 46.6% (n=291) of the

individuals. The commissures were affected in 7.9% (n=49) of the cases. Calcification was found in 3.5% (n=22) and restriction of the heart valves was found in 30.4% (n=190) of the cases (Table 6.1).

Table 6.1 Incidence of structural features

Type of feature	% (n)
Thickening of valve cusps	79.6% (n=497)
Doming of valve cusps	46.6% (n=291)
Fusion of subvalvular apparatus	27.8% (n=173)
Fusion of commissures	7.9% (n=49)
Calcification	3.5% (n=22)
Fixed leaflets	30.4% (n=190)

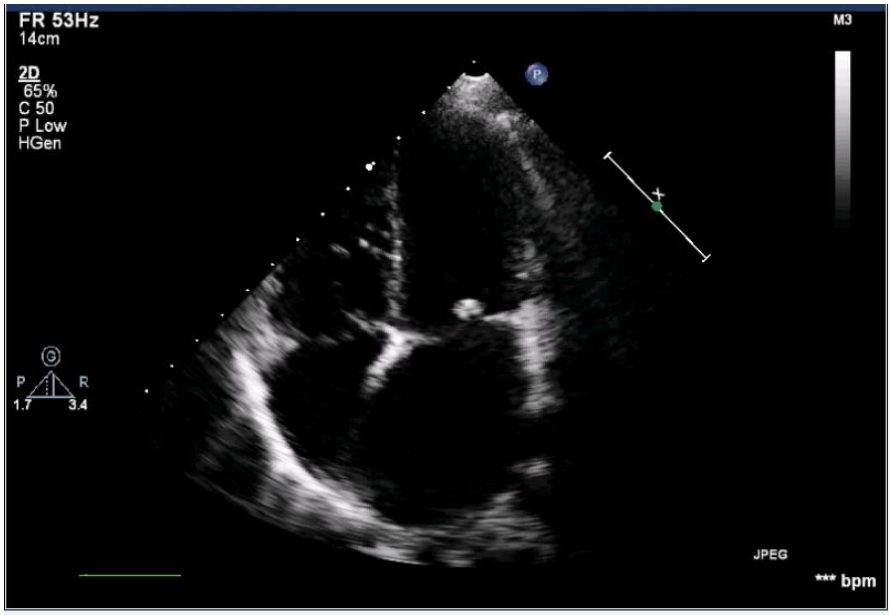


Figure 6.2Thickening of the mitral leaflets and subvalvular apparatus in a parasternal long-axis view

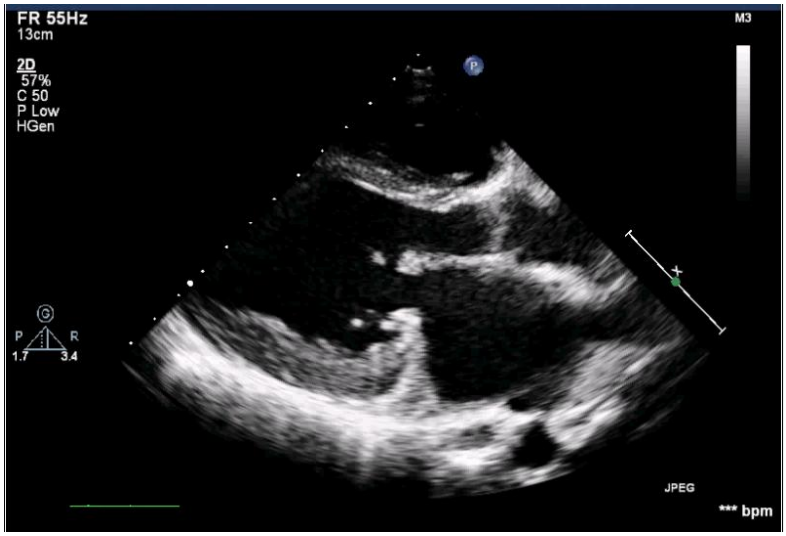


Figure 6.3 Thickening of the mitral leaflets in an apical four-chamber view

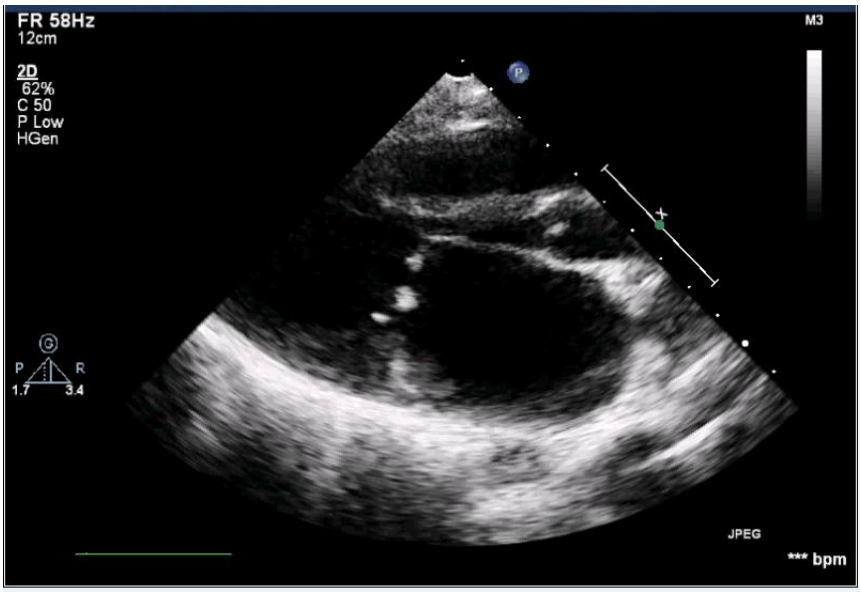


Figure 6.4 Doming of the mitral valve in parasternal long-axis view

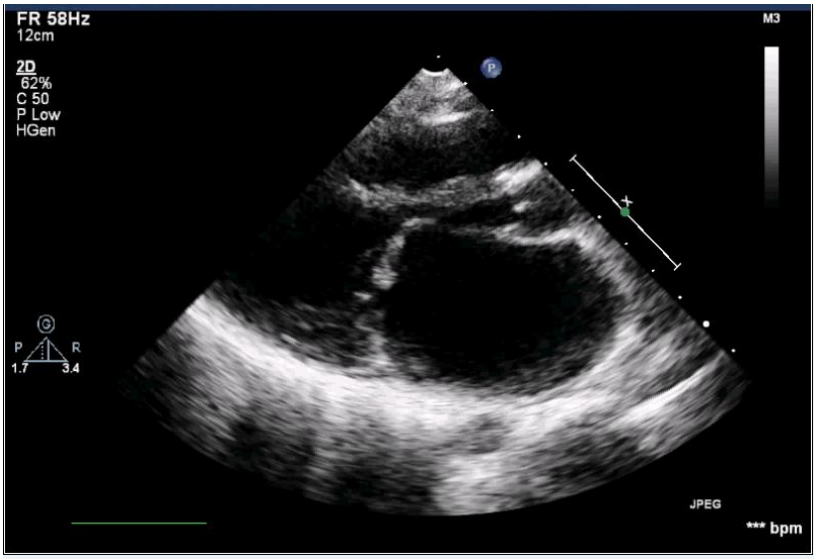


Figure 6.5 Thickening and doming of the mitral valve in parasternal long-axis view

Functional affection

A significant amount of the individuals (73.4%, n=458) had functional affection of the right sided valves. Mitral valve affection was found in 27.7% (n=173) of the individuals and 14.9% (n=93) had affection of the mitral and the aortic valve. The aortic valve was affected in 2.4% (n=15) of the cases. Affection of the mitral, aortic and tricuspid valve was found in 16% (n=100) of the individuals (Figure 6.2)

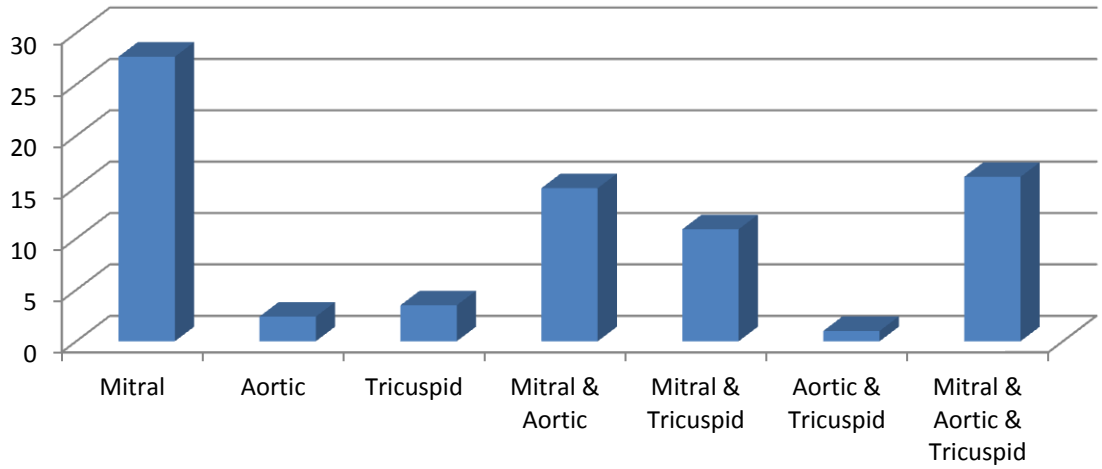


Figure 6.6 Distribution of valve affection

Mitral valve: Mitral regurgitation was found in 64.6% (n=402) of the cases. Trivial regurgitation was found in 23.1% (n=144) and mild regurgitation was found in 21.8% (n=136) of the individuals. Moderate and severe mitral regurgitation was found in 6.7% (n=42) and 11.9% (n=74), respectively (Figure 6.9).

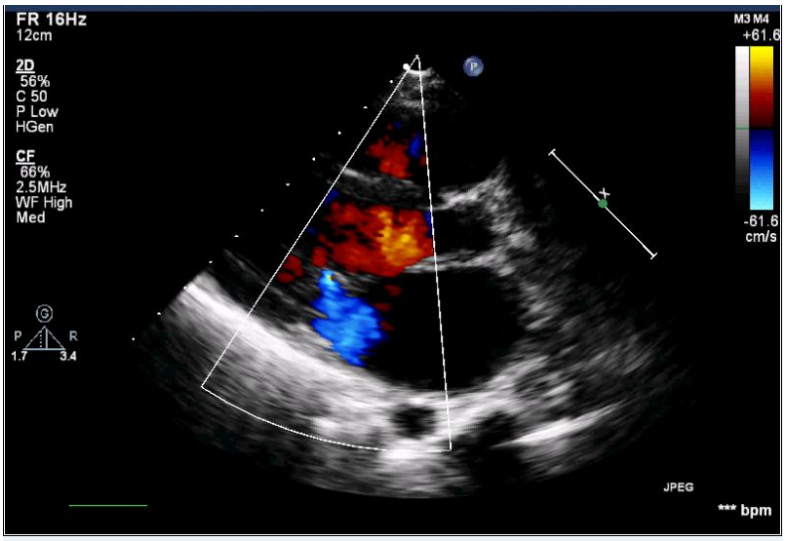


Figure 6.7 Mitral regurgitation in a parasternal long-axis view

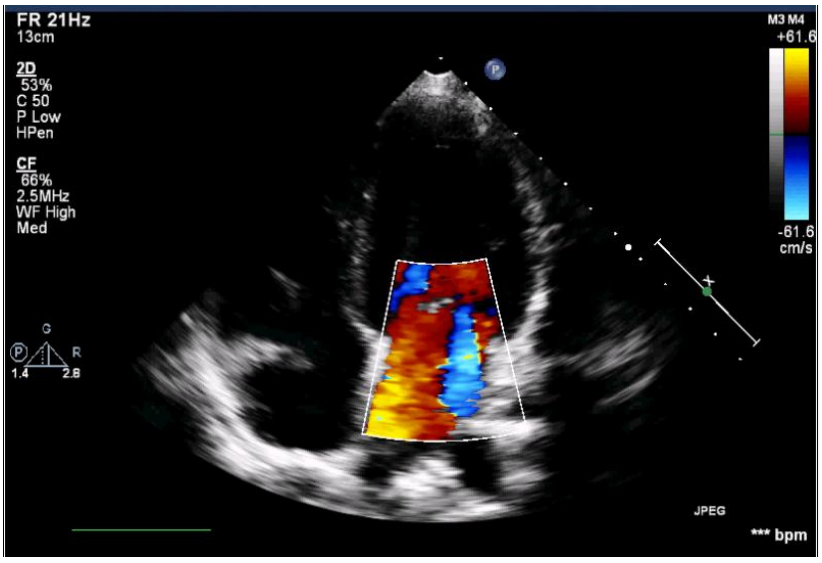


Figure 6.8 Mitral regurgitation in a apical four-chamber view

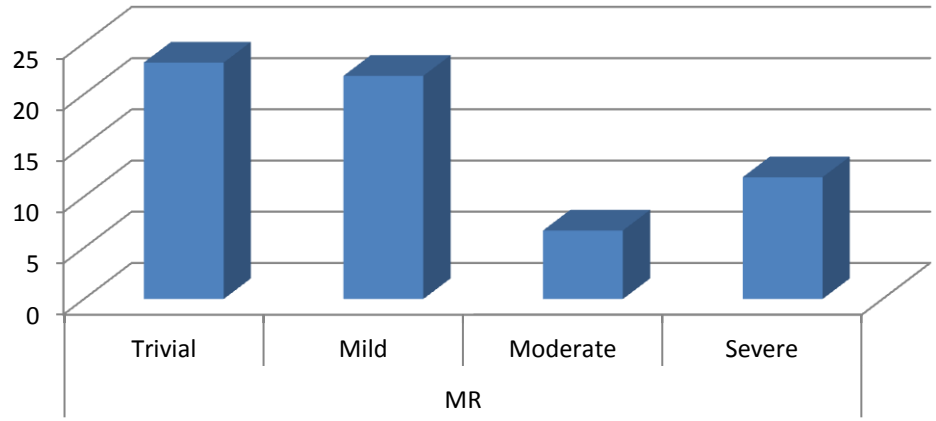


Figure 6.9 Severity of mitral regurgitation

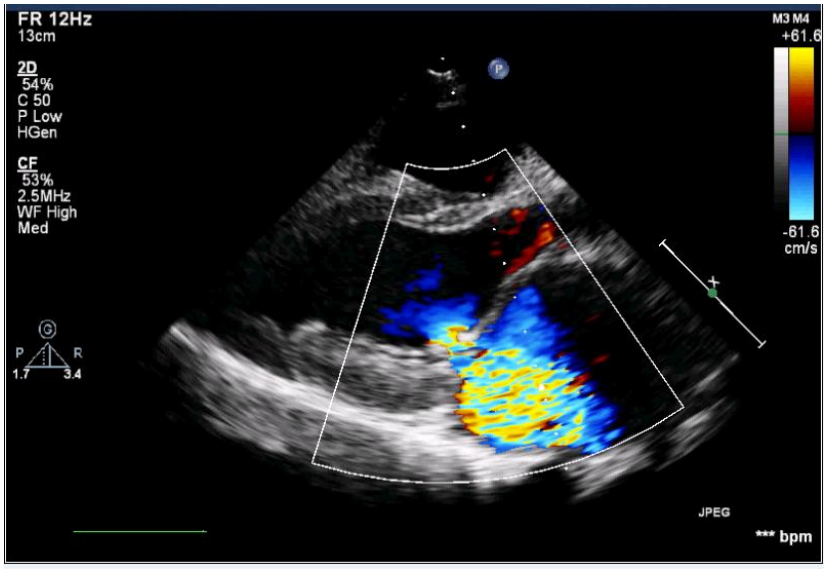


Figure 6.10 Severe mitral regurgitation in a parasternal long-axis view

Mitral stenosis was found in 26.4% (n=165) of the cases. Trivial mitral stenosis was found in 0.3% (n=2) of the cases. Mild and moderate mitral stenosis was found in 5.8% (n=36) and 5.0% (n=31) of the cases, respectively. Severe mitral stenosis was found in 14.9% (n=93) of the cases.

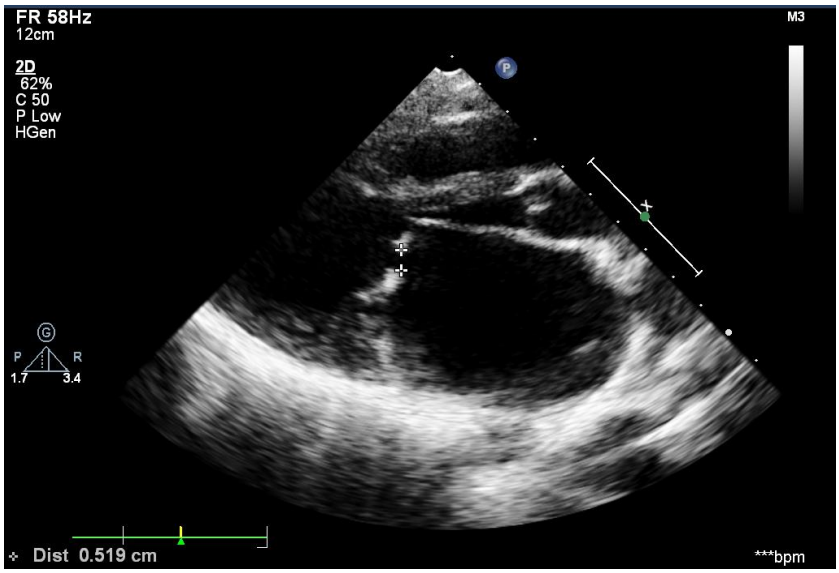


Figure 6.11 Restricted opening of a stenotic mitral valve in a parasternal long-axis view

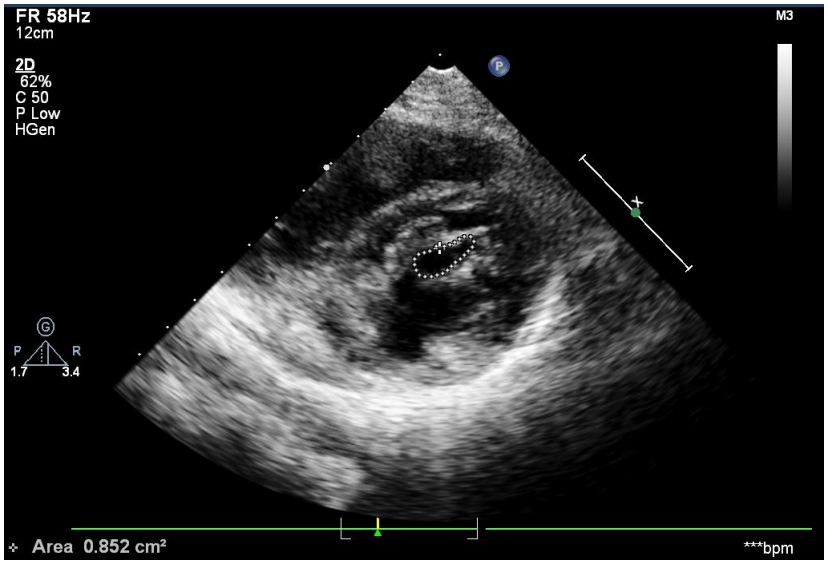


Figure 6.12 Mitral valve area in a parasternal short-axis view

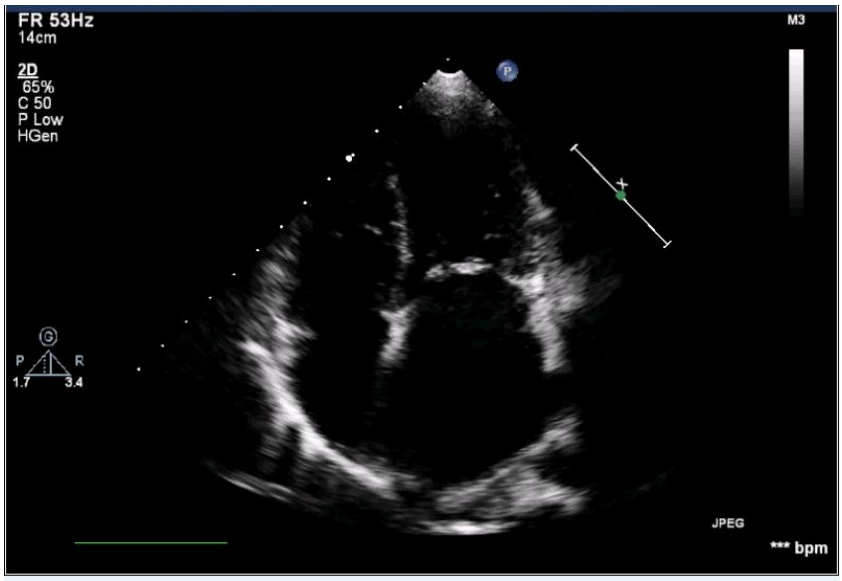


Figure 6.13 Restricted opening of stenotic mitral valve in an apical four-chamber view

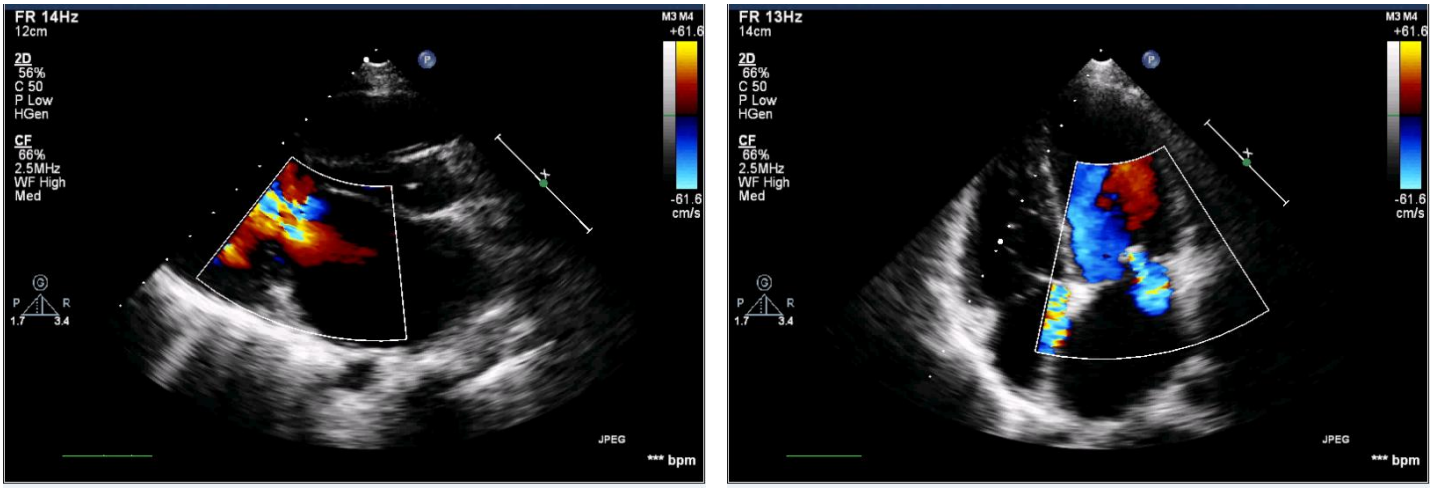


Figure 6.14 Restricted flow through stenotic mitral valve (parasternal and apical four-chamber view)

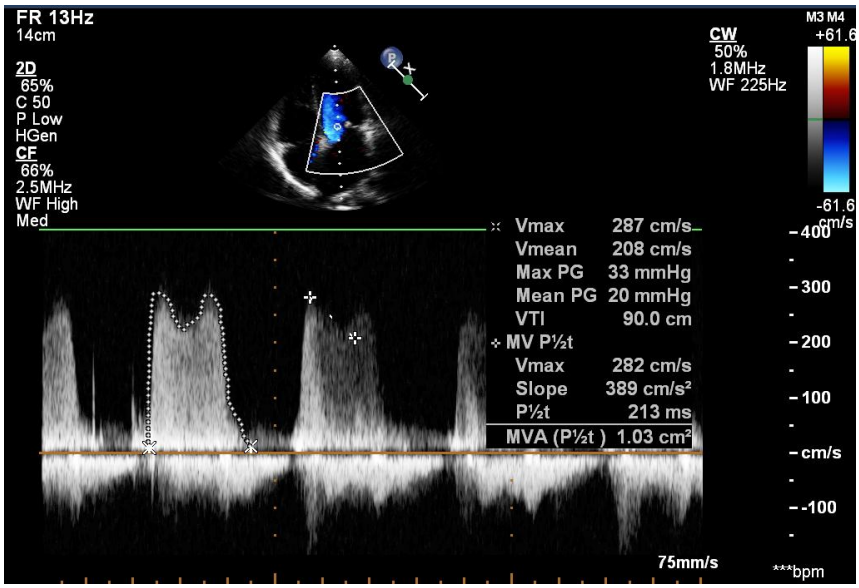


Figure 6.15 Gradient across a stenotic mitral valve (apical four-chamber view)

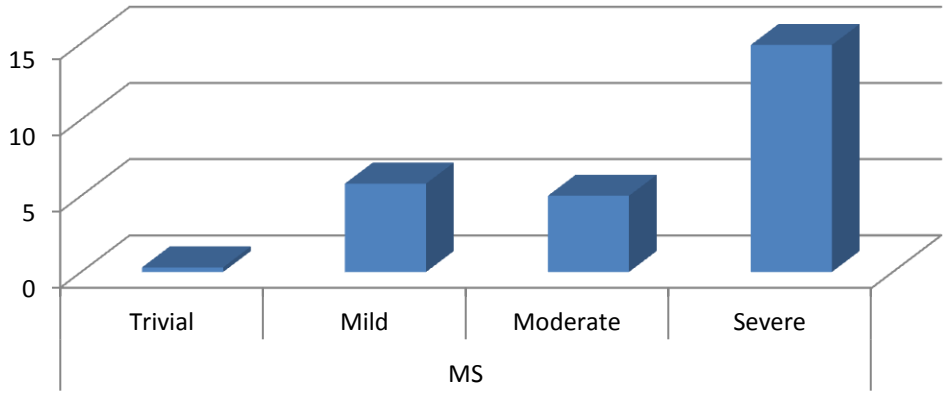


Figure 6.16 Severity of mitral stenosis

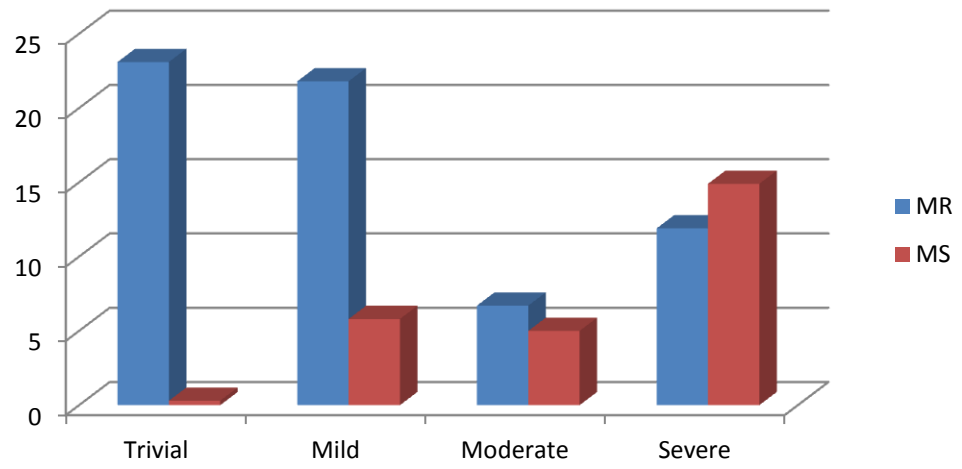


Figure 6.17 Distribution of mitral affection

Isolated mitral regurgitation was found in 22.8% (n=142) of the cases, isolated mitral stenosis was found in 2.24% (n=14) of the cases. Part of all mitral regurgitation (35.3%, n=142) and 8.49% (n=14) of all mitral stenosis cases were isolated. A combination of mitral regurgitation and stenosis was found in 3.36% (n=21) of the cases (Figure 6.18).

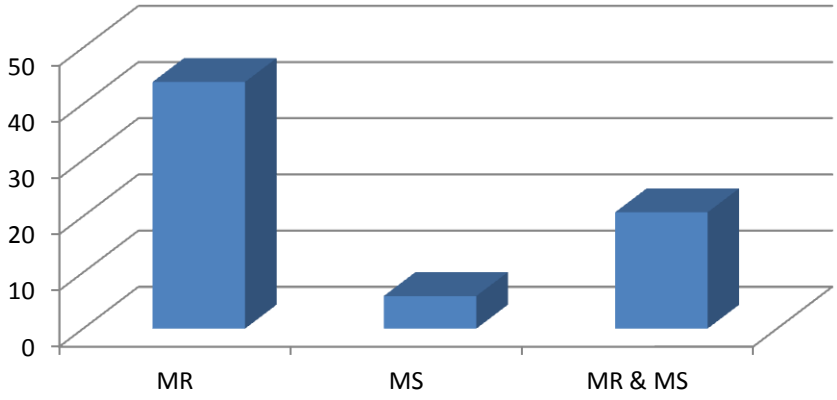


Figure 6.18 Mitral functional affection (MR= Mitral regurgitation, MS= Mitral Stenosis)

Aortic valve: Aortic regurgitation and stenosis was found in 31.4% (n=196) and 9.8% (n=61) of the cases, respectively.

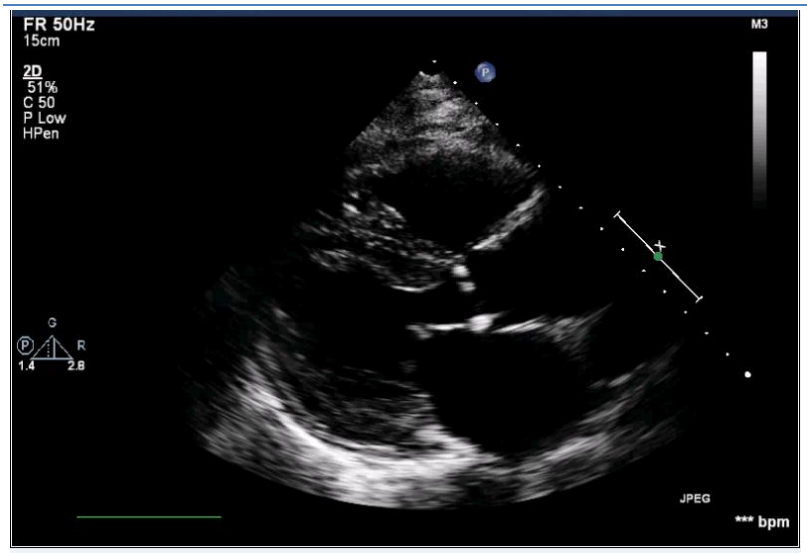


Figure 6.19 Thickening of the aortic valve in a parasternal long-axis view

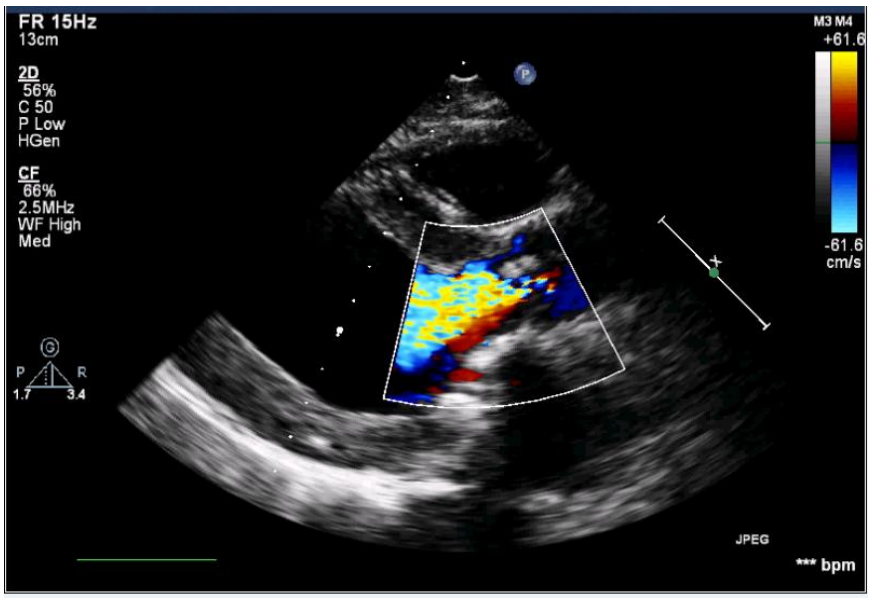


Figure 6.20 Aortic regurgitation in a parasternal long-axis view

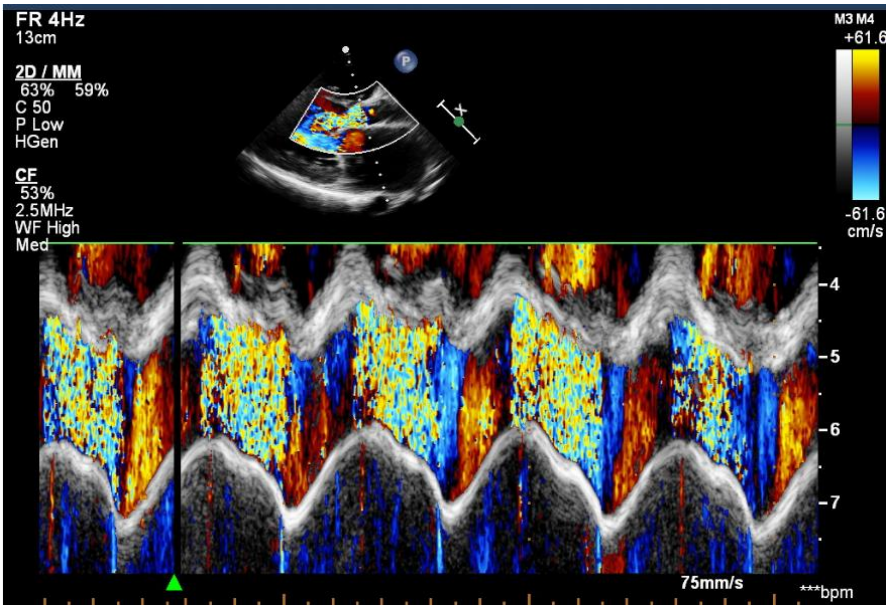


Figure 6.21 Determination of severity by analysis of the magnitude of the regurgitant jet in aortic root in M-mode (parasternal long-axis view)

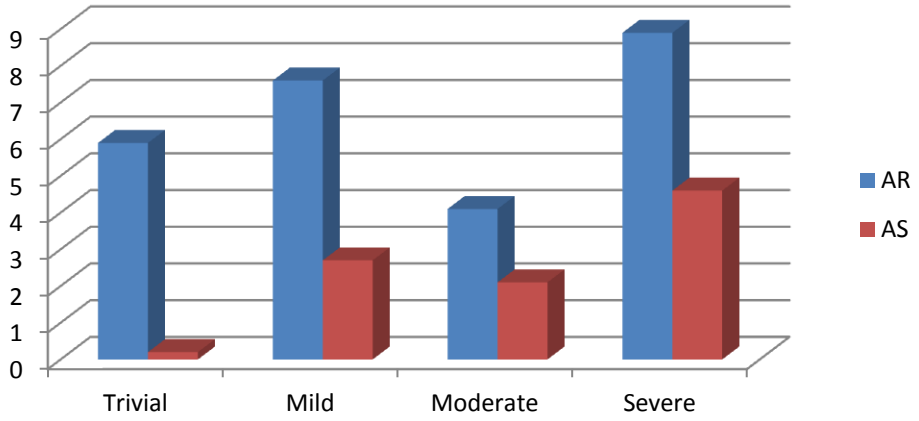


Figure 6.22 Distribution of aortic affection

Isolated aortic regurgitation was found in 2.56% (n=16) and isolated aortic stenosis was not found. A combination of isolated aortic regurgitation and aortic stenosis was found in 0.48% (n=3) of the cases. A small fraction (8.16%, n=16) of all aortic regurgitation found was isolated.

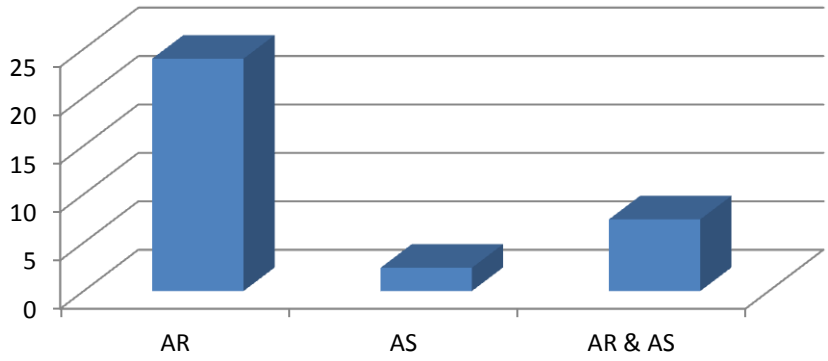


Figure 6.23 Aortic functional affection (AR= Aortic regurgitation, AS= Aortic Stenosis)

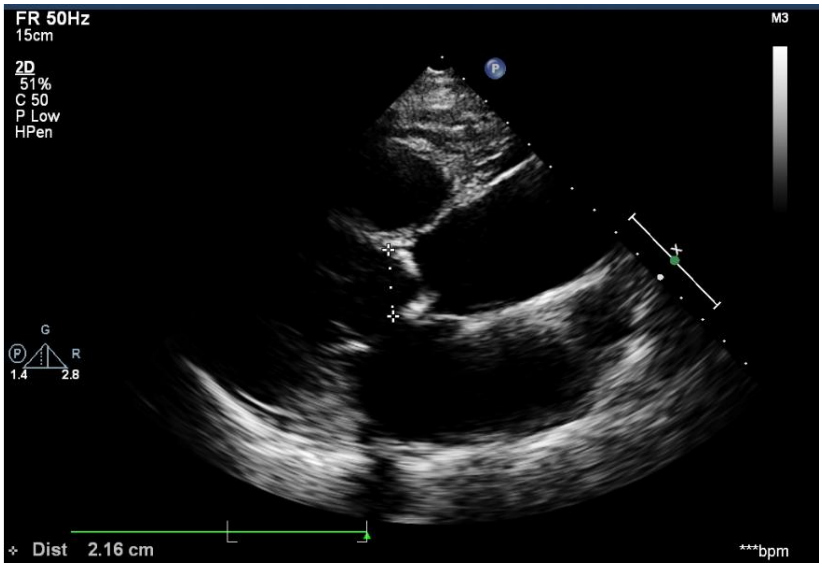


Figure 6.24 Measurement of aortic valve area in a parasternal long-axis view

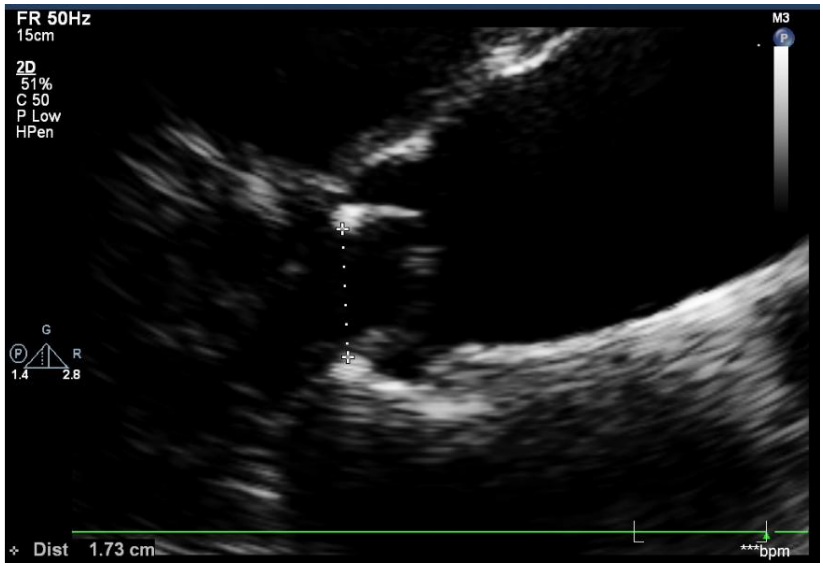


Figure 6.25 Detail: Determination of aortic valve area in thickened and stenotic valve (parasternal long-axis view)

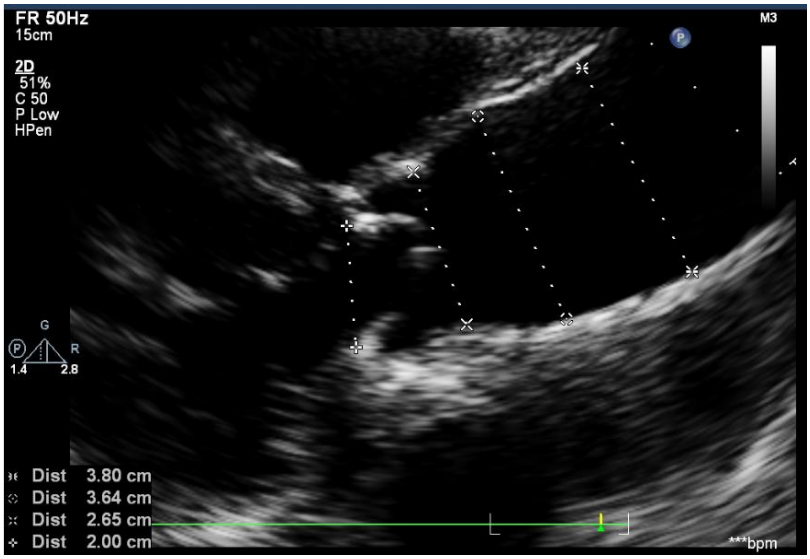


Figure 6.26 Determination of aortic valve area in relation to aortic root for determination of the severity of stenosis (parasternal long-axis view)

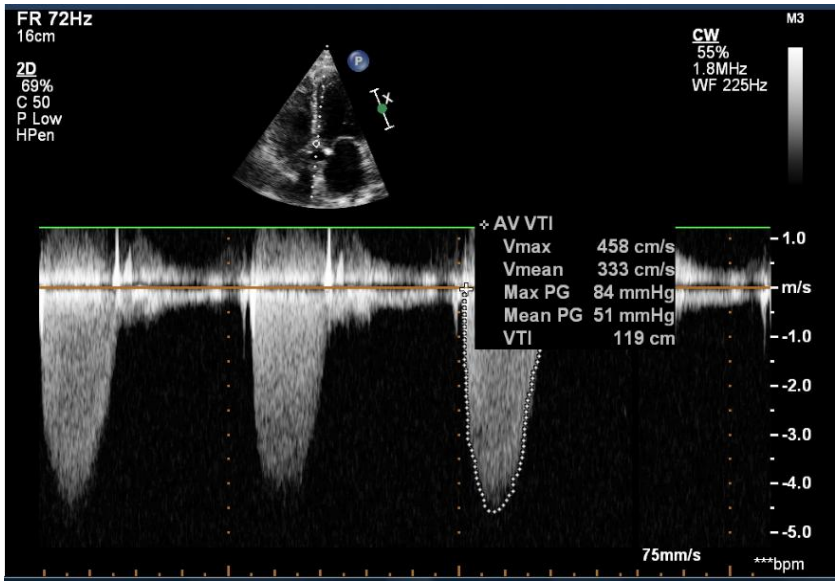


Figure 6 27 Gradient across a stenotic aortic valve (apical four-chamber view)

Tricuspid valve: Tricuspid regurgitation was found in 32.4% (n=202) and isolated tricuspid affection without functional affection of any other valves was found in 3.8% (n=24) of the cases. The majority of the cases were trivial (14,3%, n=89) while 9.3% (n=58) and 5.1% (n=32) were mild or moderate, respectively. A small number of the tricuspid regurgitation cases (1.8%, n=11) were found to be severe. Tricuspid stenosis was found in 1.1% (n=7) of the cases and no isolated tricuspid stenosis was found.

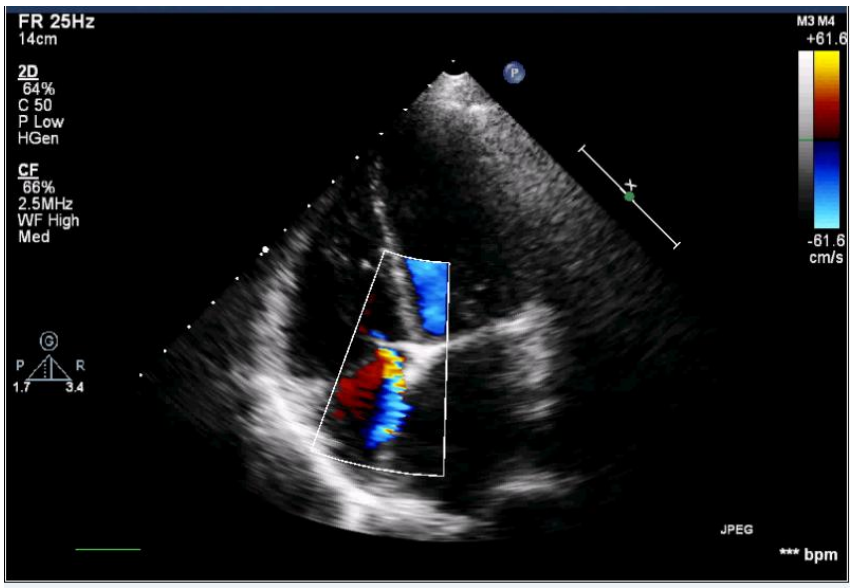


Figure 6.28 Tricuspid regurgitation in an apical four-chamber view

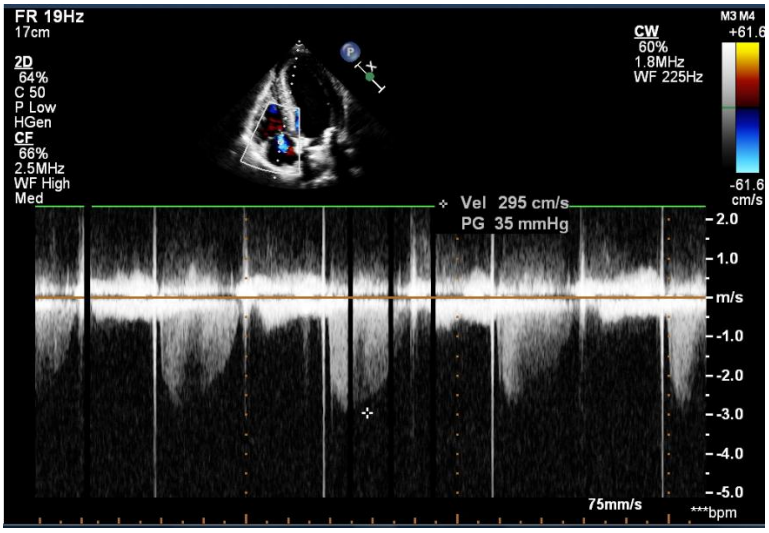


Figure 6.29 Gradient across regurgitant tricuspid valve in an apical four-chamber view

Table 6.2 Distribution of valvular affection

	Mitral % (n)		**Aortic % (n)**		**Tricuspid % (n)**	
Isolated	27.7% (n=173)		27.7% (n=173)			
	Regurgitation	**Stenosis**	**Regurgitation**	**Stenosis**	**Regurgitation**	**Stenosis**
	64.6% (402)	26.4 (165)	31.4 (196)	9.8% (61)	32.4 (202)	1.1 (7)
Trivial	23.1 (144)	0.3 (2)	6.9 (43)	0.2 (1)	14.3 (89)	
Mild	21.8 (136)	5.8 (36)	18.0 (56)	2.7 (17)	9.3 (58)	
Moderate	6.7 (42)	5.0 (31)	4.8 (30)	2.1 (13)	5.1 (32)	
Severe	11.9 (74)	14.9% (93)	10.4 (65)	4.6 (29)	1.8 (11)	

6.2.3.2 Evaluation of newly developed echocardiographic diagnostic criteria

Prevalence of subclinical RHD following the different diagnostic criteria

Following our echocardiographic criteria (criteria 4, AHC criteria) which combines functional and structural features (as described in chapter 3, pg. 89) for the diagnosis of RHD, 69.2% (n=432) of the individuals were diagnosed with RHD (Figure 6.30).

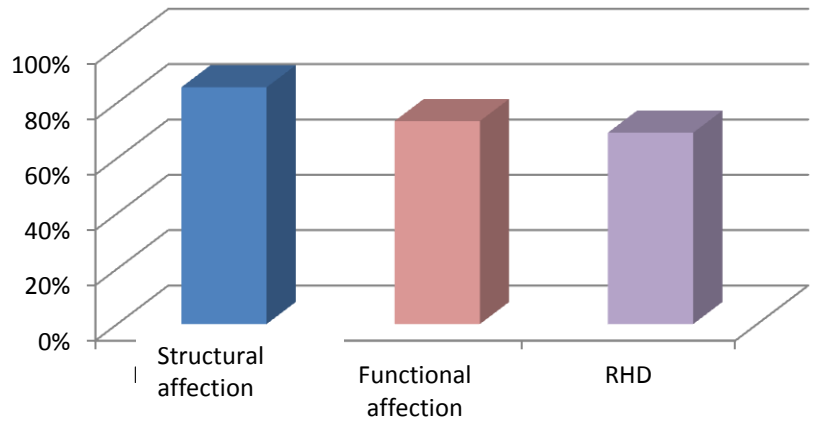


Figure 6.30 Incidence of valvular affection by the AHC criteria

Following criteria 1 (WHO 2004), which is based on the finding of mitral and/or aortic regurgitation 46.7% (n=291) would be diagnosed with RHD.

Following criteria 2 (Carapetis 2008) a total of 54.6% (n=340) would be diagnosed with RHD of which 25.9% (n=88) would have been diagnosed by the presence of pathological aortic regurgitation, aortic or mitral stenosis. However, 33.2% (n=113) of the individuals diagnosed by the criteria had pathological aortic regurgitation, aortic or mitral stenosis in combination with structural affection. A small number (0.8%, n=5) of the individuals would be diagnosed with borderline RHD in this criteria.

Following criteria 3 (Marijon 2007) which combined valvular regurgitation with at least 2 structural signs a total of 40.6% (n=253) individuals would be diagnosed with RHD. A combination of at least 2 of the structural findings but no functional affection was found in 44.6% (n=278) of the individuals (Figure 6.31).

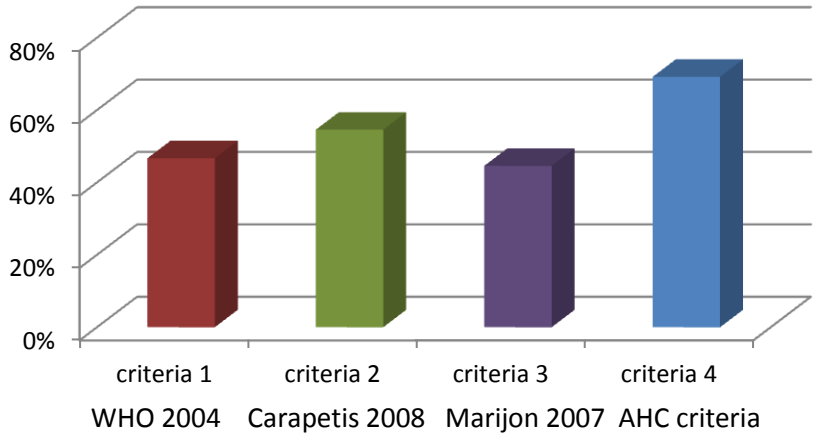


Figure 6.31 Diagnosis of RHD following the different criteria

6.2.3.3 Clinical versus echocardiographic diagnosis of subclinical RHD

The majority (81.1%, n=515) of the individuals screened did not have an audible cardiac murmur and only 18.9% (n=120) had a murmur (Figure 6.32).

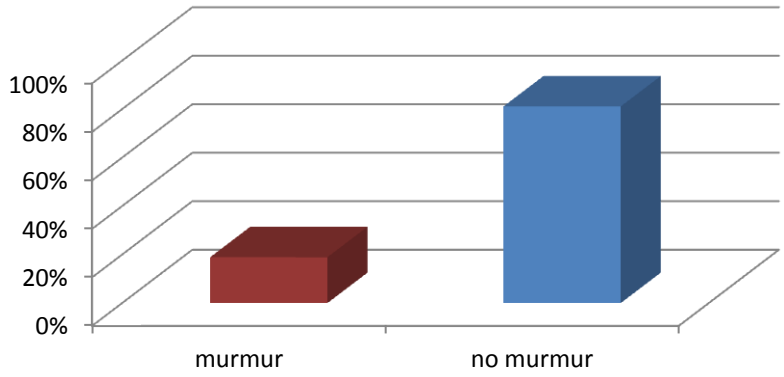


Figure 6.32 The presence of a cardiac murmur in the screened population

None of the individuals with only structural affection of the heart valves had a murmur. From the individuals with functional affection of the heart valves only 17.9% (n=113) had a murmur. The majority of the individuals (82.1%, n=519) did not have a murmur and would not have been

diagnosed by auscultation only (Figure 6.33). This shows a 4.6-fold increase of detection rate of functional lesions by echocardiography when compared to clinical examination.

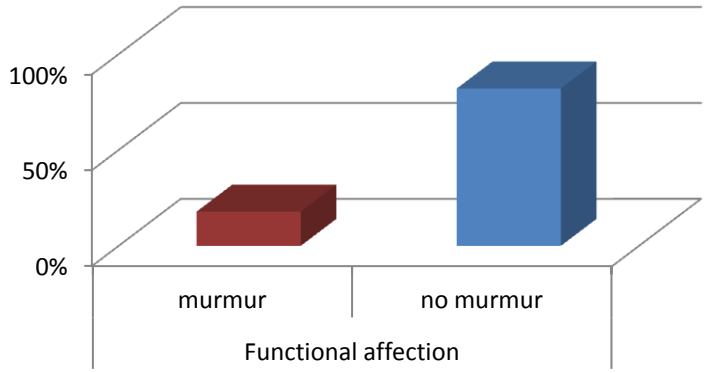


Figure 6.33 The presence of a cardiac murmur in combination with functional affection of the heart valves

From the individuals diagnosed with RHD following our echocardiographic criteria only 17.1% (n=108) had a murmur. The majority of the patients diagnosed with RHD (82.9%, n=524) did not have a murmur (Figure 6.34). This shows a 4.85-fold increase of detection rate of RHD by echocardiography when compared to clinical examination.

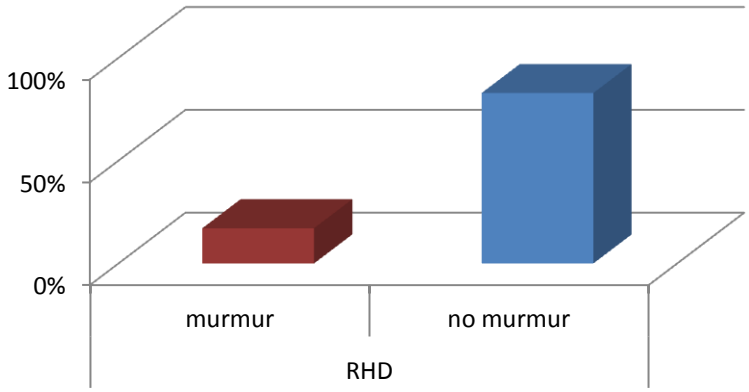


Figure 6.34 The presence of a cardiac murmur in patients diagnosed with RHD

6.2.4 Discussion

In our series only a minority of the individuals with functional affection of the heart valves and none of the individuals with isolated structural affection of the heart valves had a murmur.

From the individuals diagnosed with RHD following our echocardiographic criteria only 17.1% had a murmur, showing that 82.9% of the individuals diagnosed with RHD by echocardiography would have been missed if assessed by auscultation only. Based on these results we can conclude that functional affection of the heart valves and the presence of RHD is diagnosed 4.6 and 4.85 times more often by echocardiography than clinically, respectively. This is caused by the fact that murmurs are missed even by experienced clinicians due to many factors like the associated tachycardia, noisy surrounding, low intensity of the murmur (Saxena 2000) and the fact that mild valvular regurgitation is subclinical and in the acute phase, even moderate mitral or aortic regurgitation may not be clinically audible. Furthermore, Doppler echocardiography is more sensitive and more accurate in diagnosing valvular involvement in acute RF (Vijayalakshmi 2009) as it can detect significant valvar incompetence in the absence of auscultatory findings, during both the acute and the quiescent phases of the disease (Veasy 1993, Veasy 1994, Wilson 1995, Folger 1992).

Previous reports mention that around 40% of the mitral regurgitation and subclinical RHD cases and over 80% of the aortic regurgitation would have been missed when assessed only clinically (Vijayalakshmi 2008). Screening based only on auscultation is therefore likely to miss many cases of RHD (Carapetis 2008) and comprehensive echocardiographic assessment has been shown to identify approximately 10 times as many children with RHD as the traditional strategy of clinical screening (Marijon 2007). Our series shows a lower detection benefit with echocardiography probably due to the fact that the lesions found in earlier studies were milder, leading to a greater discrepancy between the prevalence reported by echocardiography and by auscultation (Carapetis 2008). Furthermore, the fact that we assessed a population with history of RF and expected to have a higher incidence of more prominent valvular affection might have also contributed to the presence of murmurs related to pathology. This suggests that that the detection rate by echocardiography will increasingly exceed the auscultatory findings in the screening of RHD.

Our criteria diagnosed 69.2% of the screened population with RHD, while the detection by the other criteria ranged only up to 54.6%. This might be due the fact that Criteria 1 and 2 exclude trivial regurgitation and miss therefore all trivial and milder functional affection of the valves compared to our criteria. Bearing in mind that 31.7% of the individuals screened had isolated trivial mitral regurgitation we can conclude that this criteria is not suitable for the diagnosis of subclinical RHD, which needs the best identification, as it is the most probable type of affection benefitting from treatment and prophylaxis. Although criteria 2 includes structural features for the diagnosis of RHD, these features were not required in the presence of regurgitation of mild or greater severity. This could lead to over-diagnosis of RHD in an older population where a higher incidence of functional valvular affection is expected.

In criteria 2 the presence of aortic regurgitation, mitral or aortic stenosis were considered to indicate RHD while excluding structural features. We believe that aortic regurgitation, mitral or aortic stenosis are indeed signs of RHD, but we think that they will be found in combination with structural affection and therefore, it is unneeded to create a separate criterion.

Criteria 1 and 3 do not include stenotic lesions in the diagnostic criteria while it is known that mitral and aortic stenosis are mainly caused by RHD, especially in the young population and our series shows isolated mitral and aortic stenosis in 5.8% and 2.4% of the cases. This could suggest that a great amount of RHD cases expressed by stenotic lesions would be missed in these criteria.

Furthermore, in criteria 3 a combination of 2 structural features was required for the diagnosis of RHD. Our series shows that cardiac affection was mostly related to the finding of only 1 single structural feature, suggesting that the requirement of a combination of 2 structural features will cause under-diagnosis of especially mild and subclinical cases, which require treatment and prophylaxis the most for a possible reversibility of the lesions. Our series confirms that case detection may differ importantly according to the diagnostic criteria utilized (Marijon 2009). Furthermore, based on these results we believe that all criteria under-diagnose RHD, specially mild and subclinical cases, which are mostly expected to resolve with proper treatment and prophylaxis.

Echocardiographic definition of RHD based on functional characteristics may depend on the gain settings on the ultrasound equipment, therefore, the use of Doppler criteria for abnormalities

associated with structural valve changes would be expected to improve the specific diagnosis of RHD (Marijon 2007). Also, criteria based on structural changes as defined in the combined criteria may be more likely to suggest chronic RHD lesions and is believed to improve the detection of children with subclinical RHD but without significant valve regurgitation who would not be eligible for secondary RHD prophylaxis under current international guidelines. The WHO criteria may be easier to use, however this set of criteria seems to suffer from a substantially lower sensitivity. In conclusion, adding structural features to diagnostic criteria has significant consequences in terms of case detection rates and the combined criteria seems to be better suited for screening of subclinical RHD.

Screening with sensitive and specific echocardiographic criteria for subclinical and valvular affection is highly important as it is a relatively common condition in RF (Tubridy-Clark 2007, Figueroa 2001) and RHD remains the main origin of heart valve disease in different areas. Therefore, significant subclinical valve lesions should be diagnosed and labelled and affected individuals should have long-term follow-up and secondary rheumatic fever prophylaxis (WHO 2004). However, the boundary between physiological valve regurgitation and authentic but minimal rheumatic lesions remains difficult to discern as valvular regurgitation is fairly common, showing prevalences of 2.4 to 45% for mitral (Shah 1989, Lembo 1988), up to 33% for aortic (Shah 1989, Lembo 1988), 6.3 to 95% for tricuspid (Lembo 1988, Kostucki 1986) and 21.9 to 92% for pulmonary regurgitation (Lembo 1988, Yoshida 1988).

Furthermore, subvalvular or valvular thickening and any other structural affection of the valve may develop before the leaflet retraction and thereby regurgitation, noting that structural changes of valves affected by the rheumatic inflammatory process, even before the development of pathological regurgitation likely indicate subclinical RHD, which might benefit from identification and thence secondary prevention (Marijon 2007). Adding structural features to criteria definition has been shown to increase case detection rates by up to 3-fold (Marijon 2009), leading to significant consequences in terms of case detection rates, follow-up and use of penicillin prophylaxis and thus for eventual health and long-term outcomes of RHD. Therefore, we confirm the benefit and need for the use of a combined criteria, evaluating structural and functional features of the valve, adding to the importance of different features,

contributing to improvement and updating the criteria for the diagnosis of RHD to avoid under as well as over diagnosis of the condition.

6.2.5 Conclusions

Screening based only on auscultation is likely to miss many cases of pathological valvular lesions due to RHD compared with echocardiographic screening in Egypt. Echocardiography is not only more sensitive and more accurate in confirming, diagnosing and quantifying valvular involvement in RF as it is essential for establishing the cause of the murmur. Furthermore, it is of immense value in ruling out infective endocarditis in patients with RF and RHD.

Importantly, in Eygpt, around 90% of cases of RHD are clinically silent, occurring in asymptomatic individuals without audible murmurs, suggesting that echocardiographic screening would be a desirable goal to optimize case identification and targeted secondary prevention measures. Detection of lesions is important especially because those with mild and subclinical disease stand to benefit most from secondary prophylaxis. Therefore, we confirm that echocardiography should be the diagnostic golden standard and screening tool as it is the only approach with sufficient sensitivity and specificity for the detection of subclinical carditis and RHD in the absence of auscultatory findings and overt evidence of valvular or other cardiac involvement, during both the acute and the quiescent phases of the disease. However, there are no universally agreed criteria for the echocardiographic diagnosis of RHD and the currently criterias in use differ and show different detection rates for the affected individuals.

We confirm the benefit of a criteria which combines structural and functional characteristics of RHD, and we have contributed to the improvement of the criteria which increases the detection of rheumatic valvular affection, avoiding under- as well as over-diagnosis of the condition. The use of the suggested stringent criteria will have major health care benefits as early recognition and diagnosis limits the progression to chronic subclinical valvular disease and improves the prognosis of RHD with adherence to secondary penicillin prophylaxis regimens.

6.3 Subclinical RHD in individuals with history of RF in EgyptThe aim of this study was to analyze the incidence, characteristics and determinants of RHD in a population with history of RF.

6.3.1 Patients and methods

Individuals visiting the penicillin or outpatient clinic of the NHI were assessed for the diagnosis of RHD and determination of the echocardiographic characteristics as described in chapter 3, pg 75.

The study group consisted of:

-Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement

-Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD

Demographic data

Demographic data from every subject selected were collected in the form of a standardized questionnaire (Appendix V). The questionnaire contains questions regarding the patient's demographics, family environment, risk factors, history of RF, its severity, treatment and prophylaxis.

Issues regarding the onset age of RF, symptoms, severity, presence of recurrences of RF, medication and penicillin prophylaxis were questioned on the history of RF and the presence of symptoms to confirm the presence of RF and clinical presentation. Penicillin prophylaxis schedule was divided into 3 categories, following the most used and prescribed schedules for penicillin prophylaxis, every 15 days, 3 weeks or monthly. Compliance rate for the penicillin prophylaxis was evaluated by asking the patient about the schedule, how often he ignored or postponed the injection date and if he was strict to the schedule. Compliance was then also be evaluated in relation to the schedule. A schedule of less than 9 injections a year was considered poor. A schedule of 9 to 12 injections a year was considered fair, while an excellent compliance rate was more than 12 injections a year. Many individuals were receiving prophylaxis for the first time therefore questions on schedule and compliance was not applicable.

The disease awareness as classified in 3 categories evaluated on the patients' knowledge of the RF history, symptoms and penicillin prophylaxis. The disease awareness was also be evaluated by the amount and type of questions answered on the knowledge of RF and RF history. The disease awareness was evaluated as poor when the patient had no knowledge of the reason for penicillin prophylaxis. When the patient was aware of RF or the valvular damage, and knew that penicillin is a way of prophylactic prevention but did not know the link between RF and the valvular damage the awareness was evaluated as fair. The patient's awareness was evaluated as good when he was also aware of the correlation between RF and cardiac disease (RHD). The same scale was used for the parents' awareness. Dental care was evaluated during collection of throat swabs by the general condition of the mouth and teeth.

Examination protocol

The screened individuals underwent a standardized examination protocol consisting of detailed medical history recorded by the investigator, physical examination including general analysis of the patients' health, recognition of Jones criteria and careful cardiac auscultation with the patient in the supine and left lateral decubitus positions.

Disease manifestations and auscultatory findings were evaluated according to internationally approved criteria, including the published data from World Health Organization (WHO 1988, Figueroa 1992). Detailed transthoracic echocardiography was performed with the use of a hand-carried, battery operated echocardiographic system (Vivid *i*, General Electric) with M-mode, two-dimensional, and Doppler (pulsed, continuous, and color) imaging. The following were obtained: apical two-, four-, and five-chamber views, parasternal long-axis and short-axis views at the level of the papillary muscles and the aortic valve and subcostal views.

Particular attention was paid to valve morphology on cross-sectional, two dimensional imaging and the degree and extent of mitral and aortic regurgitation, assessed by color flow Doppler imaging. The apical imaging included anterior angulation to evaluate the left ventricular outflow tract and the aortic valve. Transvalvular flow was assessed by measuring the peak velocity with continuous wave Doppler imaging. Hard copies with detailed location and type of lesions (Appendix V, page 3) and electronic records of relevant data were kept for offline re-evaluation.

The echocardiographic study was performed by the candidate and two blinded experienced cardiologists reviewed the records. The diagnosis of RHD was accepted when there was agreement of at least two cardiologists, including the researcher.

Echocardiographic criteria

Our suggested criteria for the diagnosis of RHD consists of the presence of any Doppler-detected valvular affection of the right-sided valves in association with at least one structural rheumatic valvular feature as described in chapter 3, pg. 86. Regurgitation should be defined as pathological when meeting all four criteria defined by the WHO (Table 1.3). However, including trivial affection. Mitral stenosis should diagnosed in case of flow acceleration across the mitral valve and a mean pressure gradient greater than 4 mmHg. Similarly, flow acceleration across the aortic valve with a peak velocity greater than 2 m/s is necessary for diagnosis of aortic stenosis.

The structural criteria should be based on the study of (1) quantitative leaflet morphology (typical marked thickening of the margins, greater than 4mm), (2) leaflet shortening with abnormal motion due to the posterior leaflet tip restriction), and (3) subvalvular apparatus morphology (prominent thickening, most often just below the valve, and shortening of chordal structures) (Table 3.2).

6.3.2 Results

A total of 495 individuals were recruited at the NHI penicillin clinic, excluding the individuals assessed at the cardiothoracic surgery department and/or with a history of valve replacement.

6.3.2.1 Demography

Age: The mean age was 20.66 years and the median was 20.66 years (Figure 6.35).

Gender: There was a slight female predominance (52.3%, n=259) with a male to female ratio of 1:1,1.

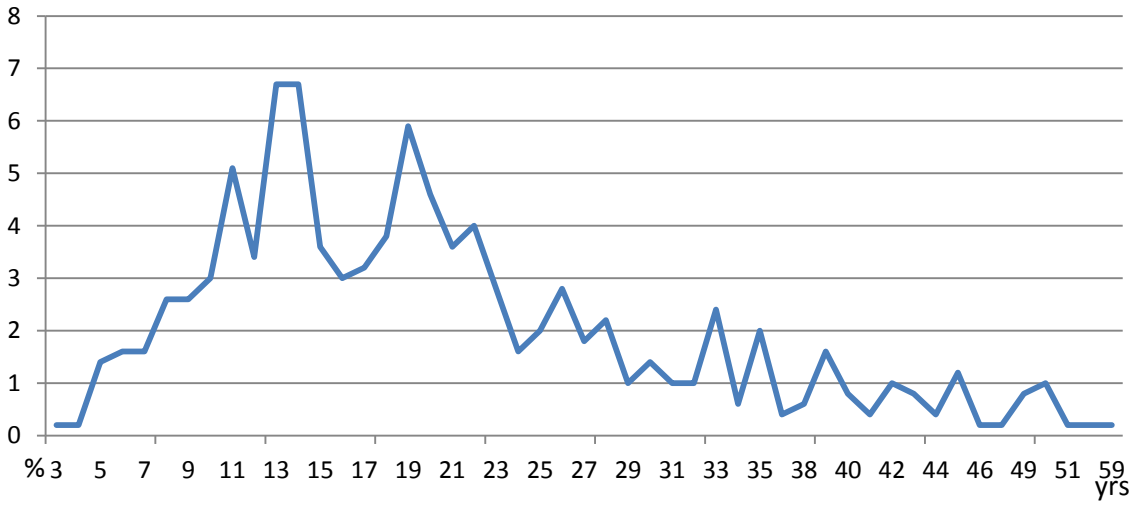


Figure 6.35 Age at the time of screening

Social factors: The number of siblings ranged up to 12, but most individuals came from families with a mean of 3.7 siblings and had good dental care.

Medical history: The majority had no cardiac risk factors and only a small fraction (2.0%, n=10) reported other risk factors like diabetes, atrial fibrillation or hypertension.

Family history: The majority of the individuals had a negative family history for cardiac diseases and RF. A small fraction of patients (6.0%, n=42) did not know if there was RF in the family.
Disease awareness: Most individuals had fair disease awareness. A small fraction of the individuals (2.7%, n=19) were too young to answer the questions on the disease awareness. A total of 182 parents were questioned about their child's health condition and the majority had a fair awareness of the disease.

Previous interventions: A small amount of the individuals had a history of previous interventions (5.3%, n=26), including balloon valvuloplasty, mitral valve repair and valve replacement. The intervention date from 3 months to 19 year (5.04 ± 4.95).

Table 6.3 Table of demographics

	% (nr)	Mean ± SD	range
Age		20.66 ± 10.61	3-59
Sex			
Male	47.4% (n=236)		
Female	52.3% (n=259)		
Nr of siblings		3.69 ± 2.04	0-12
Dental care			
Poor	4.8% (n=23)		
Fair	45.1% (n=218)		
Good	50.1% (n=242)		
Family history for cardiac diseases			
Positive	7.9% (n=36)		
Negative	92.1% (n=417)		
Family history for RF			
Positive	21.4% (n=97)		
Negative	78.6% (n=356)		
Other risk factors			
Yes	2.0% (n=10)		
No	98.0% (n=483)		
Disease awareness			
Poor	36.1% (n=168)		
Fair	38.4% (n=179)		
Excellent	25.5% (n=119)		
Parents disease awareness			
Poor	26.4% (n=48)		
Fair	41.8% (n=76)		
Excellent	31.9% (n=58)		

6.3.2.2 Echocardiographic characteristics

Structural affection

Our series showed that 81.6% (n=396) of the individuals assessed had structural affection of the heart valves. 36.1% (n=175) had 1 abnormality of the heart valves and 27.0% (n=131) had 2 different abnormalities of the valves. Thickening of the heart valves and subvalvular thickening was found in 74.2% (n=360) and 25.4% (n=123) of the individuals, respectively. Doming was found in 33.8% (n=164) of the individuals. The commissures were affected in 4.3% (n=21) of the cases. Calcification was found in 0.8% (n=4) and restriction of the heart valves was found in 14.8% (n=72) of the cases (Figure 6.36).

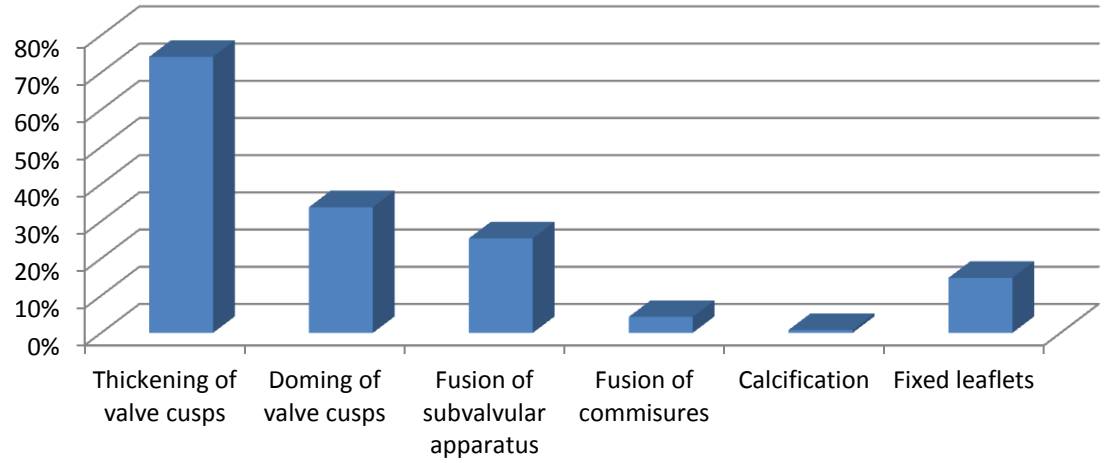


Figure 6.36 Incidence of structural features

Most individuals (66%, n=320) had functional affection of the right sided valves. Affection of the mitral valve was found in 33.2% (n=161) of the individuals and affection of the mitral and the aortic valve was seen in 12.8% (n=62). The aortic valve was affected in 2.5% (n=12) of the cases. Affection of the mitral, aortic and tricuspid valve was found in (5.6%, n=27) of the individuals.

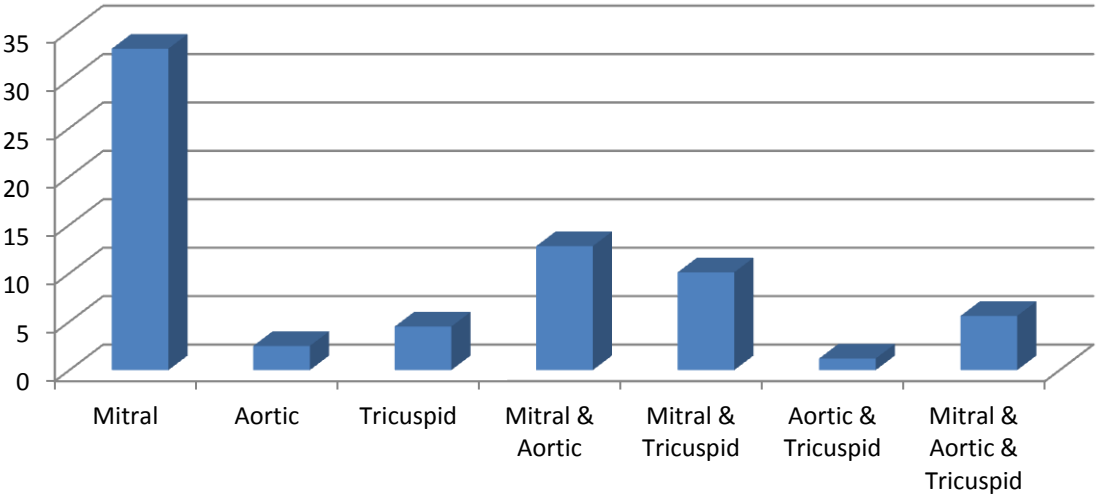


Figure 6.37 Distribution of functional affection of the valves

Mitral regurgitation was found in 58.7% (n=284) of the cases and mitral stenosis was found in 13.4% (n=65) of the cases.

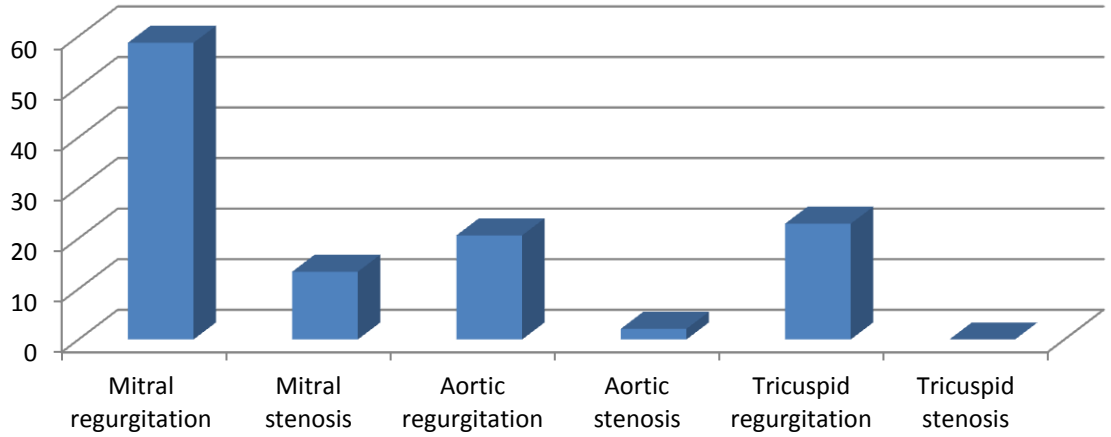


Figure 6.38 Incidence of functional affection of the valves

Isolated mitral regurgitation was found in 49.2% (n=238) of the cases, isolated mitral stenosis was found in 3.9% (n=19) of the cases. A combination of mitral regurgitation and stenosis was found in 9.5% (n=46) of the cases.

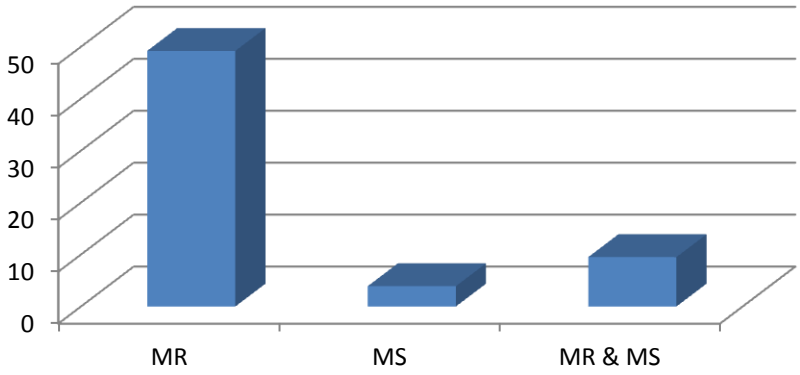


Figure 6 39 Distribution of mitral valve affection

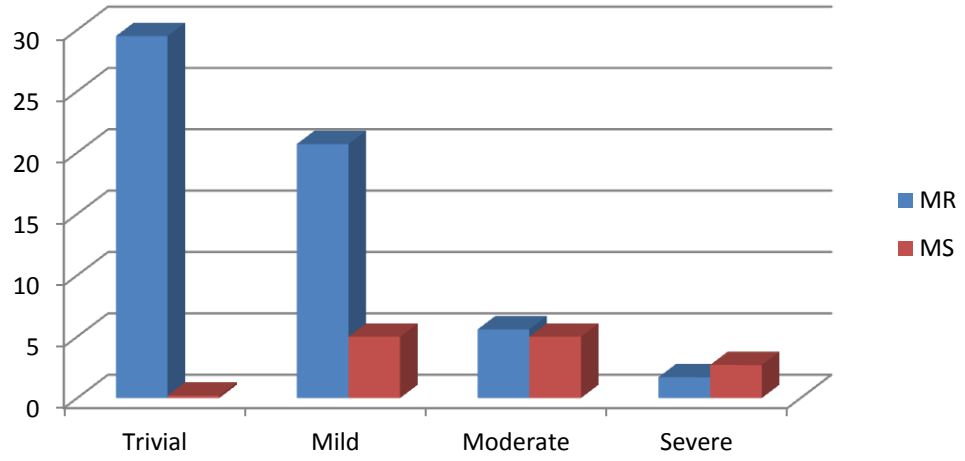


Figure 6.40 Distribution of the severity of mitral valve affection

Aortic regurgitation and stenosis was found in 20.6% (n=100) and 2.1% (n=10) of the cases, respectively. Isolated aortic regurgitation and aortic stenosis was found in 20% (n=97) and 1.4% (n=7) of the cases, respectively. A combination of aortic regurgitation and aortic stenosis was found in 0.6% (n=3) of the cases.

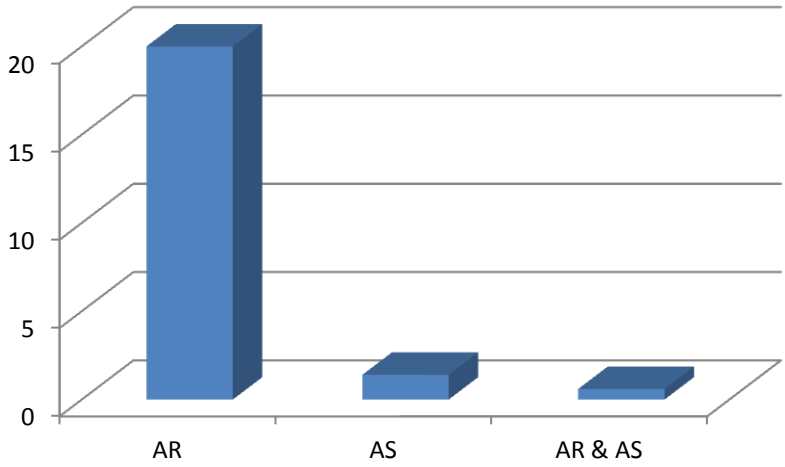


Figure 6.41 Distribution of aortic valve affection

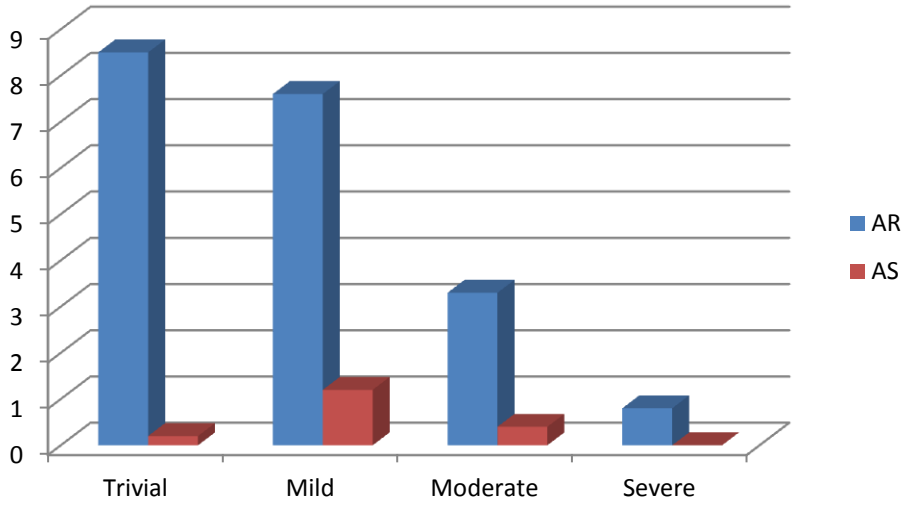


Figure 6.42 Distribution of the severity of aortic valve affection

Tricuspid regurgitation was found in 22.9% (n=111) of the cases. The majority of the cases were trivial (14.3%, n=69). Isolated tricuspid regurgitation, without the presence of any other functional affection of the heart valves was found in 3.8% (n=24) of the individuals. Tricuspid stenosis was not found in this series.

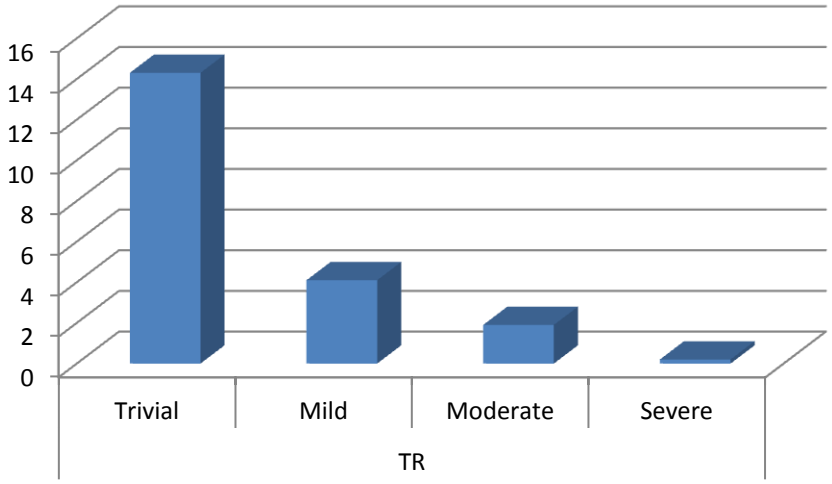


Figure 6.43 Distribution of the severity of tricuspid valve affection

6.2.2.3 Incidence of RHD

The majority of individuals had structural as well as functional affection of the heart valves Figure 6.44). Following the suggested echocardiographic diagnostic criteria 60.6% (n=294) of the individuals screened would be diagnosed with RHD.

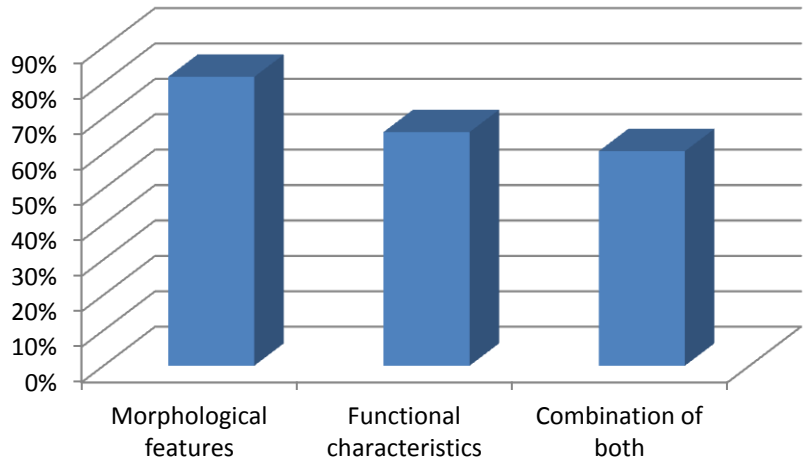


Figure 6.44 Incidence of heart pathology

6.4 Echocardiographic characteristics of RHD

6.4.1 Patients and methods

Echocardiographic data of individuals recruited at the penicillin clinic and cardiothoracic department of the NHI in Cairo as described in chapter 3, pg. 75 and diagnosed with RHD were analyzed to define the echocardiographic characteristics of RHD. The echocardiograhic findings in the different groups analyzed (chapter 6.2, pg. 122 and 6.3, pg. 136) were compared for the evaluation of the echocardiographic characteristics specific for RHD.

6.4.2 Results

In total, 69.2% (n=432) of the individuals screened at the NHI penicillin clinic, were diagnosed with RHD.

6.4.2.1 Demography

Age: The mean age of the individuals diagnosed with RHD was 25.44 years and the median was 22.0 years (Figure 6.45).

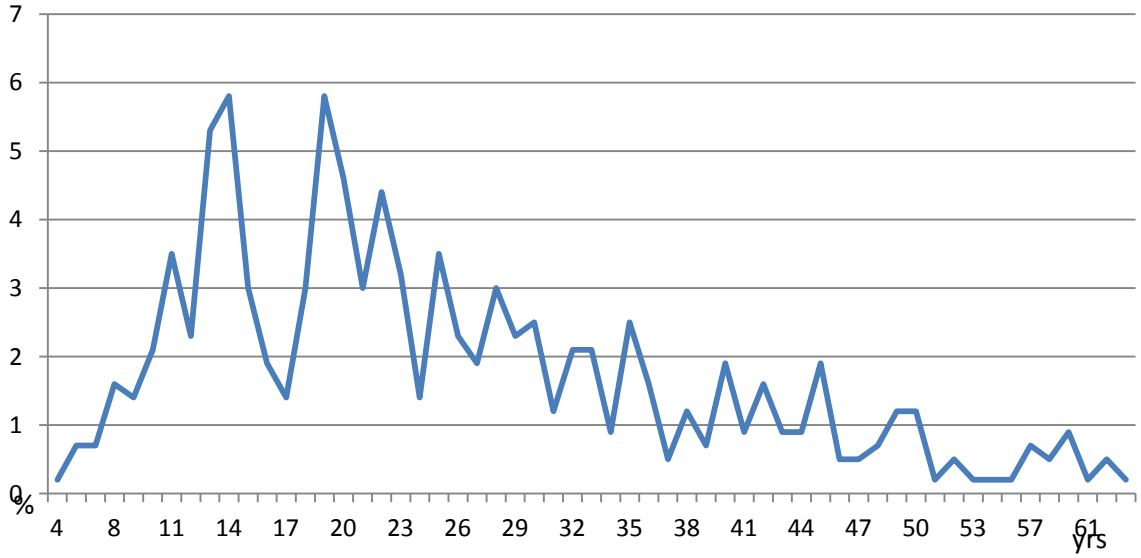


Figure 6.45 Age at the time of screening

Gender: There was a slight male predominance (51.4%, n=222) with a male to female ratio of 1:1,06.

Social factors: The number of siblings ranged up to 14, but most individuals came from families with a mean of 4.26 siblings and had fair dental care.

Medical history: The majority had no cardiac risk factors and only a small fraction (2.3%, n=10) reported other risk factors like diabetes, atrial fibrillation or hypertension.

Family history: The majority of the individuals had a negative family history for cardiac diseases and RF.

Table 6.4 Profile of patients diagnosed with RHD

	% (nr)	**Mean ± SD**	**range**
Age		25.43 ± 12.74	4-63
Sex			
Male	51.4% (n=222)		
Female	48.6% (n=210)		
Nr of siblings		4.26 ± 2.31	0-14
Dental care			
Poor	4.3% (n=18)		
Fair	51.8% (n=218)		
Good	43.9% (n=185)		
Family history for cardiac diseases			
Positive	6.7% (n=26)		
Negative	93.3% (n=363)		
Family history for RF			
Positive	18.3% (n=71)		
Negative	81.7% (n=318)		
Other risk factors			
Yes	2.3% (n=10)		
No	97.7% (n=418)		
Disease awareness			
Poor	47.8% (n=203)		
Fair	31.3% (n=133)		
Excellent	20.9% (n=89)		
Parents disease awareness			
Poor	31.3% (n=48)		
Fair	35.4% (n=35)		
Excellent	33.3% (n=33)		

Disease awareness: Most individuals had poor disease awareness. A total of 116 parents were questioned about their child's health condition and 31.3% (n=48) showed a poor disease awareness of the disease.

Diagnosis: Most individuals was diagnosed by the presence of RF symptoms (63.3%, n=273), while 36.7% (n=158) were diagnosed by cardiac symptoms or affection.

Recurrences: Most individuals reported recurrences of the RF attacks 55.6%, n=200) with the majority of the attacks reported in the last 3 to 4 weeks (98.5%, n=192), while 28.7% (n=56) reported recurrence in the last week.

Previous interventions: A small amount of the individuals had a history of previous interventions (6.3%, n=27), including balloon valvuloplasty, mitral valve repair and valve replacement. The intervention date from 6 weeks to 20 year (6.98 ± 4.97).

Penicillin prophylaxis: The majority of individuals were on penicillin prophylaxis (82.4%, n=342). The most common prophylaxis schedule was reported to be every 15-day (66.2%, n=215), while a 21-day and monthly prophylaxis routine was reported in 12.3% (n=40) and 21.5% (n=70), respectively. Good compliance to the penicillin prophylaxis was reported in 48.2% (n=157) of the cases, while 33.7% (n=110) and 18.1% (n=59) reported a fair or poor compliance, respectively.

6.4.2.2 Echocardiographic characteristics

Structural affection

Thickening of the heart valves and subvalvular thickening was found in 95.4% (n=412) and 38.5% (n=166) of the individuals, respectively. Doming was found in 56.5% (n=244) of the individuals. The commissures were affected in 11.1% (n=48) of the cases. Calcification was found in 5.1% (n=22) and restriction of the heart valves was found in 43.3% (n=187) of the cases.

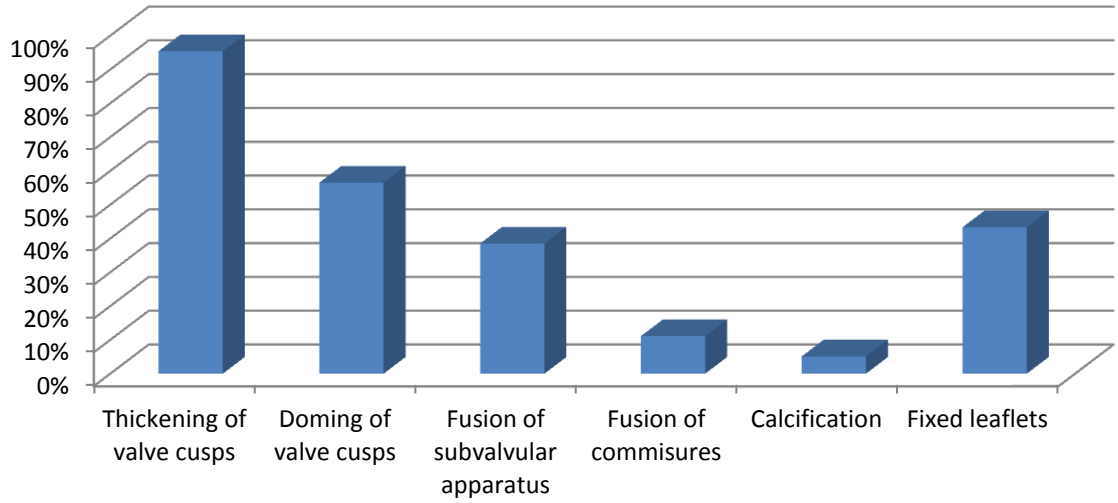


Figure 6.46 Incidence of structural features

Functional affection

Isolated mitral valve affection was seen in (35.6%, n=154) and 20.8% (n=90) had affection of the mitral and the aortic valve. The aortic valve was affected in 3.0% (n=13) of the cases. A combination of mitral, aortic and tricuspid valve affection was seen in 23.1% (n=100) of the individuals.

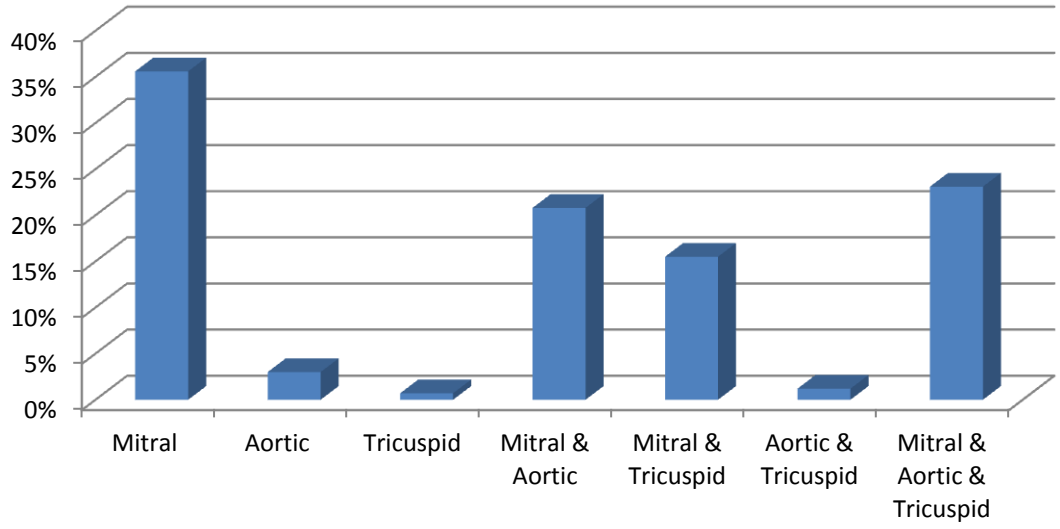


Figure 6.47 Distribution of functional affection of the valves

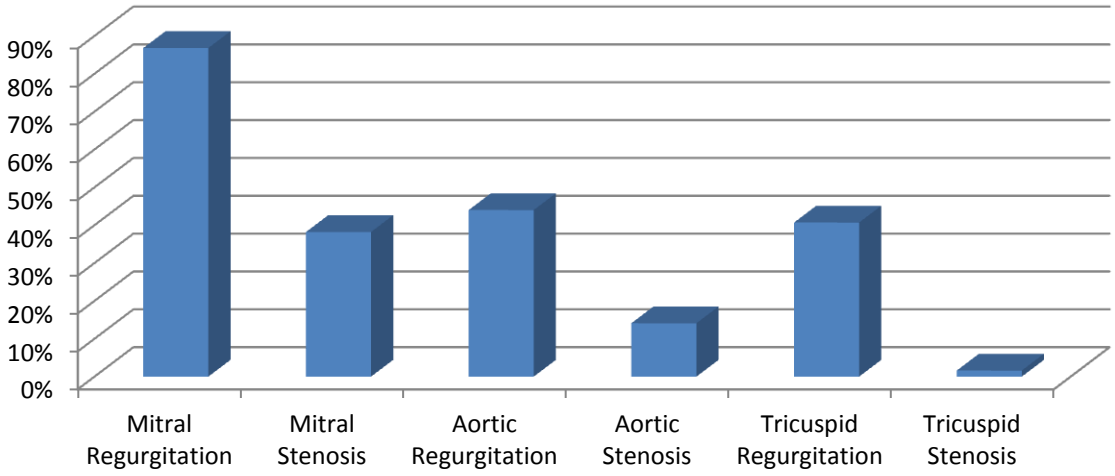


Figure 6.48 Incidence of functional valvular affection

Mitral valve: Mitral regurgitation was found in 86.8% (n=374) of the cases and mitral stenosis was found in 38.2% (n=165) of the cases. Most of the cases had trivial (28.8%, n=124) while 17.2% (n=74) had severe mitral regurgitation. Severe mitral stenosis was seen in 21.6% (n=93) of the cases, while 8.4% (n=36) and 7.2% (n=31) had mild and moderate stenosis, respectively.

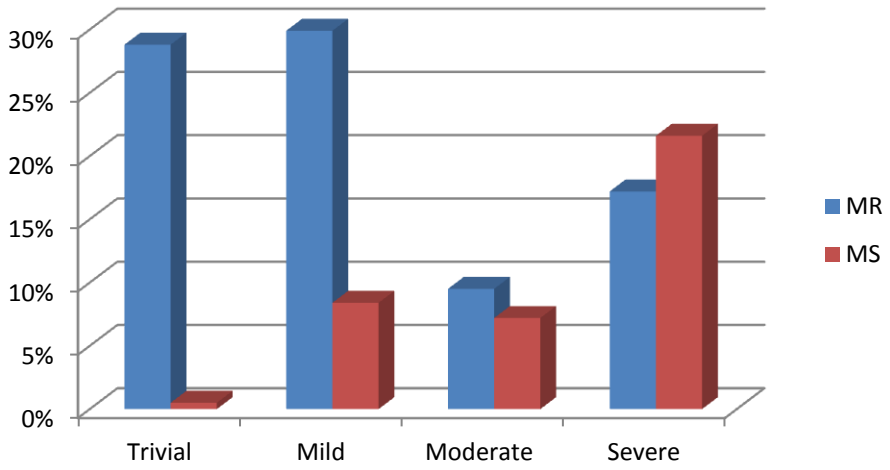


Figure 6.49 Severity of mitral valve affection

Aortic valve: Aortic regurgitation and stenosis was found in 44.0% (n=190) and 14.1% (n=61) of the cases, respectively. Severe aortic regurgitation was seen in 15% (n=65) of the cases, while 13.0% (n=56) and 6.9% (n=30) had mild and moderate regurgitation, respectively. Severe aortic stenosis was seen in 6.7% (n=29), while mild and moderate stenosis was seen in 3.0% (n=13) and 3.9% (n=17) of the cases.

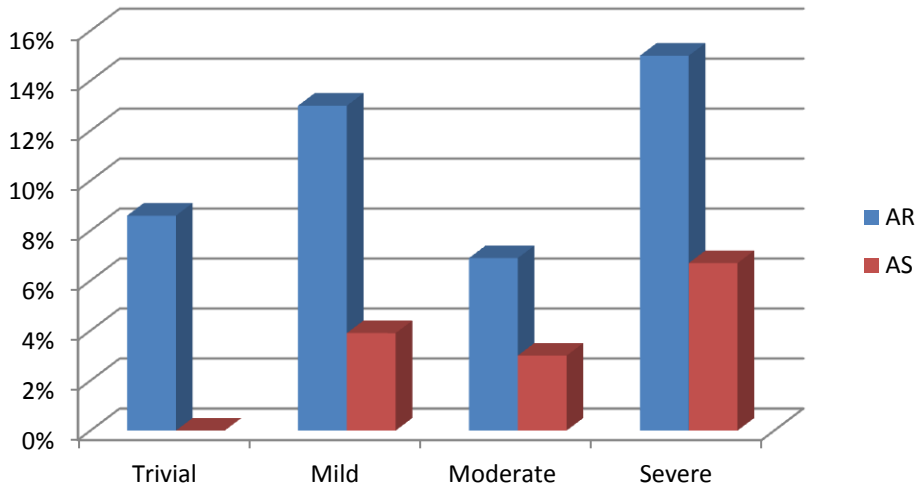


Figure 6.50 Severity of aortic valve affection

Tricuspid valve: Tricuspid regurgitation was found in 40.7% (n=176) of the cases. The majority of the cases were trivial (16.5%, n=71). Mitral stenosis was seen in 1.6% (n=7) of the cases.

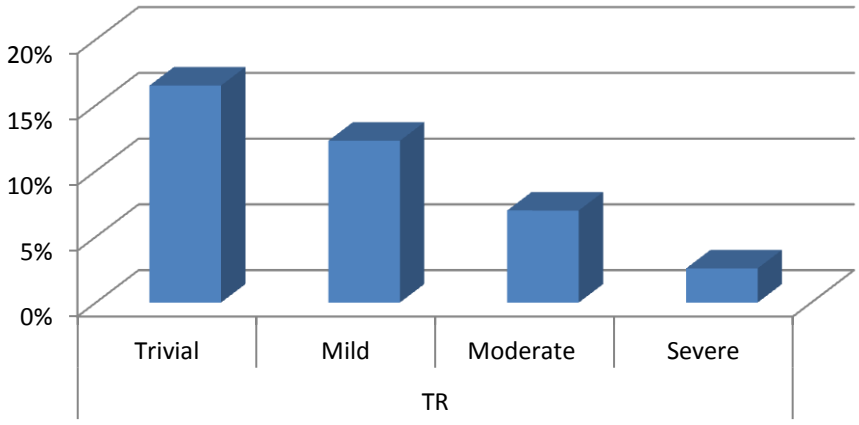


Figure 6.51 Severity of tricuspid regurgitation

6.4.2.3 Evaluation of echocardiographic findings

When comparing the echocardiograhic findings in the different groups we notice an increase in the incidence of all structural features and mixed lesions in the RHD group as shown in Figure 6.31 and Figure 6.32.

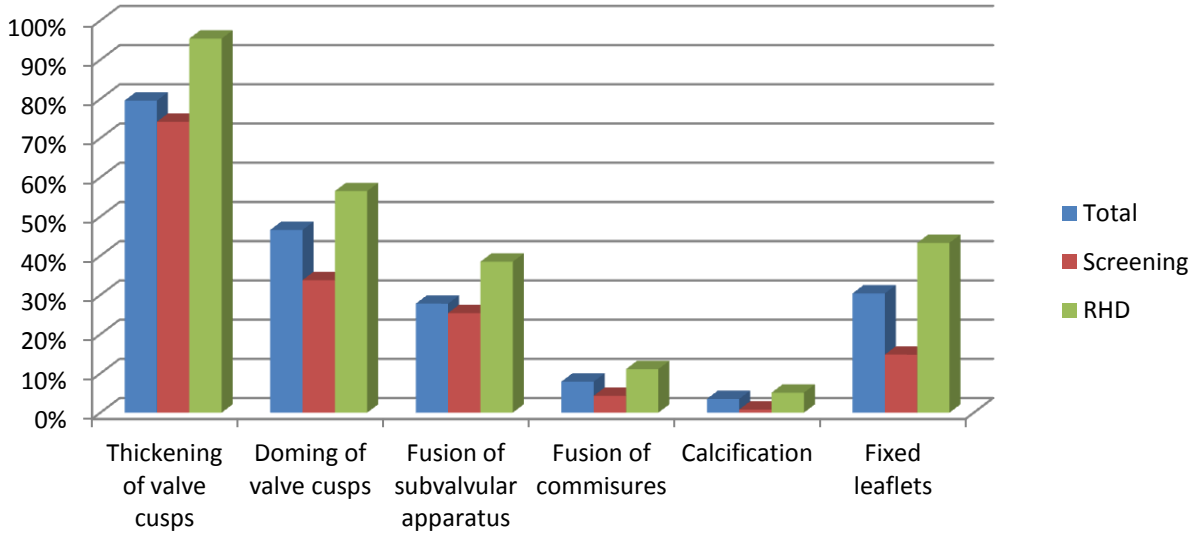


Figure 6.52 Distribution of structural affection among the groups

*Total= total group assessed, Screening= individuals screened at the penicillin prophylaxis, RHD= patients diagnosed with RHD

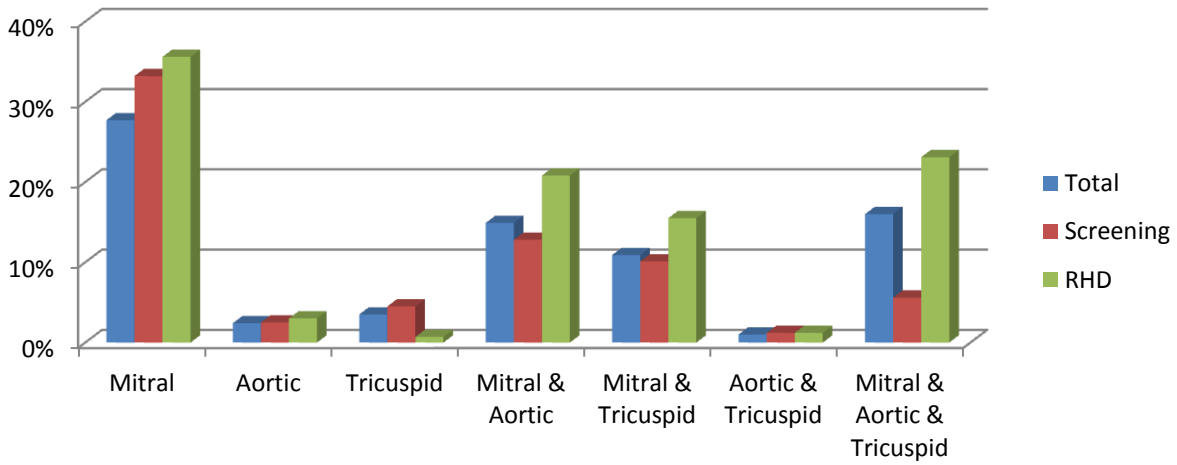


Figure 6.53 Distribution of valvular affection among the groups

In the group diagnosed with RHD there was an increase in functional affection of all valves (Figure 6.54).

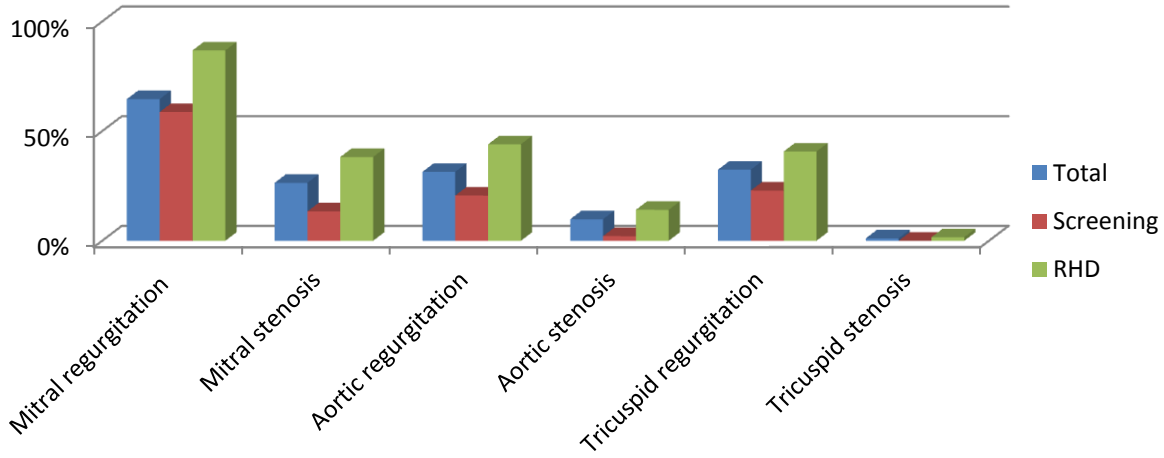


Figure 6.54 Distribution of valvular functional affection among the groups

The severity of the all functional lesions increased significantly in the group diagnosed with RHD, especially when compared with the group diagnosed with RHD at the penicillin clinic (Figure 6.55).

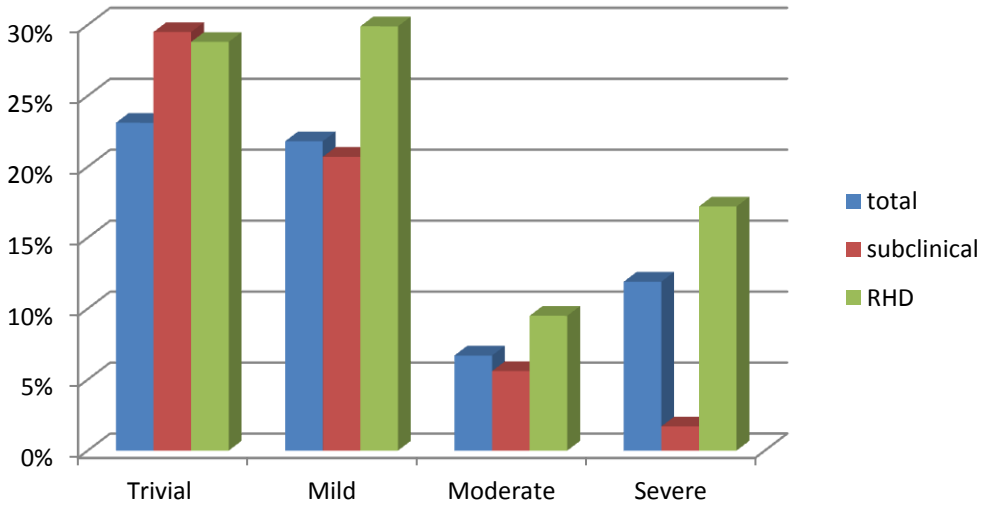


Figure 6.55 Distribution of severity of mitral regurgitation among the groups

The distribution of the severity of the different functional lesions are shown in the figures below.

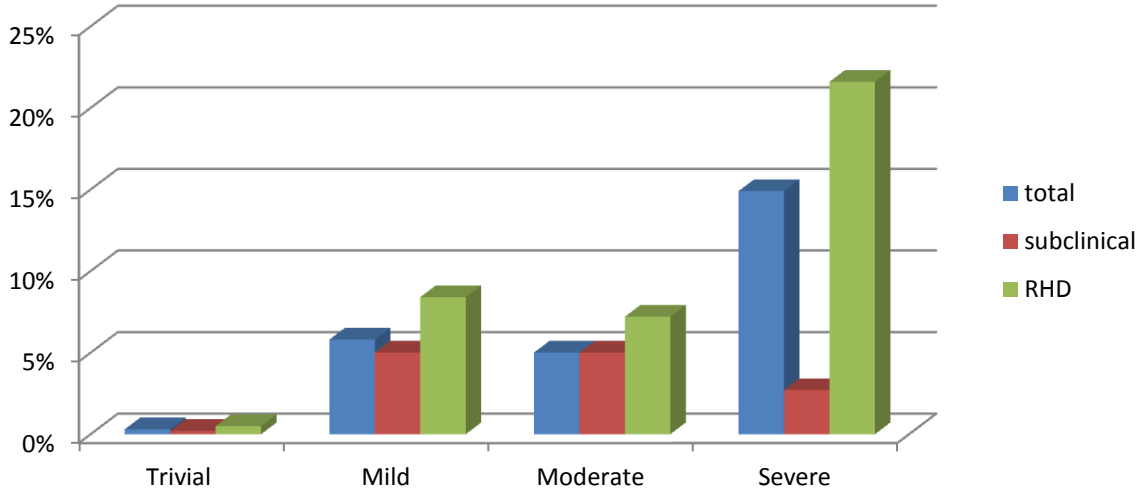


Figure 6.56 Distribution of severity of mitral stenosis among the groups

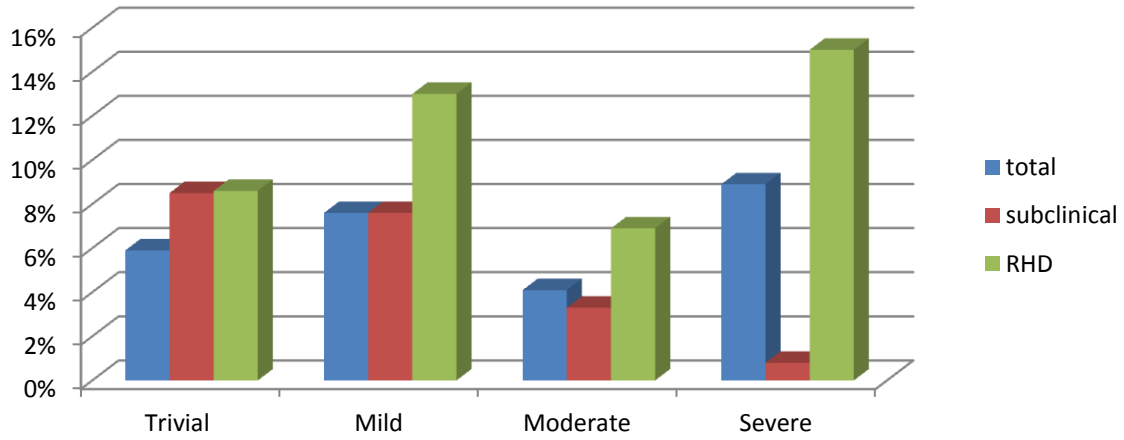


Figure 6.57 Distribution of severity of aortic regurgitation among the groups

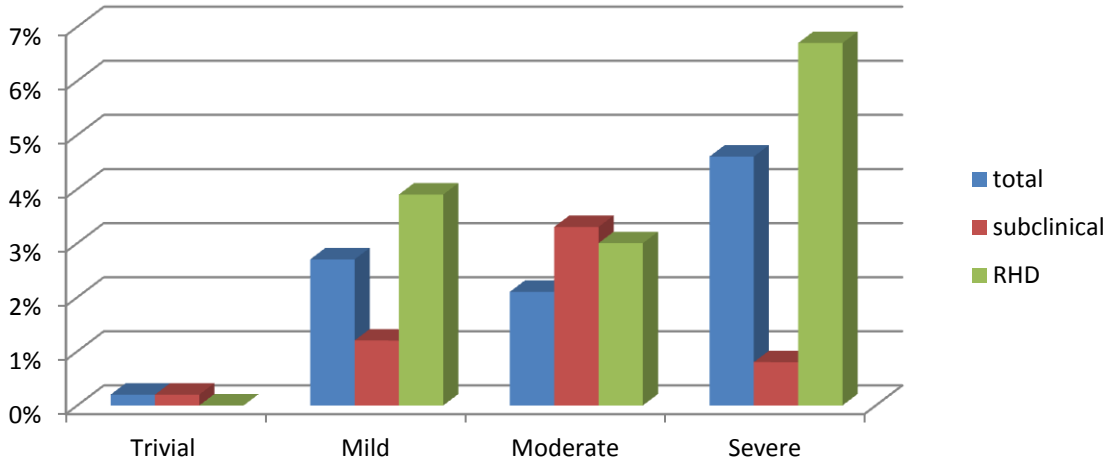


Figure 6.58 Distribution of severity of aortic stenosis among the groups

6.4.3 Discussion

The vast majority of the individuals screened had structural as well as functional affection of the heart valves. An increased thickness of the valvular leaflets was the commonest feature found, with a prevalence ranging up to 74.2% in the group screened at the penicillin clinic and 83.8% in the total screened population in concordance with earlier reports on cardiac affection as a result of RF (Vijayalakshmi 2008). However, in the group diagnosed with RHD it ranged up 95.4%, suggesting that valvular thickening seems to be a universal structural feature in RHD, highlighting the clinical meaning of this finding during echocardiography. Features like doming of the valvular leaflets, calcification and restriction of the valvular apparatus were more common in the cohort and in individuals with RHD when compared to the individuals at the penicillin clinic. Single and isolated structural features were more prevalent in the group screened at the penicillin clinic, however, in the complete cohort and individuals with RHD combinations of structural features were more common. Therefore, the presented results suggest that the amount and combination of structural features increases with progression of the disease in combination with an increase of lesion severity, showing that the cardiac affection is progressive.

In our series, the mean age of the individuals diagnosed with RHD was 25.44 with a median of 22.0 years, which is in concordance with the expectations as the peak age for RHD has been

reported to be 20 to 40 years, with the highest prevalence expected to be in individuals aged 15-35 years. (White 2010) Furthermore, RHD is the most frequent cause of heart disease among 5 to 30 year olds, and RF and RHD are responsible for approximately 25-40% of all cardiac hospital admissions in some areas, being also the most common causes of death in this age group (Markowitz 1981).

Subclinical carditis or valvular affection is known to be relatively common in RF (Tubridy-Clark 2007, Figueroa 2001) and approximately 90% of cases of RHD are believed to be clinically silent. In our series, RHD was diagnosed in 60.6% of the individuals with history of RF visiting the NHI penicillin clinic for their penicillin prophylaxis. The mitral valve was predominantly affected with regurgitation being the most common lesion showing an incidence of 64.6% in the total cohort and 86.8% in the patients diagnosed with RHD. This was in concordance with the reported affection of the mitral valve which ranges up to 98% (Woo 1983, Reddy 2004) and mitral regurgitation ranging up to 94% (Ransome 1988, Marijon 2007, Ayoub 1995, Park Myung 1996, Brook 1996, Tandon 2000, Carapetis 2008, Ramakrishnan 2009).

Mitral stenosis ranged up to 38.2% of RHD patients and 26.4% in the total group which is in concordance with some of the earlier studies reporting incidences of 11.0% (Tandon 2000) to 20% (Longo-Mbenza 1998). However, incidences have been reported to range up to 50% (Carapetis 2008, Ramakrishnan 2009). The discrepancy between the reported incidences of mitral regurgitation and stenosis might be due to age differences between the groups studied, as regurgitant lesions are more common in the young particularly in the early stages of the disease while stenosis is seen with progression of the disease. Nevertheless, most of the studies reporting the prevalence of the different valvular affection were performed on a population presenting with RHD, while a significant amount of the individuals assessed in our series had less severe valvular affection and were asymptomatic being diagnosed only by screening for subclinical RHD rather than clinical valvular disease.

Aortic regurgitation was found in around one-third of the individuals in the cohort similarly in concordance to earlier reports (Reddy 2004, Sliwa 2010, Woo 1983) while isolated aortic regurgitation, without functional affection of any other valves was rare as expected (Reddy 2004). The absence of isolated aortic stenosis in our series is not surprising, bearing in mind that

it is believed to be a rare condition (Ayoub 1995, Park Myung 1996, Brook 1996, Tandon 2000, Sliwa 2010). However, there was a significant increase of aortic regurgitation and aortic stenosis in the group diagnosed with RHD, showing also an increased severity of the lesions.
Tricuspid regurgitation was found in one-third of the cases with isolated tricuspid affection without functional affection of any other valves in 3.8% of the cases. However, tricuspid regurgitation was found in 40.7% of the individuals diagnosed with RHD, which is lower than the earlier reported affection ranging up to 56% (Tandon 2000, Reddy 2004).

The second most common finding in the individuals screened was combined affection of the mitral and aortic valves. Nevertheless, combined affection of the mitral, aortic and tricuspid was the second most common affection in the group diagnosed with RHD. Affection of both mitral and aortic valves is seen in around 27% of the RHD cases (Routray 2003, Reddy 2004) especially as combined mitral and aortic valve regurgitation while isolated mitral regurgitation or stenosis is relatively uncommon (Oli 2004) showing that mixed lesions and affection of several valves is more prevalent than isolated lesions in RHD (Reddy 2004). The incidence of the combined affections in our series is lower than reported earlier due to the fact that this study evolves a significant amount of subclinical and milder conditions, but shows a similar ranking (Vasan 1996, Routray 2003, Reddy 2004). An increase of mixed lesions as well as severity of the functional affection is found in the group diagnosed with RHD, suggesting that the more advanced the disease, the more valves affected with also increase in severity of the lesions.

6.5 Prognosis

The aim of this study was evaluation of the progression and/ or resolution of the valvular lesions for the determination of the long term prognosis under the influence of several possible prognostic determinants.

6.5.1 Patients and methods

The patients recruited in the penicillin clinic of the NHI in Cairo (as described in chapter 3, pg. 75) were reassessed 6 months after the first examination for the evaluation of the lesions, progression and/ or resolution of the cardiac condition.

The study group consisted of:

-Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement

-Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD

6.5.2 Results

A total of 167 individuals were reassessed after the initial recruitment at the penicillin prophylaxis clinic of the National Heart Institute during the follow-up, excluding the individuals assessed at the cardiothoracic surgery department and/or with a history of valve replacement.

6.5.2.1 Characterization of the Patients

Age: The age ranged from 5 to 46 with a mean± SD of 19.26±8.99 years (Figure 6.59).

Gender: There was a slight male predominance, the disease awareness was ranged trough the 3 categories and the parents' awareness was generally fair.

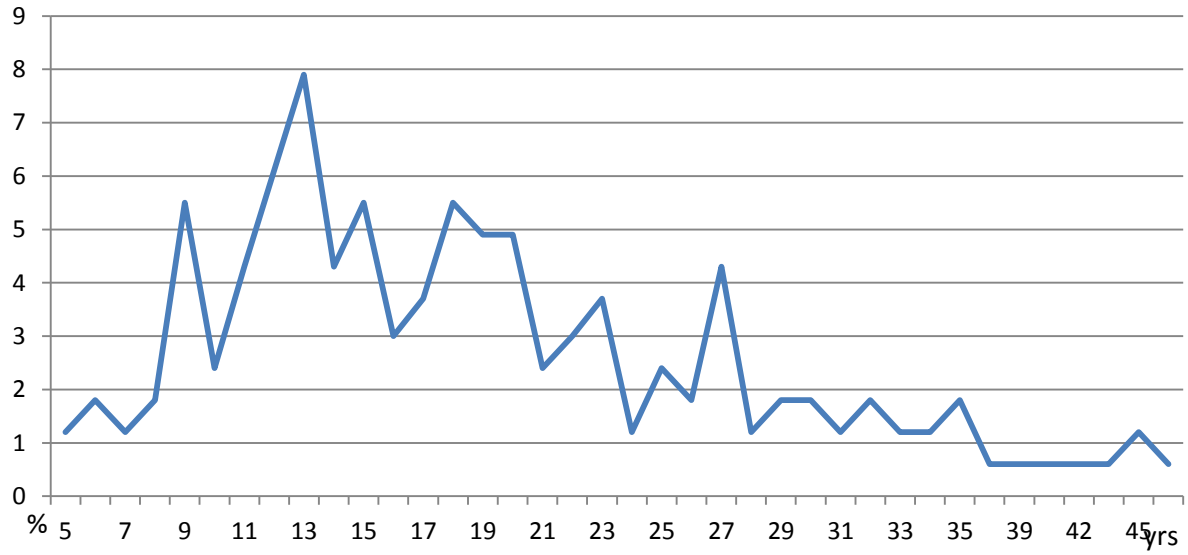


Figure 6.59 Age at the time of follow-up

Table 6.5 Patient demographics in the follow up group

	% (nr)	Mean ± SD	range
Age		19.26±8.99	5-46
Sex			
Male	53.7% (n=88)		
Female	46.3% (n=76)		
Disease awareness			
Poor	31.6% (n=49)		
Fair	37.4% (n=58)		
Excellent	31% (n=48)		
Parents disease awareness			
Poor	25% (n=16)		
Fair	40.6% (n=26)		
Excellent	34.4% (n=22)		
History for RF			
Positive	93.3% (n=153)		
Negative	6.7% (n=11)		
Age at time of RF		9.42±5.23	1-28
How long ago (mnth)		40.26±74.63	1-432
Family history for RF			
Positive	22.9% (n=36)		
Negative	77.1% (n=121)		

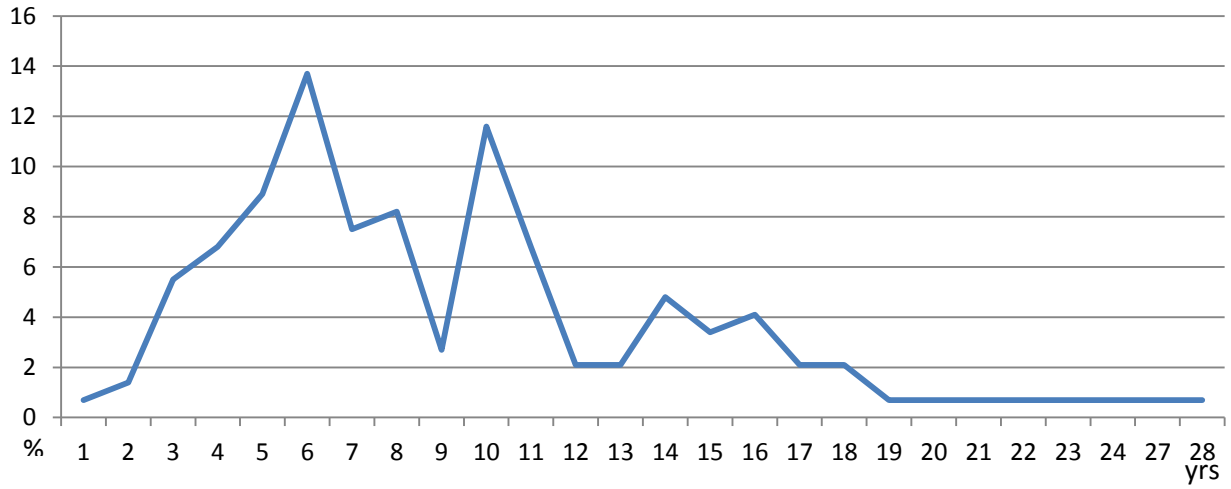


Figure 6.60 Age at the time of RF onset

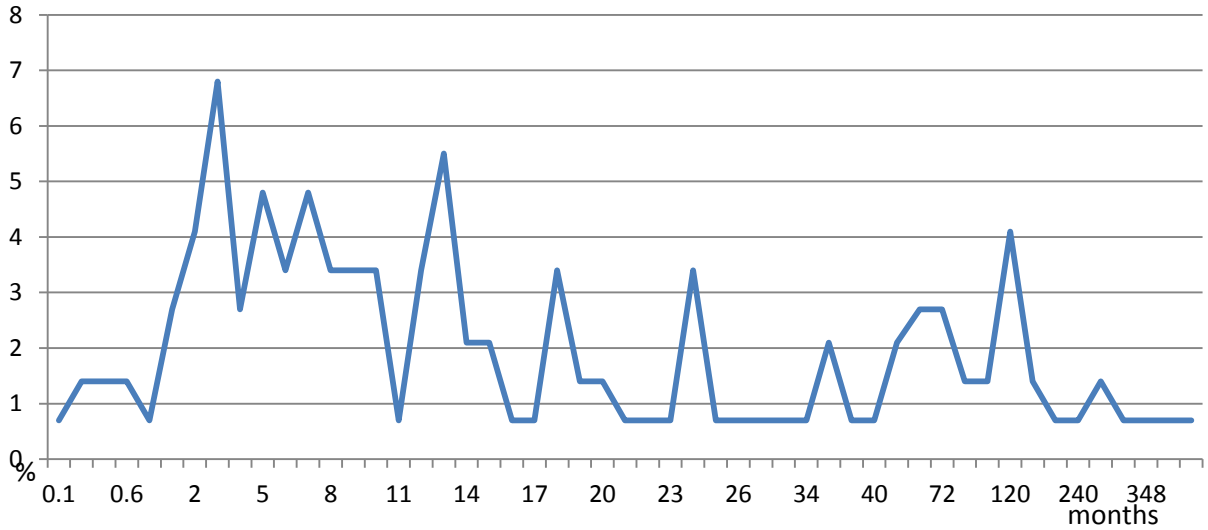


Figure 6.61 Date of RF onset

Follow-up time: Follow-up examinations were performed 7 to 12 months after the initial examination (median 8 moths). Nine patients were seen 3 times in a period ranging from 17 to 24 months (mean= 22.33 months ± SD= 2.40).

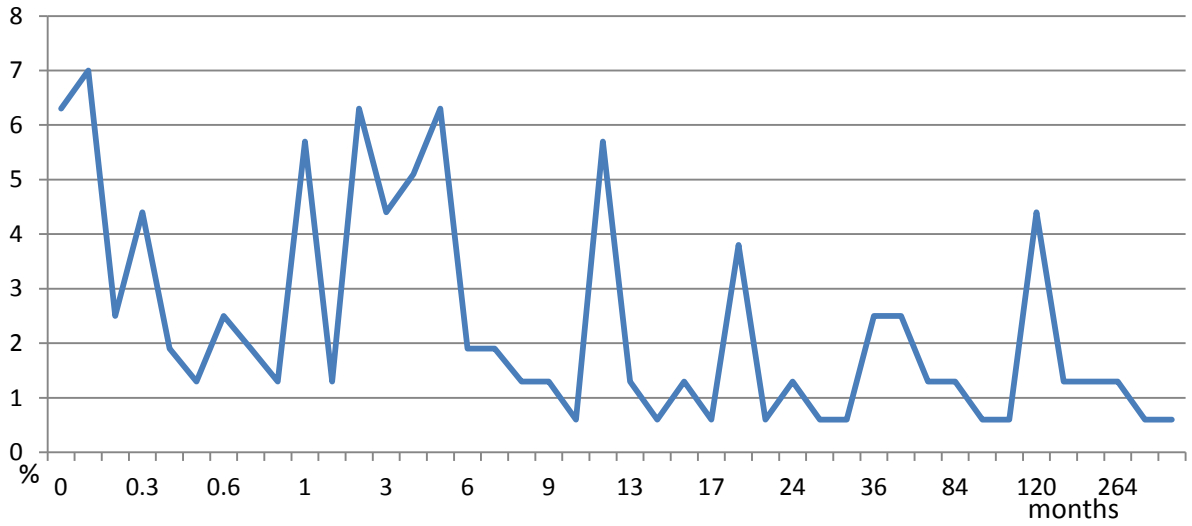


Figure 6.62 Date of start penicillin prophylaxis

Penicillin prophylaxis: The most common penicillin prophylaxis schedule was 15-day, followed by the monthly prophylaxis routine. A fraction of the patients (17.7%, n=29) was no longer on penicillin prophylaxis. The majority of them had stopped the penicillin prophylaxis in the last 6 months (59.3%, n=15). Most patients (59.3%, n=80) reported excellent compliance to the prophylaxis regimen (Table 6.5).

Table 6.6 Follow-up

	% (nr)	Mean ± SD	range
Follow-up time		11.53± 7.23	2-24
2-6 months	15.2% (n=25)		
7-12 months	51.2% (n=84)		
13-24 months	33.5% (n=55)		
Penicillin prophylaxis			
Yes	82.32% (n=135)		
No	17.7% (n=29)		
Prophylaxis schedule			
15-day	69.6% (n=94)		
21-day/ 3 weeks	9.6% (n=13)		
1-month	20.7% (n=28)		
Start date prophylaxis		27.14±61.33	0-432
Prophylaxis cessation time		1.88±10.34	0-120
Compliance			
Poor	7.4% (n=10)		
Fair	33.3% (n=45)		
Excellent	59.3% (n=80)		

6.5.2.2 Echocardiographic findings

The majority of individuals had structural as well as functional affection of the heart valves (Figure 6.70), with the most common structural feature being thickness of the valve cusps (Figure 6.63) and mitral regurgitation the most common functional feature (Figure 6.65).

Structural affection

Our series showed that 56.9% (n=95) of the individuals assessed during follow-up had structural affection of the heart valves. Most (49.5%, n=47) of the individuals with structural affection had 1 abnormality of the heart valves while 40.0% (n=38) had 2 different abnormalities of the valves. A small number of individuals (2.1%, n=2) had 5 different structural features.

Thickening of the heart valves and subvalvular thickening was found in 50.9% (n=85) and 10.2% (n=17) of the individuals, respectively. Doming was found in 17.4% (n=29) of the individuals.

The commissures were affected in 1.2% (n=2) of the cases. Calcification was found in 0.6% (n=1) and restriction of the heart valves was found in 15.1% (n=25) of the cases.

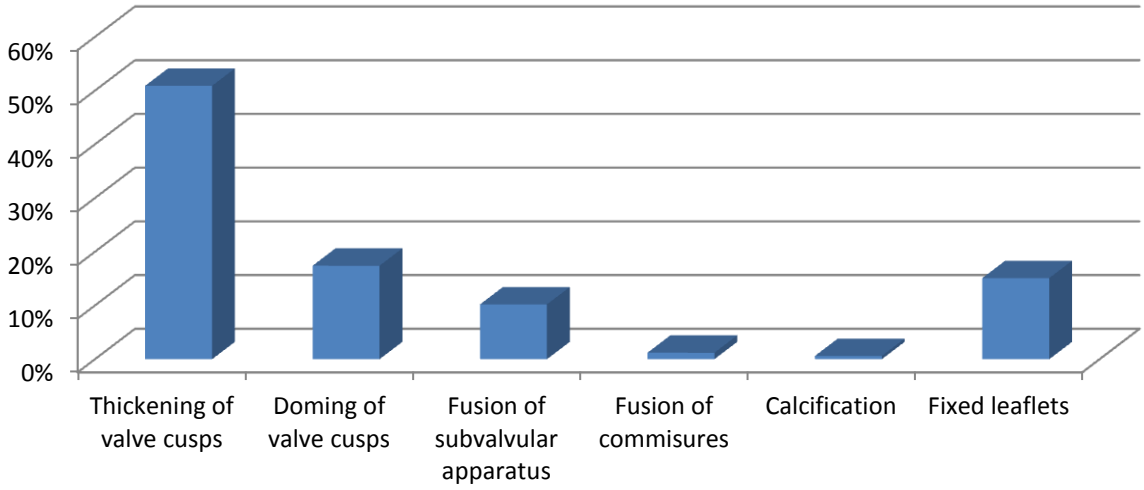


Figure 6.63 Incidence of structural features

Functional affection

Functional affection of the right sided valves was found in 61.1% (n=102) of the individuals. From the individuals with functional affection of the valves 63.4% (n=71) had mitral valve affection and 12.5% (n=14) had affection of the mitral and the aortic valve. The aortic valve was affected in 1.8% (n=2) of the cases. Affection of the mitral, aortic and tricuspid valve was seen in 2.7% (n=3) of the individuals. Mitral regurgitation was found in 61.1% (n=102) of the cases and mitral stenosis was found in 3.6% (n=6) of the cases.

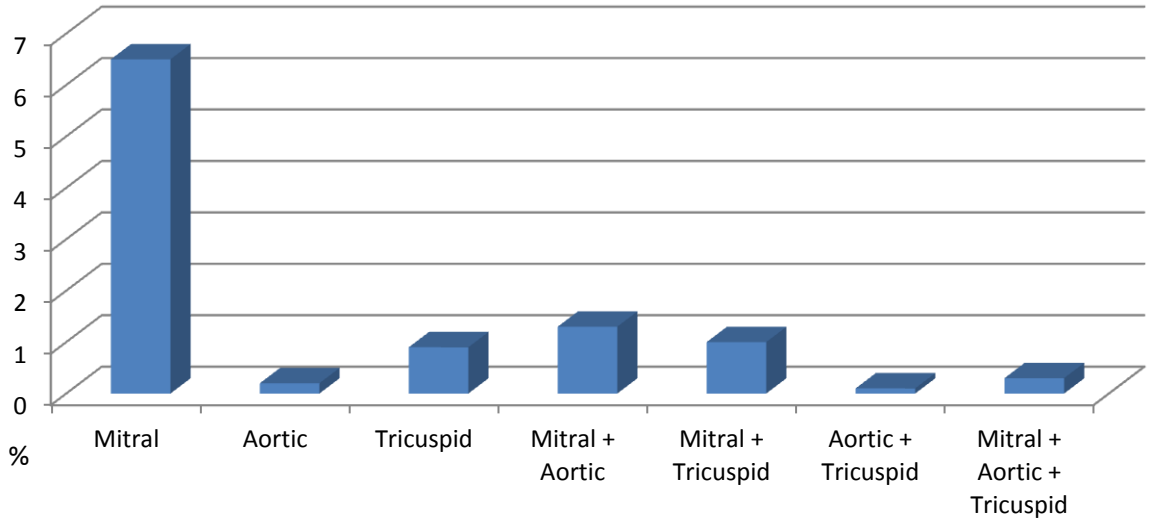


Figure 6.64 Distribution of functional affection of the valves

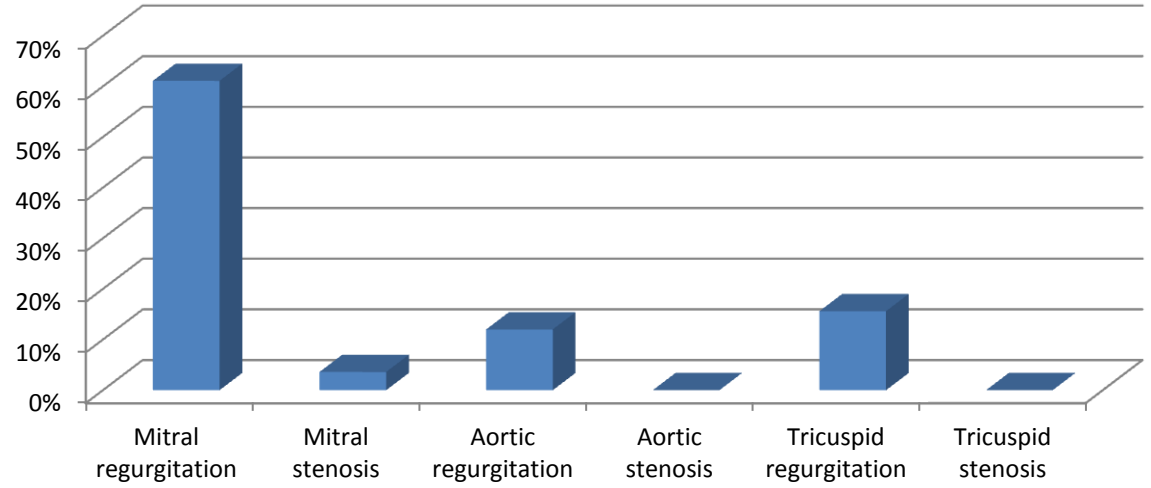


Figure 6.65 Incidence of functional affection of the valves

Isolated mitral regurgitation was found in 42.5% (n=71) of the cases, isolated mitral stenosis was found in 0.6% (n=1) of the cases. A combination of mitral regurgitation and stenosis was found in 1.2% (n=2) of the cases.

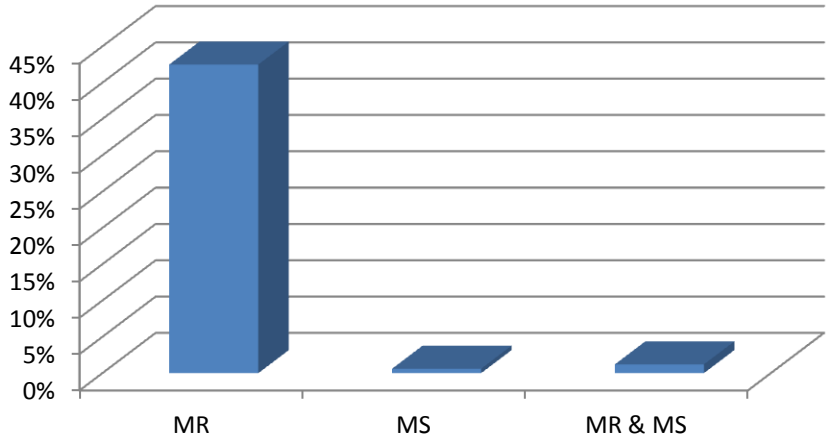


Figure 6.66 Distribution of mitral affection

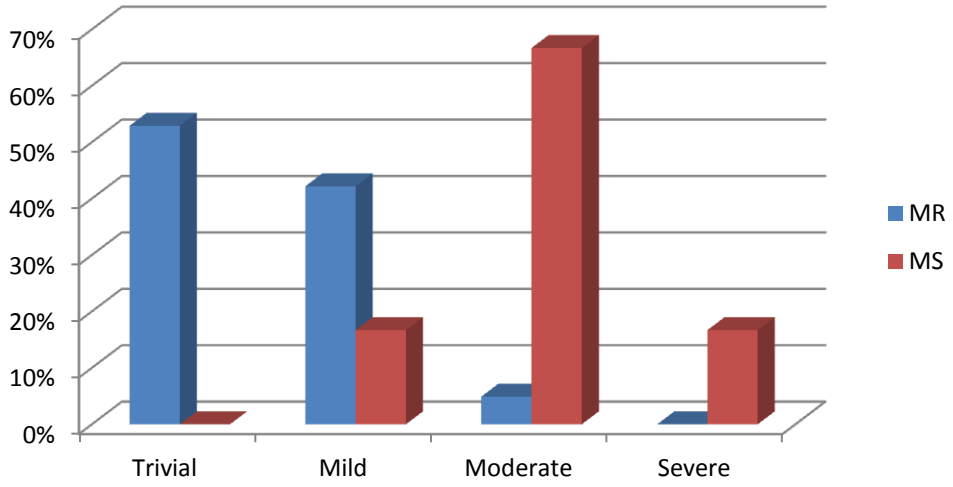


Figure 6.67 Severity of mitral affection

Aortic regurgitation was found in 12% (n=20) of the cases. No aortic stenosis was found in this series. Isolated aortic regurgitation was found in 1.2% (n=2) of the cases.

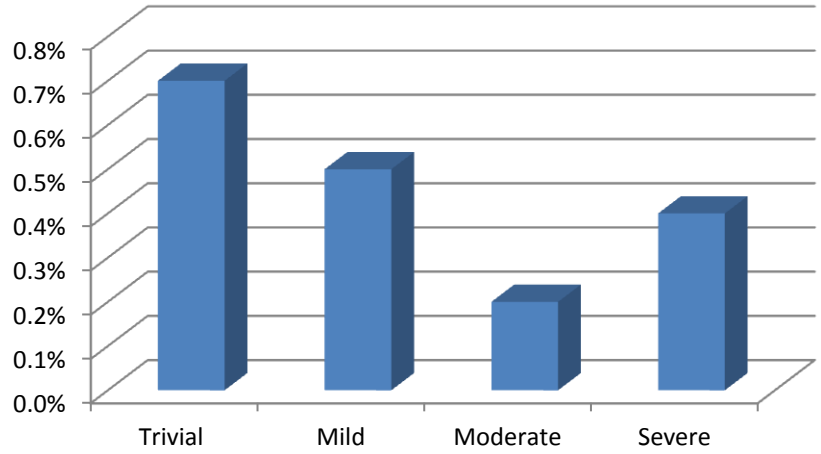


Figure 6.68 Severity of aortic regurgitation

Tricuspid regurgitation was found in 15.6% (n=26) of the cases. The majority of the cases were trivial (46.2%, n=12). 30.8% (n=8) and 23.1% (n=6) were mild or moderate, respectively. Tricuspid stenosis was not found in this series.

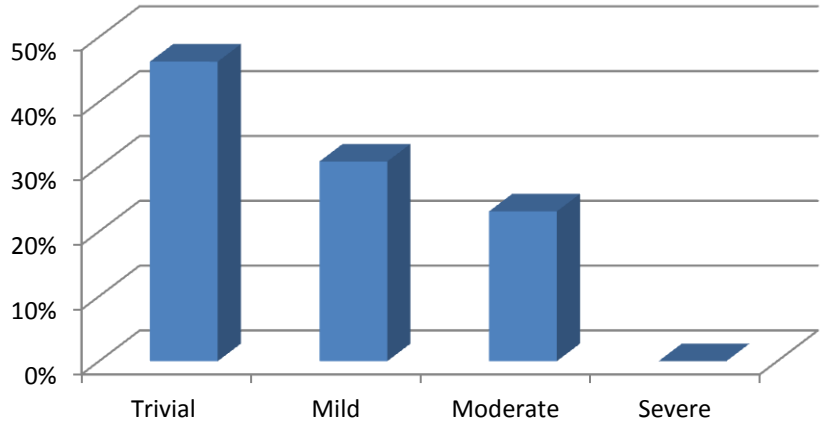


Figure 6.69 Severity of tricuspid regurgitation

RHD

Combination of structural and functional affection leading to the diagnosis of RHD was seen in 41.9% (n=70) of the individuals. From the individuals diagnosed with RHD 58.6% (n=41) were males and 37.7% (n=29) were females.

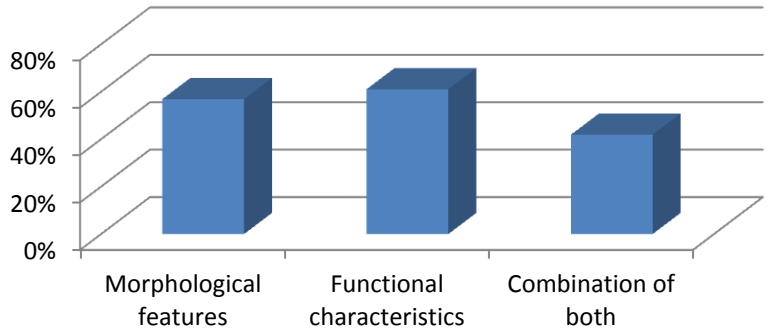


Figure 6.70 Incidence of heart pathology

6.5.2.3 Evaluation of echocardiographic findings

A part of the individuals (15%, n=25) continued free of structural affection at the time of follow-up while 25.1% (n=42) of the individuals continued free of functional affection at the time of follow-up and 37.1% (n=62) of the individuals continued free of RHD at the time of follow-up.

Development of lesions: A small fraction of the individuals (4.8%, n=8) had developed structural affection at the time of follow-up and 10.8% (n=18) of the individuals had developed functional affection at the time of follow-up. A small fraction of the individuals (5.4%, n=9) were newly diagnosed with RHD at the time of follow-up.

Maintenance of lesions: 52.1% (n=87) of the individuals had sustained the structural affection found during the first assessment at the time of the follow-up. Most individuals (50.3%, n=84) had sustained the functional affection found during the first assessment at the time of the follow-up and 36.5% (n=61) of the individuals sustained the diagnosis of RHD found during the first assessment at the time of the follow-up.

Reversibility of lesions: In 28.1% (n=47) of the individuals assessed the structural affection had resolved. In 13.8% (n=23) of the individuals assessed the functional affection had resolved.
In 21% (n=35) of the individuals assessed the characteristics leading to the diagnosis of RHD had resolved.

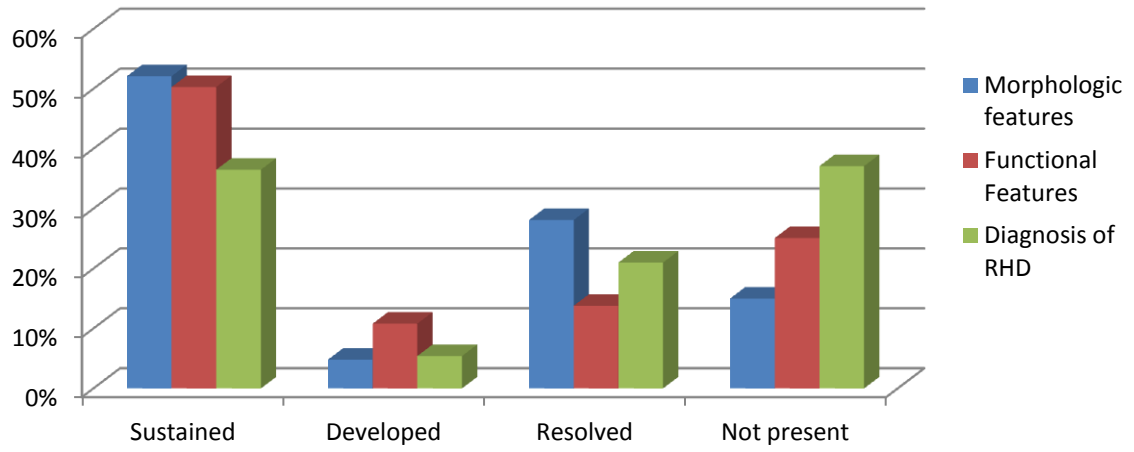


Figure 6.71 Results of follow-up

6.5.3 Discussion

Most individuals assessed during follow-up had structural or functional affection of the heart valves and the mitral valve was mostly affected, in the form of mitral regurgitation. Combination of structural and functional affection leading to the diagnosis of RHD was diagnosed in 41.9% of the individuals, with a male predominance in this group.

The follow-up time was 7 to 12 months, which is believed to be a fair latent period to reassess the development or reversibility of the valvular affection and Doppler echocardiographic findings over time and the majority of the individuals was still on penicillin prophylaxis, reporting a 15-day regimen and an excellent compliance to the prophylaxis program.

The outcome of subclinical carditis is not well established, however progression of the rheumatic valvular lesions to chronic subclinical valvular disease is around 82% (Meira 2005) while persistence or deterioration of subclinical carditis has been reported to be up to 44.7% at 3-23 months after diagnosis (Tubridy-Clark 2007) and around 60% after five years of follow up in spite of continuous penicillin prophylaxis and no evidence of recurrent disease (Figeroa 2001). In our series, half of the individuals assessed sustained structural and functional affection while over one third of the individuals assessed sustained the characteristics leading to the diagnosis of RHD. Development of valvular involvement was seen in only a small number of patients. Reversibility of the lesions leading to the diagnosis of RHD was seen in 21% of the cases which

is lower than the reversibility reported ranging up to 60-80%, with adherence to antibiotic prophylaxis (Chin 2010). However, isolated structural or functional affection were more often sustained than the combination of both and functional affection was more prone to develop than the structural affection. Importantly, structural affection was seen to resolve more often than the functional affection. Therefore, individuals were more likely to show reversibility of the diagnosis of RHD mainly due to the disappearance of structural affection.

6.6 Determinants of RHD

Variables that seem to correlate with severity of valve disease include the number of previous attacks of rheumatic fever, the length of time between the onset of disease and start of therapy, and gender. RHD has been reported to be more severe in females than in males (Cole 1976, Longo-Mbenza 1998, Mahmudi 2006) and repeated episodes of RF are believed to lead to further inflammatory damage and subsequent scarring of cardiac valves (Veasy 1997, Majeed 1992). Meanwhile, reversibility has been reported with insufficiency from acute rheumatic valve disease resolving in 60-80% of patients who adhere to antibiotic prophylaxis (Chin 2010). However, other, additional influencing factors have not yet been reported, therefore, this study aims at defining the determinants of RHD in Egypt.

6.6.1 Patients and methods

The population recruited at the penicillin clinic and cardiothoracic department of the NHI in Cairo was evaluated as described in chapter 3, pg. 78 to determine the factors influencing RHD.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

6.6.2 Results

In total, we analyzed the data of 639 individuals, excluding individuals with a history of valve replacement.

Age: The individuals that had structural (p=0.002) as well as functional (p=0.00) and RHD affection (p=0.00) of the cardiac valves tended to be older at the time of screening.
All individual with functional affection tended to be older at the time of assessment. The significance was as follows: mitral regurgitation (p=0.00), mitral stenosis (p=0.00), aortic regurgitation (p=0.00), aortic stenosis (p=0.00), tricuspid regurgitation (p=0.00), tricuspid stenosis (p=0.002). Furthermore, individuals that developed functional or RHD affection of the heart valves tended to be younger at the time of follow-up (p=0.021 and p=0.045, respectively) while individuals that sustained characteristics of RHD at the time of follow-up tended to be older at the time of screening (p=0.037).

Gender: There was a slight female predominance (50.8%, n=269) in the group diagnosed with structural affection (p=0.033) while there was a slight predominance of males (50.5%, n=231) in the group diagnosed with functional affection (p=0.011) and RHD (51.3%, n=221) (p=0.004) (Figure 6.72).

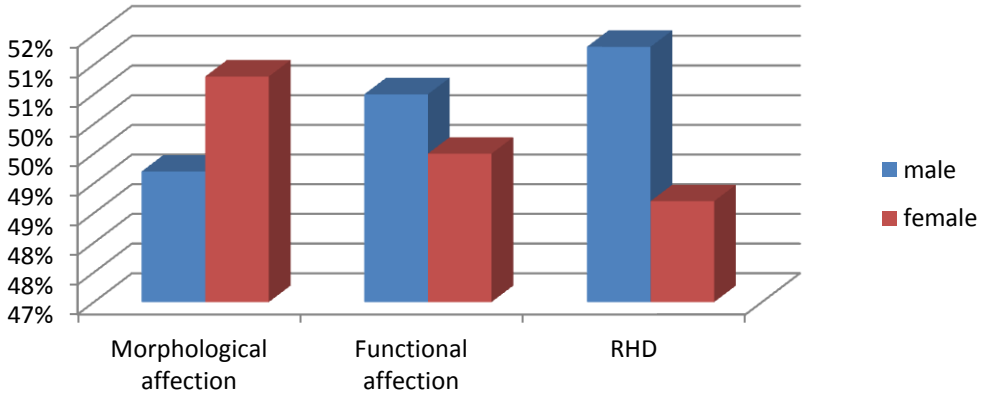


Figure 6.72 Difference in affection between the gender

Affection of the mitral and aortic valve was more common in females, found in 41.9% (n=98) and 4.3% (n=10) of the females while a combination of mitral and aortic valve 24.9% (n=60) and aortic and tricuspid valve affection (1.7%, n=4) was more common among males (p=0.036) (Figure 6.73).

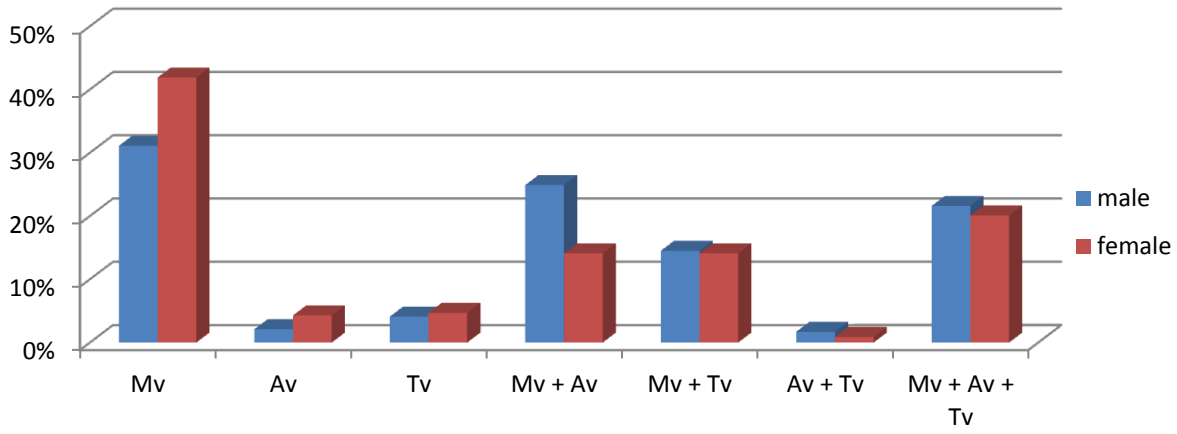


Figure 6.73 Distribution of valvular affection by gender

There was a slight male predominance in the maintenance of the functional affection (59.8%, n=52) and characteristics of RHD (44.8%, n=39) during follow-up (p=0.020 and p=0.032, respectively). Nevertheless, there was a female predominance (45.5%, n=35) in the individuals that did not have characteristics of RHD during the first assessment and follow-up (p=0.017).

Family history: No correlation was found between structural affection and family history of RF. Affection of mitral (51.9%, n=41) as well as a combination of the mitral and aortic valve affection (25.3%, n=20) was more common in patients with a positive family history of RF (p=0.001).

In patients with a negative family history of RF the most common affections were of the tricuspid valves (5.4%, n=19), a combination of mitral and tricuspid valve (16.9%, n=60) and mitral and aortic and tricuspid valve (21.8%, n=77). However, no correlation was found between a family history of RF and the presence of functional or RHD affection.

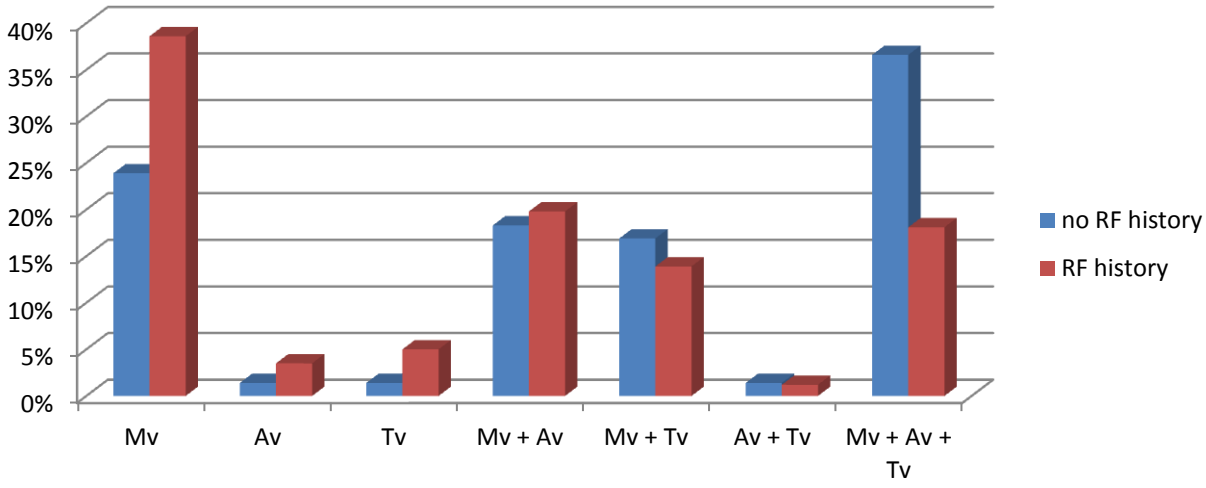


Figure 6.74 Distribution of valvular affection by history of RF

History of RF: The majority of the individuals diagnosed with structural affection (86.6%, n=459) as well as well functional affection (84.7%, n=387) had a positive history of RF (p=0.041 and p=0.00, respectively). Furthermore, the majority of the individuals (84.2%, n=363) diagnosed with RHD had a positive history of RF (p=0.00) (Figure 6.75).

Single valve lesions were more common in patients with history of RF, while combinations of lesions were more common among patients without history of RF.

Only 10.7% (n=11) of the individuals that did not undergo tonsillectomy and 28.6% (n=12) of the individuals that did undergo tonsillectomy did not have structural affection of the heart valves during the first assessment as well as the follow up (p=0.007). The majority of the individuals diagnosed with structural affection (80.0%, n=381) did not undergo tonsillectomy (p=0.050). Only a small fraction of the individuals diagnosed with RHD had undergone tonsillectomy (18.3%, n=72). The majority of the individuals diagnosed with RHD (81.7%, n=322) did not undergo tonsillectomy (p=0.006). No correlation was found between functional affection and tonsillectomy.

No correlation was found between functional affection nor the diagnosis of RHD and the presence of carditis. However, 16.7% (n=2) of the individuals without history of RF and 3.9% (n=6) of the individuals with history of RF had newly developed structural affection of the heart

valves during follow-up (p=0.049). A small fraction of the individuals with no history of RF (8.3%, n=1) and 38.2% (n=58) of the individuals with history of RF did not have echocardiographic characteristics of RHD during the first and follow-up assessment (p=0.038).

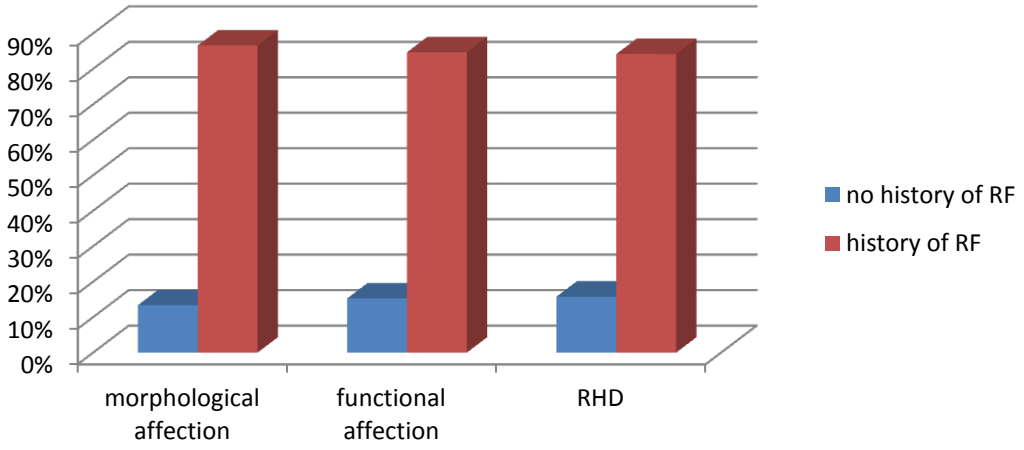


Figure 6.75 Valvular affection in the presence of RF history

Age of RF: The individuals that had structural affection of the valves tended to be older at the time of the first RF attack (p=0.031). They also reported a longer time since the RF attack (p=0.00). Furthermore, individuals that remained having structural affection at the time of follow-up also reported RF longer time ago (p=0.032) while individuals that showed regression of the structural affection tended to report the RF attack a shorter time ago (p=0.050). Importantly, individuals with mitral stenosis were older at the time of the first RF attack (p=0.004). The individuals that had functional affection of the valves reported a longer time since the RF attack (n=0.00). Furthermore, individuals with mitral regurgitation (p=0.031), mitral stenosis (p=0.00), aortic regurgitation (p=0.012), aortic stenosis (p=0.00) and tricuspid regurgitation reported RF longer time ago (p=0.004).

Recurrences: Most individuals with functional affection (56.8%, n=218), structural (57%, n=260) or RHD characteristics of the heart valves (55.6%, n=200) reported recurrences of RF (p=0.042, (p=0.006) and p=0.008, respectively). Most individuals that did not report recurrences of the RF symptoms (47.6%, n=30) were diagnosed with RHD during the first as well as the

follow up assessment while 28.9% (n=26%) of the individuals that reported recurrences were diagnosed with RHD at the time of the first as well the follow-up assessment (p=0.018).

Type of diagnosis: The majority of the individuals diagnosed with structural affection (68.7%, n=364) were diagnosed by the RF symptoms while 31.3% (n=166) of the individuals were diagnosed by the cardiac symptoms. From the individuals diagnosed with RF symptoms 18.1% (n=25) did not have structural affection of the valves during the first assessment and follow-up (p=0.018). Most individuals diagnosed with functional affection (65%, n=297) were diagnosed by the RF symptoms while 35% (n=160) of the individuals were diagnosed by the cardiac symptoms. Nevertheless, the majority of the individuals diagnosed with RHD (63.3%, n=273) were diagnosed by the RF symptoms while 36.7% of the individuals were diagnosed by the cardiac symptoms. However, a fraction of the individuals reportedly diagnosed by cardiac symptoms, had no signs of structural (4.5%, n=4), functional (6.2%, n=10) or RHD affection (6.4%, n=12) on echocardiography and 7.7% (n=2) of the individuals diagnosed by cardiac symptoms remained of functional or RHD affection during the first assessment as well as follow-up compared to 26.8% (n=37) and 41.3% (n=57) of the individuals diagnosed by RF symptoms (p=0.001, respectively).

Sustenance of functional affection during follow up was seen in 69.2% (n=18) of the individuals diagnosed due to cardiac symptoms and 47.8% (n=66) of the individuals diagnosed by the RF (p=0.045). The RHD characteristics found during the first assessment resolved in 38.5% (n=10) of the individuals diagnosed by cardiac symptoms and in 18.1% (n=25) of the individuals diagnosed by RF characteristics (0.020).

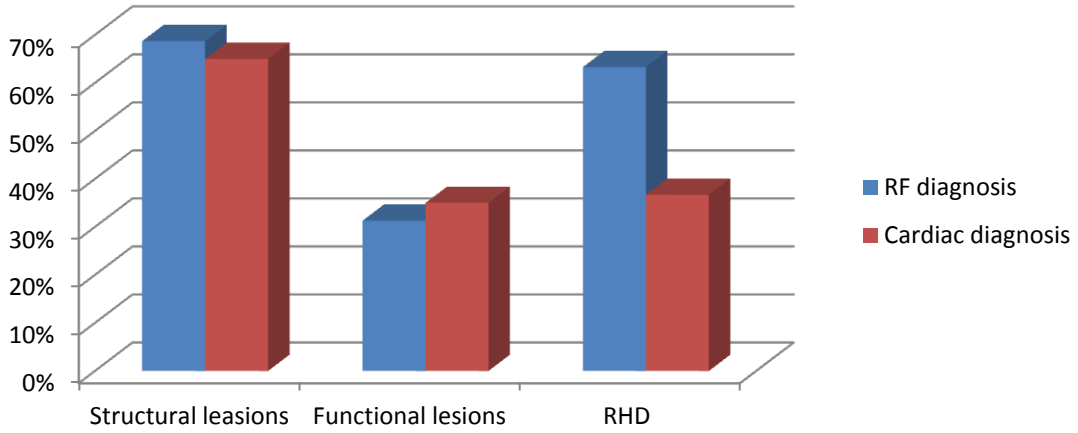


Figure 6.76 Distribution of valvular lesions by diagnosis

Penicillin prophylaxis: The majority of the individuals with structural (83.4%, n=427) as well as functional affection of the heart valves (82.5%, n=363) were on penicillin prophylaxis. On the other hand, the majority of the individuals on penicillin prophylaxis had structural (84.2%, n=427) or functional (71.6%, n=363) affection of the heart valves. Furthermore, the majority of the individuals not on penicillin prophylaxis were diagnosed with structural (93.4%, n=85) or functional affection (84.6%, n=77) of the heart valves (p=0.021 and p=0.010, respectively) (Figure 6.77).

The majority of the individuals diagnosed with RHD (82.4%, n=342) were on penicillin prophylaxis. Most (67.5%, n=342) individuals on penicillin prophylaxis had a diagnosis of RHD while 32.5% (n=165) had not. Nevertheless, 80.2% (n=73) of the individuals not on penicillin prophylaxis were diagnosed with RHD (p=0.015). However, individuals that showed development of RHD at the time of screening tended to have a 21-day or monthly penicillin schedule (p=0.028).

Newly developed functional affection was found in 33.3% (n=4) of the individuals not on penicillin prophylaxis and in 9.2% (n=14) of the individuals on penicillin (p=0.010). However, there was no correlation between the penicillin schedule and compliance and the presence of structural, functional or RHD affection of the heart valves.

Individuals with mitral and aortic stenosis tended to be less compliant to the penicillin prophylaxis (p=0.00 and 0.002, respectively) while individuals with tricuspid regurgitation and those that had developed structural affection at the time of follow-up tended to have a 21-day or monthly penicillin schedule (p=0.01 and p=0.020, respectively).

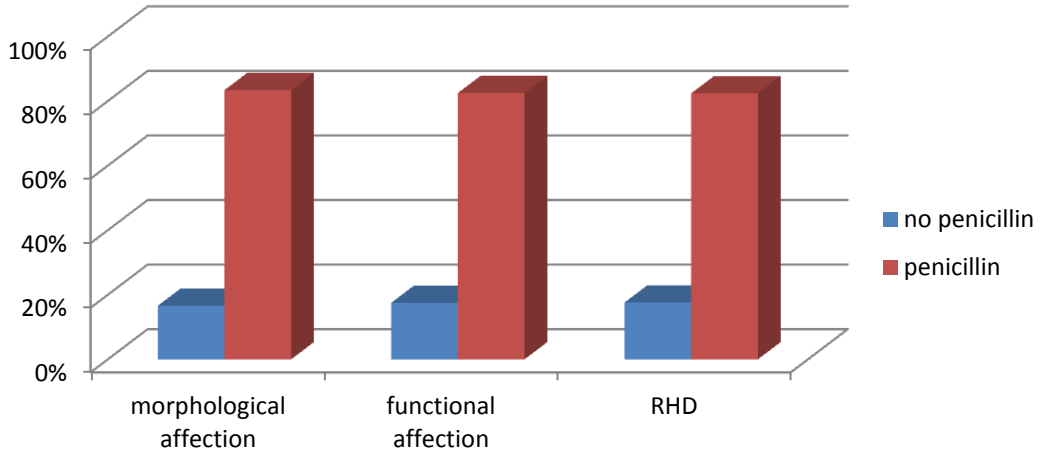


Figure 6.77 Effect of penicillin prophylaxis on valvular affection

Follow-up time: Individuals that sustained structural affection at the time of follow-up tended to have a longer follow-up time (p=0.023) while individuals that showed regression of the structural affection (p=0.014) or development of functional affection of the heart valves tended to have a shorter follow-up time (p=0.011).

6.6.3 Discussion

Variables that have been reported to correlate with severity of valve disease include the number of previous attacks of rheumatic fever, the length of time between the onset of disease and start of therapy, and gender. However, in our series, younger patients seemed to be more at risk for the development of structural, functional and RHD features while older patients are more at risk of sustaining the found RHD features which might have been developed at a younger age. Furthermore, it seems that RF attacks at a younger age are a risk factor for the development of

RHD. Nevertheless, mitral stenosis was more common in individuals reporting an older age at the time of the first RF attack.

There was a slight female predominance in the group diagnosed with structural affection of the heart valves while there was a male predominance in the individuals diagnosed with functional lesions and RHD. Single valve lesions of the mitral or aortic valve were seen more often among females, while males tended to have combinations of valvular affection. Nevertheless, males tended to sustain functional features as well as the diagnosis of RHD suggesting a more severe course in males in contradiction with earlier reports of an increased severity in females with higher incidence of mitral stenosis and cardiac affection (Cole 1976, Longo-Mbenza 1998, Mahmudi 2006).

Mitral valve or a combination of mitral and aortic valve affection was more common in patients with a positive family history of RF, while in patients with a negative family history there was more affection of the tricuspid valve, alone or in combination with mitral and/or aortic valve. This could indicate that familiar predisposition for RF affects the phenotype of the valvular affection.

The majority of the individuals with structural and RHD affection reported recurrences of RF suggesting its role in RHD. However, there was no relationship between the presence of recurrences and the development or reversibility of the valvular lesions. This is surprising as recurrences have been reported to be a risk factor for the development of mitral stenosis (Thomas 1971) and the severity of valve regurgitation has been shown to be greater in recurrences of carditis, possibly owing to repeated attacks of valvulitis that culminate in greater valvular damage and due to the self-perpetuating nature of regurgitant lesions (Vasan 1996) leading to worsening of rheumatic valve affection (Jaffe 1988, Vasan 1996, Carapetis JR 2000) and the development or deterioration of RHD (Bisno 2005, Carapetis 2005[2], McDonald 2005, Manyemba 2003). However, it has also been suggested that valvular lesions may progress despite the lack of symptomatic recurrence (Tompkins 1972).

Individuals diagnosed by cardiac symptoms were more likely to sustain the found functional affection. Importantly, a longer time since the first RF attack seems to be an indicator for sustenance of structural and structural affection while a shorter time seems to be a good prognostic factor for reversibility of the features. This is logic as lesions present for a longer time

tend to be sustained while the lesions that were more prominent to resolve would have done so a longer time ago. Furthermore, structural features seem more prone to resolve than functional lesions, especially short after the development.

A fraction of the individuals reportedly diagnosed due to cardiac symptoms did not have sign of any cardiac affection during the first assessment as well as during follow-up. This might suggest that in this group the RHD characteristics had resolved in the time between the cardiac diagnosis and our first assessment. However, a diagnosis of RF based on cardiac symptoms is a risk factor for sustenance of functional valvular affection which might suggest that clinical functional affection is less probable to resolve after established. The fact that the cardiac diagnosis of RF seems to correlate with reversibility of the features leading to the diagnosis of RHD might be due the disappearance of the structural features, as the functional affection tend to persist. However, this might also indicate that diagnosis leads to medical treatment and the use of penicillin prophylaxis and contributes therefore to the reversibility of the cardiac lesions.

Importantly, the majority of the individuals with structural, functional affection or RHD were on penicillin prophylaxis and there was no correlation between the penicillin schedule and compliance and the presence of valvular affection. However, individuals not on penicillin prophylaxis had more often structural and functional affection of the heart valves and the diagnosis of RHD.

Furthermore, a penicillin schedule of 21-days or 1 month was related to development of structural features and RHD and non-compliance to prophylaxis was related to development of functional affection. This was expected as a 21-day or monthly penicillin schedule was shown to be a risk factor for the development of structural and RHD features, which enforces the view that a 15-day penicillin prophylaxis should be encouraged. Furthermore, cessation of penicillin prophylaxis is a risk factor for the development of functional valvular lesions which is logic as secondary penicillin prophylaxis has been shown to prevent development and worsening of RHD (Veasy 1997, Majeed 1992). Nevertheless, mitral and aortic stenosis was related to non-compliance to penicillin prophylaxis. Therefore, we reinforce the opinion that non-compliance to penicillin prophylaxis is a risk factor for the development of RHD and highlight the focus on adherence to secondary prophylaxis regimens with long-term, regular delivery of penicillin to

patients with RHD or a history of RF to prevent recurrences of the attacks and the worsening of valve lesions. Importantly, most individuals diagnosed with RHD had poor disease awareness, which especially in the form of a missing history of RF was shown to be a risk factor for the development of structural features and for sustaining the diagnosis of RHD. This might suggest that individuals that are not aware of RF do not receive the needed treatment and prophylaxis and are therefore more at risk for the development and progression of RHD.

6.7 Conclusions

In conclusion, we have shown that in Egypt functional affection tends to develop more often and is also more difficult to resolve than structural features. In Egypt, cardiac affection seems more severe in males as there is a male predominance in the individuals with functional and RHD characteristics, showing also an increase in the amount of mixed lesions. Young age at the time of presentation as well as at the time of the first RF attack is related to a tendency of development of structural as well as RHD features. Meanwhile, older patients diagnosed with RHD tend to sustain the valvular affection. The longer ago the first RF attack, the less probable it is that the valvular affection will resolve. Furthermore, clinical functional affection is less likely to resolve.

Patient information is highly desired in Egypt as a lower disease awareness and non-compliance to penicillin result in the development of structural as well as functional valvular lesions and sustenance of RHD features. Our series confirms the predominance of mitral valve affection, with regurgitation being the most common feature. However, in RHD, combination of lesions is more predominant. Nevertheless, the amount of mitral stenosis was comparable to earlier studies. The presented results also confirm the need to encourage a penicillin regimen of 15-days in Egypt, as a longer interval between the injections was shown to be a risk factor for the development of structural and RHD features. Long-term follow-up is necessary, nonetheless, to determine the outcome in young children with subclinical echocardiographic evidence of valvar disease. The serial use of echocardiography in patients with RHD is helpful in finding the progression or regression of the disease and will help us determine the development, characteristics, progression and reversibility of valvular lesions.

CHAPTER 7

IMMUNE RESPONSE IN RF AND RHD

7.1 Background

Different infection parameters are used to confirm the suspicion of RF differentiating between general infection parameters and streptococcal specific parameters. The initial routine laboratory assessment consists of the major inflammatory parameters and acute phase indicators such as ESR and CRP which correlate with the magnitude and severity of inflammation as well as disease activity (Lane et al, 2002, Wagner-Weiner 2002). The ESR and CRP are both used in the diagnosis of RF in the Jones criteria however, the levels and distribution of these infection parameters in different areas, as a response to RF, especially in a quiescent phase of the disease is unknown. A major role is suggested for inflammatory cytokines in mediating RF and RHD (Guilherme 2009) as they appear to play a crucial role in triggering immunologic and inflammatory reactions in RF and have been shown in peripheral blood of acute RF and active RHD patients (Yegin 1997, Miller 1989, Morris 1993, Narin 1995, Samsonov 1995) as well as valvular tissue, suggested to mediate the heart lesions (Guilherme 2009).

Furthermore, cellular infiltration with a predominance of $CD4^+$ T-cells (Guilherme 1995) and some $CD8^+$ T-cells is found in patients that present rheumatic activity (Guilherme 2001). However, the immune response and signs of immune activation vary showing different characteristics of the cellular as well as the humoral response.

We hypothesized that there is still an ongoing immunological reaction in apparently quiescent phase of the disease and therefore the inflammatory parameters are expected to remain raised in RF and RHD. The aim was to analyze the inflammatory parameters and presence of pro- and anti-inflammatory cytokines to evaluate the signs of immune activation in RF and RHD in Egypt for the recognition of potential inflammatory parameters to monitor RF activity and determine the role of inflammatory cells and cytokines in mediating RF and RHD.

7.2 Haemal immune response in RF and RHD

7.2.1 Patients and methods

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI were recruited as described in chapter 3, pg. 75.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

Blood sample collection and assays

Blood was collected by venipuncture into 1 siliconised EDTA, 1 non-additive and 1 citrate ESR tube as described in chapter 3, pg 88. A complete CBC, ESR analysis, qualitative determination and semi-quantitative determination of the ASOT and CRP was done. Serum and plasma was obtained and stored.

Analysis of blood samples

Complete blood count (CBC): Analysis was performed by Sysmex KX-21N (Automated Hematology Analyzer, SYSMEX CORPORATION). Parameters in whole blood mode included the CBC (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT) and a 3-Part Differential leukocyte count (Lymph %, #, Neut %, #, Mixed %,#).

ESR analysis: The ESR analysis was performed following the Wintrobe method.

A calibrated Wintrobe tube graduated from 0-100mm (0-10cm) and with a capacity of 1 ml of blood was placed in a sedimentation rack and filled to the 0 mark with 1 ml of well-mixed anticoagulated blood (collected in a ESR citrate tube) using a long-stemmed pipet and left to incubate in a vertical position for exactly one hour. At the end of one hour, the distance the erythrocytes have fallen from the plasma meniscus (at the zero mark) in the blood samples is measured and recorded in millimeters (mm) using the markings on the tube.

ASOT- Latex Test: The manual ASOT-Latex Test is an agglutination test for the direct qualitative and semi-quantitative detection of clinically significant levels of anti-streptolysin O antibodies (ASO) in human serum which are produced by growing strains of Group A haemolytic streptococci (and by most strains of groups C and G). The assay is performed by testing a suspension of latex particles coated with streptolysin O antigen against unknown serum. The presence or absence of a visible agglutination, indicates the presence or absence of ASO in the samples tested. The reagent is adjusted in the way that presence of an ASO title of 200 IU/mL or higher in the serum gives a visible agglutination of the latex particles without previous sample dilution.

Qualitative determination

- One drop of reagent was placed on the field of the slide
- One drop of undiluted serum was added with a pipette
- The drops were mixed well with the flat end of the pipette
- The slide was rocked for 2 minutes
- Result was read immediately under direct light
- In case of positive test, a semi-quantitative test was performed

Semi-quantitative determination

- Dilutions (1:2, 1:4, 1:8, 1:16, 1:32) of the positive samples were prepared with 0.9% of sodium chloride solution (saline)
- One drop of reagent was placed on the field of the slide
- One drop of diluted serum was added with a pipette
- The drops were mixed well with the flat end of the pipette
- The slide was rocked for 2 minutes
- Result was read immediately under direct light
- The serum titer was defined as the highest dilution showing a positive result.
- The ASO concentration was then be estimated from the last dilution with visible agglutination. The approximate ASO concentration was obtained by multiplying the titer by the limit of sensitivity (200 IU/mL)

CRP Latex Test: The CRP Latex test is based on the immunologic reaction between the CRP and the corresponding antibody coated on the surface of latex particles. The CRP reagent contains polystyrene latex particles coated with human anti CRP antibody. When the reagent is mixed with serum containing CRP at a level equal or greater than 6 mg/l, the particles agglutinate without previous sample dilution. A smooth homogenous milky suspension indicates a CRP concentration of less than 6 IU/ml (as observed with the negative control). Agglutination indicates a CRP content more than 6 IU/ml.

Qualitative determination

- One drop of reagent was placed on the field of the slide
- One drop of undiluted serum was added with a pipette
- The drops were mixed well with the flat end of the pipette
- The slide was rocked for 2 minutes
- Result was read immediately under direct light
- In case of positive test, a semi-quantitative test was performed

Semi-quantitative determination

- Dilutions (1:2, 1:4, 1:8, 1:16, 1:32) of the positive samples were prepared with 0.9% of sodium chloride solution (saline)
- One drop of reagent was placed on the field of the slide
- One drop of diluted serum was added with a pipette
- The drops were mixed well with the flat end of the pipette
- The slide was rocked for 2 minutes
- Result was read immediately under direct light
- The serum titer was defined as the highest dilution showing a positive result.
- The CRP concentration was then be estimated from the last dilution with visible agglutination. The approximate CRP concentration may be obtained multiplying the titer by the limit of sensitivity (6 mg/L)

Cytokine Luminex assay:

Serum samples: Serum samples require a 4-fold dilution. However, after several trials we have decided to use neat serum due to low concentrations of the cytokines.

Standards: The standards were made of standard Cocktails 1 and 2 with Calibrator Diluent RD6-40 (for serum samples). They were allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Assay procedure: The filter-bottomed microplate was pre-wet by filling each well with 100 µL of Wash Buffer. The liquid was removed through the filter at the bottom of the plate using a vacuum manifold designed to accomodate a microplate. A diluted microparticle mixture was resuspend by vortexing andv50 µL of the microparticle mixture was added to each well of the microplate. 50 µL of Standard or sample was added per well and incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. Each well was filled with Wash Buffer (100 µL) and using a vacuum manifold device designed to accommodate a microplate, the liquid was removed. This wash procedure was performed 3 times. 50 µL of diluted Biotin Antibody Cocktail was added to each well and left to incubate for 1 hour at room temperature on the shaker set at 500 ± 50 rpm. The wash procedure was repeated 3 times. 50 µL of diluted Streptavidin-PE was added to each well and incubated for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm followed by 3 washings. The microparticles were resuspend by adding 100 µL of Wash Buffer to each well and left to incubate for 2 minutes on the shaker set at 500± 50 rpm. The results were read within 90 minutes using a Luminex analyzer.

Statistical analysis

The statistical analysis was performed as described in chapter 3, pg 100.

7.2.2 Results

7.2.2.1 Inflammatory parameters in RF and RHD

A total of 309 blood samples were collected from the individuals recruited. A raised WBC of >11 K/uL was found in 10.3% (n=35) and a raised lymphocyte count of >4 was found in 49.5% (n=164) of the individuals.

An RBC <4 M/uL was found in 10.5% (n=35) of the individuals. An HGB <12 g/dL was found in 42.5% (n=134) of the individuals, with 18.4% (n=61) showing an MCV <76.0 fL and 51.2% (n=162) with an MCH <27.0 pg. 2.56% (n=8) of the individuals had an HGB <9 g/dL. A PLT count <140 K/uL was found in 5.4% (n=18), while 2.1% (n=7) had a PLT count >450 (Table 7.1).

Table 7.1 CBC results

	Mean ± SD	**range**
WBC	7.63 ± 3.35	3.20-28.80
LYM	18.90 ± 18.61	1.20-61.70
MID	0.55 ± 0.22	0.30- 1.40
GRAN	4.68 ± 3.48	0.90- 18.0
RBC	4.65 ± 0.63	2.34- 6.96
HGB	12.23 ± 1.60	7.0- 16.10
HCT	37.10 ± 4.79	20.0- 48.40
MCV	80.08 ± 6.63	51.20-93.50
MCH	26.43 ± 2.82	16.20- 32.60
MCHC	33.03 ± 1.61	28.00-43.50
RDW	15.80 ± 14.21	11.90-262.00
PLT	255.01 ± 84.78	66.00- 756.00
MPV	4.42 ± 5.52	7.20-13.30
ESR	29.28 ± 26.22	2.0-132.00
ASOT	74.91 ± 124.155	0-800
CRP	7.92 ± 20.48	0-96.00

A positive CRP (≥ 12 IU/ml) was seen in 16.6% (n=51) of the individuals. Most individuals (65%, n=199) had a negative CRP (Table 7.2).

Table 7.2 CRP results

CRP	
0	65% (n=199)
6	18.3% (n=56)
12	6.5% (n=20)
24	3.6% (n=24)
48	2.3% (n=7)
96	4.2% (n=13)

30.6% (n=95) of the individuals had an ESR ≤10 mm/h, while 20.5% (n=64) had an ESR between 11 and 20 mm/h. In total, 51.1% (n=159) of the individuals had an ESR ≤20 mm/h.

7.2.2.2 Relation between inflammatory parameters

The relation between the ESR rate and CRP and the WBC, LYM count, RBC, HGB and HCT is described in Table 7.3. No correlation was found between the ASOT and the WBC, the LYM count, RBC count, HGB and HCT.

Table 7.3 Relation between inflammatory parameters

	Increase		**Decrease**	
ESR	WBC	(p=0.00)	LYM	(p=0.029)
			RBC	(p=0.00)
			HGB	(p=0.00)
			HCT	(p=0.00)
CRP	WBC	(p=0.00)	LYM	(p=0.034)
	ESR	(p=0.00)	RBC	(p=0.02)
			HGB	(p=0.00)
			HCT	(p=0.01)

7.2.2.3 Cytokine parameters in RF and RHD

The serum cytokine levels were as indicated in Table 7.4 and the relation between the cytokines is described in table 7.5.

Table 7.4 Cytokine levels

	Mean ± SD pg/mL	**Minimum pg/mL**	**Maximum pg/mL**	**Normal values pg/mL***
IL-1b	0.22 ± 0.68	0.01	8.17	0.16 ± 0.17
Il-1ra	803.5 ± 1677.6	0.02	15825.7	829 ± 292
IL-2	0.25 ± 0.18	0.10	1.97	
IL-4	0.30 ± 0.58	0.01	2.26	3.34 ± 0.84
IL-6	0.92 ± 2.66	0.01	26.54	0.39 - 12.3
IL-8	4.99 ± 14.57	0.01	117.8	9.51
IL-10	0.57 ± 0.43	0.04	2.02	9.2 ± 1.5
TNF-α	0.67 ± 0.74	0.10	4.41	2.03 - 10.5

* Reported in earlier studies

Table 7.5 Relation between cytokines

	Increase		**Decrease**	
IL-1b	IL-2	(p=0.00)	IL-1ra	(p=0.00)
	IL-6	(p=0.00)	IL-10	(p=0.00)
	TNF-α	(p=0.035)		
IL-1ra	IL-4	(p=0.00)	IL-2	(p=0.00)
	IL-8	(p=0.00)	IL-6	(p=0.00)
	IL-10	(p=0.00)		
	TNF-α	(p=0.00)		
IL-2	IL-6	(p=0.00)	IL-4	(p=0.00)
			IL-10	(p=0.00)
IL-4	IL-8	(p=0.00)	IL-6	(p=0.00)
	IL-10	(p=0.00)		
	TNFa	(p=0.00)		
IL-6	TNFa	(p=0.00)	IL-10	(p=0.00)
IL-8	TNFa	(p=0.00)		
	IL-10	(p=0.00)		

7.2.2.4 Factors influencing the haemal parameters in RF and RHD

No correlation was found between the level of cytokines found in the serum and the gender, family history for RF, the age of the RF attack, the penicillin schedule and compliance was not related to the serum cytokine level.

Age: Older patients had a higher WBC count (p=0.005), ESR (p=0.00), CRP (p=0.045) as well as a higher IL-1b (p=0.027), IL-2 (p=0.002) and IL-6 (p=0.00). However, older patients had a lower the platelet count (p=0.001) Furthermore, there was no correlation between the age and the ESR, LYM count, RBC count, HGB and HCT.

Gender: Males had higher blood hemoglobin (p=0.000) and hematocriet (p=0.001) than females. 35.5% (n=54) of the males and 56.2% (n=90) of the females had a hemoglobin ≤12 g/dL. 3.2% (n=5) of the males and 1.8% (n=3) of the females had a hemoglobin ≤9 g/dL. Females tended to have a higher ESR (p=0.005) and platelet count (p=0.018) than males. No correlation was found between the gender and the WBC, lymphocyte count, MCV and ASOT.

History of RF: Individuals with a positive history of RF had a higher lymphocyte count (p=0.038) and platelet count (p=0.00). No correlation was found between a history of RF and the WBC, hemoglobin, hematocriet, MCV, ESR, ASOT and CRP. A correlation was found for a positive history of RF was IL-6 (p=0.010) as patients with a positive history of RF showed lower levels of IL-6.

Date of RF: A positive correlation was found between the WBC count and the date of the first RF attack (p=0.032). The longer ago the first RF attack was the higher the WBC count. There was no correlation between the date of the first RF attack and the ESR, LYM count, RBC count, HGB and HCT. There was also no correlation with the CRP.
A correlation was found between the ASOT and the date of RF onset. The less time since the RF attack the higher the ASOT (p=0.004). The longer ago the RF attack had been the higher the IL-1b (p=0.012), the IL-2 (p=0.008) and the IL-6 (p=0.020).

Familial predisposition: Individuals with a negative family history for RF tended to have a higher hemoglobin (p=0.043) and hematocriet (p=0.034). No correlation was found between the family history for RF and the WBC, lymphocyte count, MCV, platelet count, ESR, ASOT and CRP.

Recurrences: Individuals who reported recurrences of RF tended to have a lower WBC (p=0.009) than those who did not. No correlation was found between the presence of recurrences of RF and the other inflammatory and streptococcal parameters. Individuals that reported recurrences showed a lower IL-1b (p=0.027), IL-2 (0.003), IL-6 (p=0.005) and TNFa (p=0.015).

Diagnosis: Individuals who were diagnosed by the RF symptoms tended to have a higher lymphocyte (p=0.010) and platelet count (p=0.001) and a lower CRP (p=0.044) than those diagnosed by the cardiac symptoms. Individuals that were diagnosed by RF symptoms had lower IL-6 levels (p=0.039). There was no correlation between the type of diagnosis and the ESR nor the ASOT.

Prophylaxis: Individuals on penicillin prophylaxis tended to have a lower WBC (p=0.037) and CRP (p=0.013) and a higher platelet count (p=0.00). No correlation was found between the use of penicillin prophylaxis and the other inflammatory and streptococcal parameters. There seemed to be a correlation between the compliance to the prophylaxis and the platelet count (p=0.004), with individuals with a higher compliance showing higher platelet counts. Patients on penicillin prophylaxis had a lower of serum IL-6 (p=0.021).

7.2.3 Discussion

Inflammatory parameters

The inflammatory parameters are affected by many factors including the patient's age, gender, dietary options, medication, etc (Lane 2002). Our series shows a raised WBC in 10.3% and a raised lymphocyte count in 49.5% of the individuals screened. The raised WBC, known as leukocytosis is a sign of infection and a raised lymphocyte count, lymphocytosis, is usually a sign of a viral infection and has also been shown in 84% of the ARF patients (Mahmudi 2006). Penicillin is known to lower leukocyte count, which means that in the absence of penicillin prophylaxis the lymphocytosis would have been expected to be higher.

Leukocytosis with neutrophilia and mild to moderate anaemia are found in inflammation and concurrent anaemia has been reported to be found in up to 62% of the ARF cases (Mahmudi

2006) and 8% of the cases of newly diagnosed RHD. (Sliwa 2010) In our series 42.5% of the individuals had an HGB <12 g/dL with 18.4% showing an MCV <76.0 fL and 51.2% with an MCH <27.0 pg.

The most common causes of microcytic anemia are iron deficiency due to inadequate dietary intake or chronic disease. The MCH value is diminished in hypochromic anemias mainly caused by vitamin B6 deficiency from a low iron intake, diminished iron absorption, or excessive iron loss. Nevertheless, anemia can also be caused by infections or other diseases and therapeutic drugs. The found anemia could however not be caused by the penicillin prophylaxis, which is known to cause hemolytic anemia and therefore macrocytic changes of the RBC. Importantly, concurrent anaemia has been reported in combination with RHD (Sliwa 2010). In our series, anemia was more common among females, as 56.2% had an HGB ≤12 g/dL, however, males tended to have more often a more severe anemia with 3.2% showing HGB ≤9 g/dL. A small number of individuals (2.56%, n=8) had an HGB ≤9 g/dL which is usually associated with severe carditis.

A normal platelet count in a healthy individual is between 150 and 450 K/uL. In our series a PLT count <140 K/uL was found in 5.4%, while 2.1% had a PLT count >450. Low platelet counts may be caused by systemic viral or bacterial infection and increases bleeding risks.

Penicillin is known to impair and inhibit platelet function by inhibiting platelet aggregation response and release reaction as it inhibits some essential mechanisms involved in platelet activation. However, penicillin is not known to influence the platelet count (Schulz 2010, Rao 1983).

The normal upper limit of ESR for persons aged 50 years and younger is 15 mm/h in men and 20 mm/h in women with a mean of normal values around 9.6 mm/h for men and 10.4 mm/h for women younger than 20 years (Volanakis 2001). Acute phase reactants are always elevated at the onset of acute RF and the erythrocyte sedimentation rate (ESR) is elevated in the first weeks of the disease. Elevated ESR (>40 mm/hr) has been reported in up to 90% of the ARF cases (Mahmudi 2006) with higher levels among patients with cardiac involvement. In our series, 48.9% of the individuals screened had a positive ESR with a mean of 29.28 mm/h (Shearn

1986), showing that there is a remaining acute phase inflammation as the ESR correlates with increased levels of acute phase reactants, in particular, fibrinogen.

The C-reactive protein (CRP) is elevated at the onset of the acute phase and tends to disappear at the end of the second or third week; however both ESR and CRP are affected by anti-inflammatory medications. A positive CRP has been found in up to 85% of the ARF patients and in 16.6% of the individuals screened in our series. The mean CRP was 7.92 IU/ml, showing a mild magnitude of the inflammation and disease activity. This may be the result of the latent period since the RF attack or the treatment and penicillin prophylaxis, as CRP levels change quickly in response to inflammation and fall quickly with appropriate treatment bearing in mind that 84.4% (n=521) were on prophylaxis. However, 11.3% of the individuals reported the RF attack being in the last 2 months, while 48.5%, and 68.5% were in the last 12 and 24 months, and most individuals reported recurrences of RF which may explain the found elevated ESR and CRP.

Elevated or rising ASO titers provide reliable confirmation and identification of a recent preceding GAS infection in patients suspected of RF as these titers rise approximately 1 week and peak 3 to 6 weeks after the infection and may persist for several months after even uncomplicated GAS infections. However, approximately 20% of patients with RF may not have this antibody, although raised ASO titers have been shown to be elevated in 87% of the ARF patients (Mahmudi 2006). The fact that only 30.9% of the screened individuals in our series had raised ASO titers might be due to the fact that the majority of the RF attacks had been months ago and the titers might have resolved and the fact that over 80% of the individuals were on penicillin prophylaxis.

Our series shows that a higher LYM and RBC count, HGB and HCT is related to a lower ESR and CRP. A higher CRP was related to a higher ESR, which was expected as the ESR as well as the CRP are indicators of inflammation and a relationship between the findings is obvious. The age of the patient was also related to the inflammatory parameters with older patients showing a higher WBC count, ESR and CRP and a lower platelet count. These results are surprising as only

the ESR is known to be related to age, being higher in patients older than 50 years. As expected, males had higher blood hemoglobin and hematocriet than females. However, females tended to have a higher ESR and platelet count than males. The ESR is known to be higher in women than in men but there is no gender difference in the platelet count.

Individuals with a positive history of RF had a higher lymphocyte and platelet count. The high lymphocyte count is a result of the inflammatory process, but the raised platelet count cannot be explained by a history of infection. A longer time since the first RF attack was related to a higher WBC count. This is contradictory to our expectations, as the WBC is supposed to lower after the acute inflammatory event. Importantly, the shorter the time since the RF attack the higher the ASOT, which is logic as the ASOT lowers after the infection while remaining positive for several months.

Penicillin prophylaxis seems to result in a lower WBC and CRP and a higher platelet count and a better compliance to the prophylaxis also tends to result in a higher platelet count. Individuals who reported recurrences of RF tended to have a lower WBC than those who did not. This is strange because the WBC would be expected to be higher as a result of recurrences of the inflammatory process.

Individuals who were diagnosed by the RF symptoms tended to have a higher lymphocyte and platelet count and a lower CRP than those diagnosed by the cardiac symptoms.

Meanwhile, individuals with structural and RHD features tended to have a lower platelet count. The finding of structural or cardiac affection seems therefore, to be related to a lower platelet count which is dependent on the use and compliance to the penicillin, suggesting that non-compliance is a risk factor for both. Importantly, individuals with a lower ASOT level tended to remain free of structural affection of the valves during follow up.

Furthermore, reversibility of functional affection is related to a lower WBC which is related to the age, the time since the RF attack and the use of penicillin, therefore it seems that younger age, a shorter time since the RF attack and use and compliance to penicillin are promoters for reversibility of functional affection. Strangely, individuals with a higher WBC tended to remain free of functional affection at the time of follow-up ($p=0.003$).

In conclusion, a lower platelet count seems to be a risk factor for cardiac affection and the presence of structural features and the diagnosis of RHD and a high ASOT seems also to be a risk factor for valvular affection. Reversibility can be expected in individuals with a lower WBC, which can be achieved by use and better compliance to the penicillin prophylaxis. Meanwhile, non-compliance or a lower compliance to the penicillin prophylaxis is a risk factor for the presence of structural features.

Haemal cytokine profile

The levels of IL-1 found in our series is comparable to earlier studies in healthy controls. This is strange as IL-1 has been reported to increase during active rheumatic carditis (Morris 1993), persisting for up to 48 weeks (Miller 1989) and in ARF and RHD patients (Morris 1993) which can be explained by the clinical significance of IL-1 due to its activities as a stimulator of T-cells and being in combination with other cytokines an important mediator of inflammatory reactions.

No data on IL-2 levels in serum of healthy controls has been found, but we believe that plasma levels are comparable to serum. Therefore, the significantly lower IL-2 levels compared to healthy controls is surprising (Mazzone 1999) especially because IL-2 plays a role in anti-inflammatory reactions and has been reported to be increased in ARF and RHD patients (Yegin 1997).

We have found lower levels of IL-4 compared to healthy controls. Importantly, IL-4 is of clinical importance in inflammatory diseases and autoimmune diseases since it inhibits the production of inflammatory cytokines such as IL-1, IL-6 and TNF-α by monocytes and of TNF by T-cells. In addition, the lack of IL-4 in valvular tissue has been suggested to perpetuate and/or exacerbate the production of inflammatory cytokines TNF-α and IFN-γ (Guilherme 2005[3]). However, in our series, most of the cytokine levels were lower than reported in control subjects.

The IL-6 levels found in our series is in the range reported in healthy controls, although a very wide range from 0.39 up to12.3 pg/mL has been reported (Morikawa 2003, Yoshida 2002, Straub 2000, Özbek 2003, Grey 1996, Al-Awadhi 1999, Bas 2004, Steddon et al, 2004).

The found IL-8 was significantly lower than the average serum levels reported in controls (Özbek 2003). This was not expected as significant elevations in the plasma levels of IL-8 have been observed in active phase of RHD patients suggesting that IL-8 might have a role in

the pathogenesis of RF (Yegin 1997). Furthermore, IL-8 is believed to be a marker for different inflammatory processes as it directly affects B-cells through a specific mechanism that is different from IFN-γ and IFN-α.

TNF-α levels were significantly lower in our series (Elkind 2002, Wang 2003, Erdem 2003, Mizia-Stec 2003, Halasz 2003, Diez 2002, Yoshida 2002, Mishima 2001, Wang 1999, Özbek 2003, Grey 1996, Celik 1995, Kalt 2006) which is surprising as TNF-α mediates part of the cell mediated immunity against obligate and facultative bacteria and parasites and is required for normal immune responses and has been reported to be elevated in patients with ARF (Narin 1995, Samsonov 1995, Yegin 1997, Miller 1989, Morris 1993, Narin 1995, Yegin 1997) and RHD. Furthermore, TNF-α is suggested to play an important role in the initiation of multisystemic inflammatory response in RF and in the pathogenesis of RHD (Yegin 1997, Settin 2006).

In our series, only IL-6 and IL-10 levels were in the range reported in healthy controls (Andersson 2000, De Vita 2000), which might suggest that they are elevated in comparison to the other cytokines.

The surprising low levels of cytokines found in our series, especially IL-2, IL-4, IL-8 and TNF-a might suggest that decreased levels of these cytokines mediates rheumatic lesions. We expected IL-1, IL-6 and TNF-α to be increased as they are believed to be produced to eliminate the bacteria (Ramasawmy, unpublished observations) and an increase of TNF-a, IL-1 and IL-2 has been described in the peripheral blood of acute RF and active RHD patients (Miller 1989, Morris 1993, Narin 1995, Samsonov 1995, Yegin 1997).

Nevertheless, IL-6 and TNF-α are considered well-known inducers of acute phase reactants and show significant correlation with the C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) (Yegin 1997). TNFa levels have also been shown to correlate with the IL-6 levels and have been suggested to be the major cytokine in RF leading to IL-6 and IL-8 production (Yegin 1997). However, in a previous study in Egypt, no increase in the production of IL-6 and IL-10 was found in acute rheumatic carditis (Hafez 2001).

7.3 Valvular immune activation in RF and RHD

7.3.1 Patients and methods

Individuals admitted at the Cardio-thoracic department of the NHI, Imbaba, Cairo and scheduled for valve replacement due to RHD were recruited as described in chapter 3, pg. 75.

Sample collection

Excised valves from the individuals requiring valve replacement were collected during the procedures. The valvular tissue was stored in formalin directly after collection and then embedded in paraffin. The samples were transported to the HSC for histology and immunohistochemistry analysis.

The condition of the normal valves is expected to be the same and there was no significant difference in the results found. We have therefore reported the combined results for an easier overview and comparison of the results. The reason that we have only used 2 control valves is due the fact that it is very difficult to collect healthy human valvular tissue.

Immunohistochemistry Analysis

Immunostaining of the valves for determination of inflammatory cells, ECM proteins and cytokine profile was performed on paraffin-embedded tissues using immunoperoxidase. The following primary antibodies were used: CD8 (BD Bioscience), CD4 (BD Bioscience), Collagens I, III, IV (Novotec), NFKB (Chemicon), CD45 (Vector laboratories), CD68 (DAKO), IL-10 and IL-12 (Abcam).

Prior to immunoperoxidase staining, 5μm thick paraffin wax sections of decalcified non coronary root of heart valve tissue were dewaxed and rehydrated into water, washed in phosphate buffered saline (PBS) for 5 minutes, then the slides were immersed in 0.1M citrate buffer (pH 6) and microwaved for 10 minutes before blocking for endogenous peroxidases using 0.3% hydrogen peroxide in PBS for 15 minutes. Sections were washed twice in PBS and blocked with 3% bovine serum albumin (W/V) (BSA) in PBS for 30 minutes. Sections were incubated separately for 1hour with primary antibodies. Negative control consisted of 3% BSA in PBS. Primary antibodies was then removed by washing the sections 3 times in PBS followed by a second layer of biotinylated goat anti-mouse immunoglobulins (GAM IgG-Vector laboratories)

diluted 1/250 in PBS for 1hour. Sections were then washed 3 times in PBS before 1 hour incubation with Avidin-Biotin Complex (ABC-Vector laboratories). Reactivity was detected using diaminobenzidine tetrahydrochloride (DAB tablets, Sigma) (25mg/ml) and hydrogen peroxide (0.01% W/V). Sections were then counter stained with Mayer's haematoxylin and viewed on a Ziess Axioskop microscope. Digital micrographs were taken using a Nikon DMX1200 camera. The samples were scored semi-quantitatively for the expression of the antigens present within the valve tissue.

7.3.2 Results

Human Rheumatic Valves and Control Valves

Valve tissues were collected at the time of surgery. We obtained 24 human rheumatic valves from patients (n=24, mean age, 35.42 years, range, 14 to 63 years, male, 13, female, 11) at the time of surgical valve replacement. We compared them to 2 control valve tissue (n=2, gender: male, age: 56 and 62 years) obtained by autopsy.

7.3.2.1 Inflammatory parameters in valvular tissue

$CD4^+$

A total of 19 valves were stained for $CD4^+$ which was present in all (n=19) RHD valves, ranging from 0 to 15 cells/mm^2. The total density of $CD4^+$ T-cells was 1.18±1.34 cells/mm^2 in the rheumatic valves and 1.95±0.63 cells/mm^2 in control valves (p=0.9). The localization of the $CD4^+$ was studied in 15 valves and $CD4^+$ was the most prominent in the spongiosa (Table 7.6).

Table 7.6 Localisation of CD4+

	%	N
Ventricularis	33.3%	4
Spongiosa	100%	19
Fibrosa	33.3%	5
Localisation		
Ventricularis (only)	0%	0
Spongiosa (only)	60%	9
Fibrosa (only)	0%	0
Ventricularis + spongiosa	6.7%	1
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	26.7%	4
Spongiosa + fibrosa	6.7%	1

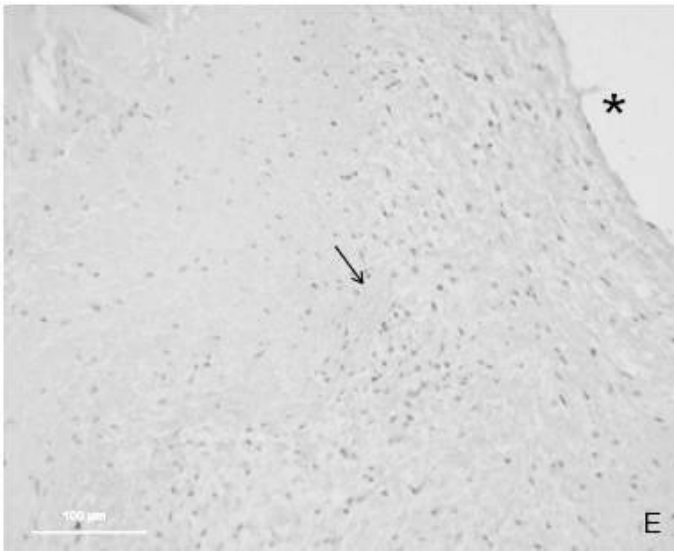


Figure 7.1 $CD4^+$ staining in RHD valve (E). * Fibrosa, arrow = $CD4^+$ staining in spongiosa

$CD8^+$

A total of 17 valves were stained for $CD8^+$ which was present in 88.2% (n=15) of RHD valves, ranging from 0-12 cells/mm^2. The total density of $CD8^+$ T-cells was 0.91 ± 1.30 cells/mm^2 in the rheumatic valves and 3±1.41 cells/mm^2 in control valves (p=0.84). $CD8^+$ was absent in 1 valve (5.9%). The localization of the $CD8^+$ was studied in 12 valves, which was found predominantly isolated in the spongiosa (33.3%, n=4) (Table 7.7).

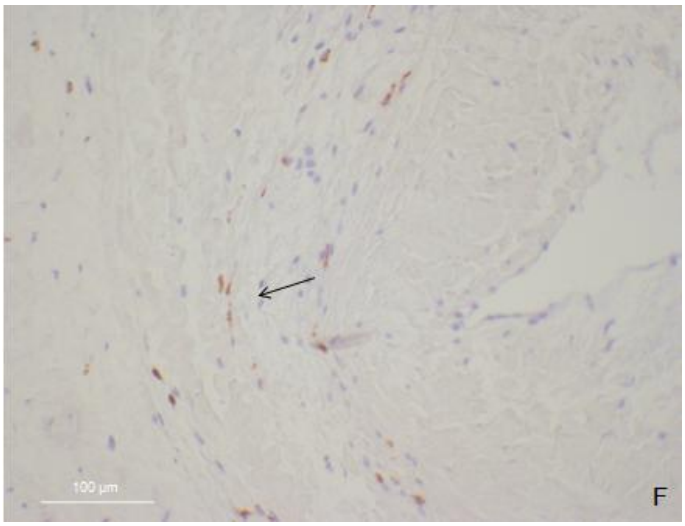


Figure 7.2 $CD8^+$ staining in RHD valve (E). arrow = $CD8^+$ staining in spongiosa

Table 7.7 Localisation of CD8+

	%	N
Ventricularis	16.7%	2
Spongiosa	91.7%	11
Fibrosa	58.4%	7
Localisation		
Ventricularis (only)	0%	0
Spongiosa (only)	33.3%	4
Fibrosa (only)	8.3%	1
Ventricularis + spongiosa	8.3%	1
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	8.3%	1
Spongiosa + fibrosa	41.7%	5

$CD45^+$

A total of 11 valves were stained for $CD45^+$ which was present in all (n=11) RHD valves, ranging from 0 to 49 cells/mm^2. The total density of $CD45^+$ T-cells was 2.29 ± 2.30 cells/mm^2 in the rheumatic valves and 14.0±9.97 cells/mm^2 in control valves (p=0.7). The localization of the $CD45^+$ was studied in 8 valves, which was found predominantly isolated in the spongiosa (37.5%, n=3) (Table 7.8).

Table 7.8 Localisation of $CD45^+$

	%	N
Ventricularis	50%	4
Spongiosa	100%	8
Fibrosa	37.5%	3
Localisation		
Ventricularis (alone)	0%	0
Spongiosa (alone)	37.5%	3
Fibrosa (alone)	0%	0
Ventricularis + spongiosa	25%	2
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	25%	2
Spongiosa + fibrosa	12.5%	1

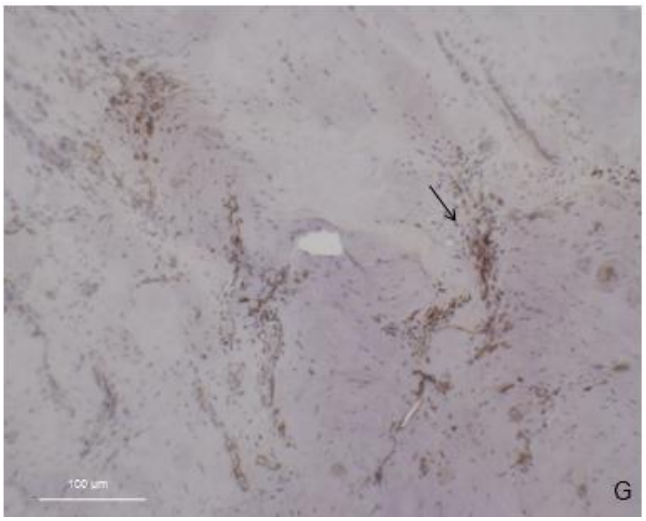


Figure 7.3 $CD45^+$ staining in RHD valve (G). arrow = $CD45^+$ staining in spongiosa

$CD68^+$

$CD68^+$ was present in 79.2% (n=19) of the 24 valves stained, ranging from 0 to 47 cells/mm^2. The total density of $CD68^+$ T-cells was 5.58 ± 4.05 cells/mm^2 in the rheumatic valves and 12.1±5.51 cells/mm^2 in control valves (p=0.4). The localization of the $CD68^+$ was studied in 10 valves, which was found predominantly isolated in the spongiosa (37.5%, n=3) (Table 7.9).

Table 7.9 Localisation of $CD45^+$

	%	N
Ventricularis	50%	4
Spongiosa	100%	8
Fibrosa	37.5%	3
Localisation		
Ventricularis (alone)	0%	0
Spongiosa (alone)	37.5%	3
Fibrosa (alone)	0%	0
Ventricularis + spongiosa	25%	4
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	25%	2
Spongiosa + fibrosa	12.5%	1

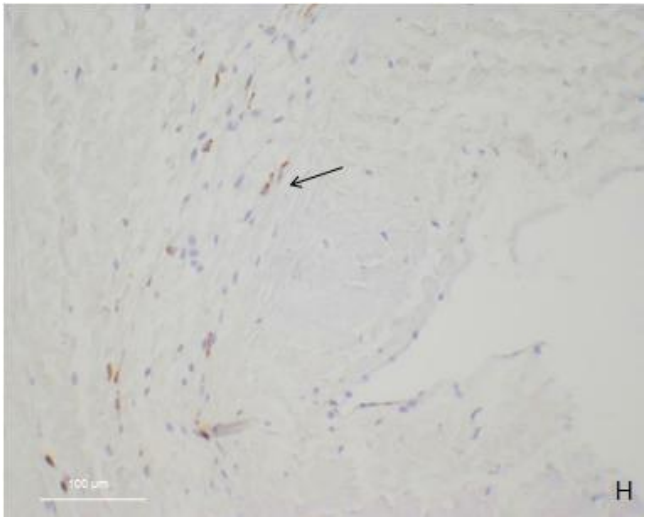


Figure 7.4 $CD68^+$ staining in RHD valve (H). arrow = CD68 staining in spongiosa

NFKB

A total of 7 valves were stained for NFKB. The total density of NFKB-positive cells was 20.5±9.80 cells/mm^2 in the rheumatic valves and 19.6±15.6 cells/mm^2 in control valves (p=0.92), as shown in Table 7.10.

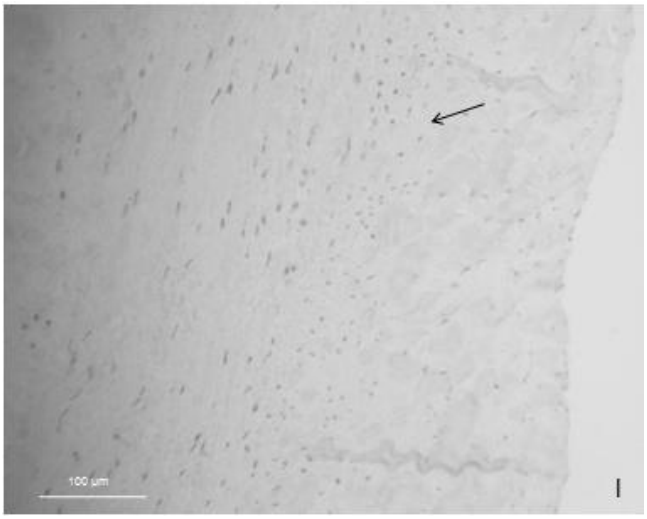


Figure 7.5 NFKB staining in RHD valve (I). arrow = NFKB staining

Table 7.10 Summary of the found results

Valve	CD 4	CD 8	CD 45	CD 68	NFKB
RHD	1.18±1.34	0.91±1.30	2.29 ±2.30	5.58 ±4.05	20.5±9.80
Controls	1.95±0.63	3±1.41	14.0±9.97	12.1±5.51	19.0±15.6
(p-value)	0.9	0.84	0.7	0.4	0.92

* results are presented as number of positive cells/field (MEAN±SD)

IL-1α

In the 20 valves stained IL-1α was present in 75% (n=15) with a total density of 2.56± 2.67 cells/mm^2 in the rheumatic valves valves. IL-1α was most found in the spongiosa (75%, n=15) (Table 7.11).

Table 7.11 Localization of IL-1α

	%	N
Ventricularis	35%	7
Spongiosa	75%	15
Fibrosa	50%	10
Localisation		
Ventricularis (only)	0%	0
Spongiosa (only)	26.7%	4
Fibrosa (only)	0%	0
Ventricularis + spongiosa	6.7%	1
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	40%	6
Spongiosa + fibrosa	26.7%	4

IL-1b

In the 20 valves stained IL-1b was present in 90% (n=18) with a total density of 2.70 ± 2.27 cells/mm^2 in the rheumatic valves. IL-1α was the most found in the spongiosa and fibrosa (75%, n=15) (Table 7.12).

Table 7.12 Localization of IL-1b

	% (N)
Ventricularis	65% (n=13)
Spongiosa	75% (n=15)
Fibrosa	75% (n=15)
Localisation	
Ventricularis (alone)	11% (n=2)
Spongiosa (alone)	0% (n=0)
Fibrosa (alone)	5.5% (n=1)
Ventricularis + spongiosa	5.5% (n=1)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	55.6% (n=10)
Spongiosa + fibrosa	22.2% (n=4)

IL-2

In the 20 valves stained IL-2 was present in all valves with a total density of 2.93 ± 2.85 cells/mm^2 in the rheumatic valves. IL-2 was the most prominent in the spongiosa (95%, n=19) (Table 7.13).

Table 7.13 Localisation of IL-2

	% (N)
Ventricularis	55% (n=11)
Spongiosa	95% (n=19)
Fibrosa	30% (n=6)
Localisation	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	20% (n=4)
Fibrosa (alone)	5% (n=1)
Ventricularis + spongiosa	10% (n=2)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	45% (n=9)
Spongiosa + fibrosa	20% (n=4)

IL-4

In the 20 valves stained IL-4 was present in 95% (n=19) with a total density of 1.67 ± 1.36 cells/mm^2 in the rheumatic valves. IL-4 was the most found in the spongiosa (95%, n=19) (Table 7.14).

Table 7.14 Localisation of IL-4

	% (N)
Ventricularis	50% (n=10)
Spongiosa	95% (n=19)
Fibrosa	55% (n=11)
Localisation	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	36.8% (n=7)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	0% (n=0)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	52.6% (n=10)
Spongiosa + fibrosa	10.5% (n=2)

IL-6

In the 20 valves stained IL-6 was present in all with a total density of 2.21 ± 2.43 cells/mm^2 in the rheumatic valves. IL-6 was the most found in the spongiosa (95%, n=19) (Table 7.15).

Table 7.15 Localisation of IL-6

	% (N)
Ventricularis	40% (n=8)
Spongiosa	95% (n=19)
Fibrosa	30% (n=6)
Localisation	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	47.4% (n=9)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	21.1% (n=4)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	21% (n=4)
Spongiosa + fibrosa	10.5% (n=2)

IL-8

In the 20 valves stained IL-8 was present in all with a total density of 1.89 ± 1.99 cells/mm^2 in the rheumatic valves. IL-8 was mostly found in the fibrosa (40%, n=8) (Table 7.16).

Table 7.16 Localisation of IL-8

	% (N)
Ventricularis	35% (n=7)
Spongiosa	25% (n=5)
Fibrosa	40% (n=8)
Localisation	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	50% (n=10)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	15% (n=3)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	20% (n=4)
Spongiosa + fibrosa	15% (n=3)

Table 7.17 Summary of the found cytokine results

Valve	IL-1α	IL-1b	IL-2	IL-4	IL-6	IL-8
RHD	2.56 ±2.67	2.70 ± 2.27	2.93 ±2.85	1.67 ±1.36	2.21± 2.43	1.89± 1.99

* results are presented as number of positive cells/field (MEAN±SD)

IL-10

In the 24 valves stained IL-10 was present in 96% (n=23) of the RHD valves with a total density of 4.46 ± 3.18 cells/mm^2 among the valves, ranging from 0 to 76 cells/mm^2 and 5.25±1.06 cells/mm^2 in control valves (p=0.5). In the 19 valves analyzed for localization IL-10 was mostly found in the spongiosa of the other valves (94.7%, n=18) (Table 7.18).

Table 7.18 Localisation of IL-10

	% (N)
Ventricularis	31.6% (n=6)
Spongiosa	94.7% (n=18)
Fibrosa	31.6% (n=6)
Localisation	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	61.1% (n=11)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	5.6% (n=1)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	27.8% (n=5)
Spongiosa + fibrosa	5.6% (n=1)

IL-12

In the 20 valves stained IL-12 was present in 96% (n=23) with a total density of 4.13 ± 3.32 cells/mm^2 among the RHD valves, ranging from 0 to 35 cells/mm^2 and 4.13±2.07 cells/mm^2 in control valves (p=0.42). In the 19 valves analyzed for localization IL-12 was mostly found in the spongiosa (85%, n=17) (Table 7.19).

Table 7.19 Localisation of IL-12

	% (N)
Ventricularis	60% (n=12)
Spongiosa	85% (n=17)
Fibrosa	45% (n=9)
Localisation	
Ventricularis (alone)	5.3% (n=1)
Spongiosa (alone)	26.3% (n=5)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	21.1% (n=4)
Ventricularis + fibrosa	5.3% (n=1)
Ventricularis + spongiosa + fibrosa	31.6% (n=6)
Spongiosa + fibrosa	10.5% (n=2)

Table 7.20 Summary of the found IL-10 and IL-12 results

Valve	**IL-10**	**IL-12**
RHD	4.46±3.18	4.13±3.32
Controls	5.25±1.06	4.13±2.07
(p-value)	0.5	0.42

* results are presented as number of positive cells/field (MEAN±SD)

7.3.2.2 Relation between inflammatory cells and valvular cytokines

The relation between the inflammatory cells and the valvular cytokines is shown in table 7.21.

Table 7.21 Relation between inflammatory cells and valvular cytokines

	Increase	
CD4^{+}	CD8+	(p=0.001)
	IL-4	(p=0.036)
CD45^{+}	IL-10	(p=0.039)
CD68^{+}	IL-6	(p=0.041)
VWF	CD4+	(p=0.011)
	CD8+	(p=0.006)
	CD 45+	(p=0.00)
	IL-4	(p=0.025)
	IL-12	(p=0.020)
IL-1b	IL-6	(p=0.045)
	IL-12	(p=0.035)
IL-2	IL-6	(p=0.010)
	IL-12	(p=0.010)
IL-6	IL-12	(p=0.010)
IL-8	IL-10	(p=0.018)
IL-12	IL-1b	(p=0.035)
	IL-2	(p=0.010)
	IL-4	(p=0.021)
	IL-6	(p=0.037)
	IL-10	(p=0.00)

7.4 Determinants of RF and RHD

7.4.1 Factors influencing immune activation in valvular tissue

The longer ago the RF attack, the higher the IL-6 (p=0.020) and a higher ASOT was related to a high IL-1α (p=0.010). Males tended to have a higher IL-4 expression in the valvular tissue than females (p=0.021) and individuals with a positive family history of RF tended to have higher valvular IL-4 levels (p=0.038). A high IL-8 was related to a higher lymphocyte count (p=0.018) and hemoglobin (p=0.014). No correlation was found between a positive history for RF, the use of penicillin and valvular CD and IL and ECM levels.

7.4.2 *The influence of inflammatory parameters on valvular affection*

A negative correlation was found between the platelet count and the presence of structural affection (p=0.010) and diagnosis of RHD (p=0.027) showing that individuals with structural and RHD features tended to have a lower platelet count.

No other correlation was found between the presence of structural, the functional affection and/or the diagnosis of RHD and the inflammatory parameters. No correlation was found between the inflammatory factors and the maintenance, development or regression of the features leading to the diagnosis of RHD during follow up.

There was no correlation between the inflammatory factors and the maintenance, development or regression of the structural features during follow up. Individuals with a lower ASOT level tended to remain free of structural affection of the valves during follow up (p=0.008).

The functional affection found during the first assessment tended to resolve in the individuals with a lower WBC (p=0.020). Strangely, individuals with a higher WBC tended to remain free of functional affection at the time of follow-up (p=0.003).

7.4.3 Serum cytokines

Individuals with structural valvular findings tended to have a higher level of serum TNF-α (0.025) while individuals with functional valvular findings tended to have a higher IL-6 serum level (p=0.046) and the higher the TNF-α level (p=0.012). In addition, idividuals with the diagnosis of RHD tended to have a higher TNF-α (p-0.003).

Table 7.22 Influence of serum cytokines on valve tissue immune activation

	Increase	
IL-1b	IL-4 valve	(p=0.029)
IL-8	IL-4 valve	(p=0.010)
IL-10	IL-12 valve	(p=0.011)

7.4.4 Valvular cytokines

An increase in elastin was correlated to an increase in $CD4^+$ (p=0.001) and $CD8^+$ (p=0.028).

No correlation was found between the amount of valvular expression (CD, IL and ECM) and thickening of the leaflet, calcification, mitral regurgitation and aortic stenosis. Affection of the subvalvular apparatus was correlated with higher levels of valvular VWF (p=0.025)

7.5 Discussion

Our study population had a mean of 35 years and a normal gender distribution, which is fair when aiming to avoid valvular mechanisms other than RHD as degenerative, aging or gender dependent factors.

In our series, $CD4^+$ and $CD45^+$ were found in all RHD valves while $CD8^+$ was present in 88.2% of the valves. The presence of $CD4^+$ and $CD8^+$ T-cells in valvular tissue has been demonstrated (28,29) and chronic inflammatory cell infiltration with $CD4^+$ T-cells has been observed in both calcified and non calcified mitral valves from RHD patients. The highest number of both $CD4^+$ and $CD8^+$ T-cells has been reported in patients presenting with rheumatic activity which indicates expansions of auto reactive T-cells in the heart during the acute episode and decrease in auto reactive T-cells numbers after the acute episode remaining important to maintain the heart lesions. (Sampaio 2007) Furthermore, the high frequency and the persistence of T-cell oligoclonal expansions in the damaged heart valves seems to be associated with the progression of the disease (Guilherme 2001), probably due to the T-cell recognition of several heart tissue proteins exposed by local lesions (Figueroa 2002). This indicates that both T-helper/inducer and T cytotoxic/ suppressor lymphocytes are involved in the development of lesions in rheumatic valvular inflammatory heart disease. Nevertheless, the presence of activated inflammatory cells

indicates ongoing inflammatory reaction, with an almost certain interaction with the overlying endothelium. Pathologically increased activated lymphocytic infiltrates in association with an enhanced expression of adhesion molecules can be regarded as a clear cut indication of a persisting chronic inflammatory process.

The predominance of $CD4^+$ T-cells and macrophages at the site of heart lesions in RHD (Raizada 1985, Kemeny 1989) is apparently caused by the fact that adhesion molecules such as VCAM-1 facilitate the extravasation of $CD4^+$ and $CD8^+$ T-cells into the valves, after being up-regulated on the vascular endothelium of the valve of ARF patients (Guilherme 1995), suggesting a direct role for these cells in the pathogenesis of RHD (Raizada 1985, Kemeny 1989). Therefore, it is believed that $CD4^+$ T-lymphocytes are the major effectors of heart lesions and display a degenerate pattern of antigen recognition (Guilherme 2004[2]). Predominance of macrophages has also been demonstrated in immunohistochemical studies (Raizada 1985, Kemeny 1989) and $CD8^+$ and $CD68^+$, glycoproteins expressed on monocytes/macrophages, were found in 88.2% and 79.2% of the RHD valves analyzed in our series, respectively.

It is known that Th1 cytokines play an important role in certain organ-specific autoimmune diseases (Libiau 1995, Falcone 1999) and seem also to be predominant in heart lesions, especially valvular lesions (Guilherme 2004[2]) In our series IL-2, IL-6 and IL-8 was found in all RHD valves while IL-1α was found in 75% of the RHD valves. IL-1b, IL-4, IL-10 and IL-12 were found in over 90-95% of the RHD valves confirming a major role of inflammatory cytokines in rheumatic valvular lesions.

An increased expression of IL-1b has been demonstrated in stenotic aortic valves in association with an infiltrate of leukocytes. In contrast, control valves have been reported to show only scattered leukocytes and no staining for IL-1b. IL-1 is expressed mainly by leukocytes, but can also be seen in local myofibroblasts, fibroblasts (Fini 1994, Siwik 2000), and smooth muscle cells (Galis 1994, Galis 1995) and belongs to a family of cytokines that play an important role in the regulation of acute inflammation. The expression of IL-1b in leukocytes and myofibroblasts in stenotic valves suggests an activation of these cells. Thus, activated leukocytes may, by secretion of IL-1, promote an inflammatory microenvironment in stenotic aortic valves that, in turn, leads to activation of local interstitial cells. Therefore, IL-1 is believed to be a

prototypical pro-inflammatory cytokine (Dinarello 1984, Dinarello 1996, Bazan 1996) and might be associated with RHD.

Furthermore, the production of IL-1, TNFa and IL-2 has been correlated with progression of the Aschoff nodules in heart lesions during the acute phase of RHD, localized mainly in the endocardium, subendocardium or perivascular regions of the myocardial interstitium (Fraser 1997). Moreover, an increased expression of the interleukin-2 receptor has been shown on leukocytes in stenotic aortic valves (Olsson 1994).

In our series IL-4 was found in 95% of the fragments analyzed. This is surprising as earlier reports of sparse production of the regulatory cytokine IL-4 by valve-infiltrating intralesional mononuclear cells (under 10% IL-4-positive cells) in the majority of valve fragments of both acute RF and chronic RHD patients, in combination with large numbers of IL-4-positive cells (over 50%) in the myocardium fragments analyzed suggest that low numbers of IL-4-producing cells in the valvular tissue might contribute to the progression of valvular RHD lesions (Guilherme 2004[2], Guilherme 2005[3]). However, in our series IL-4 showed the lowest amount of positive cells among the tested cytokines. Importantly, it is believed that RHD lesions are mediated by inflammatory cytokines with low numbers of IL-4 producing cells in the valvular tissue contributing to the development and progression of valvular tissue damage in RHD, and perpetuating or exacerbating the production of inflammatory cytokines (TNF-α and IFN-γ) leading to permanent damage in the valves (Guilherme 2005[3], Guilherme 2004[2], Guilherme 2004).

IL-10 and IL-12 positive cells were the most prominent in our series in concordance to earlier reports that show that IL-10 positive cells, a predominantly Th-2 regulatory cytokine, are consistently predominant in both myocardium and valvular tissue (Guilherme 2005-3, Guilherme 2004-2, Guilherme 2004) suggesting that despite the presence of Th2 cells, they are probably not sufficient to down-regulate the deleterious effect of Th-1 cytokines, predominant mainly in the valves (Guilherme 2004[2]).

Normally, no infiltration is expected in normal valve tissue. However, in our series the control valves showed infiltration of all cells analyzed. This might be due to the fact that both valves were from individuals with 56 and 62 years of age, and that these valves could have been in a degenerative process, which might be caused by identical mechanisms as valvular damage due to

accelerated damage in RHD. It is very difficult to collect healthy human valvular tissue for analysis, as most of the collected tissue might be affected by other mechanisms which might be similar to the end result of RHD.

When compared to normal valves, the RHD valves showed a lower expression of all cells except NFKB and an equal expression of IL-12, although with a bigger SD, showing that the amount of IL-12 positive cells varies in the different valves. The biggest difference was found in the amount of $CD8^+$, $CD45^+$ and $CD68^+$, although, there was no significant correlation between the control and the RHD valves.

Mediators of valvular affection

A lower ASOT level at the time of assessment is related to absence of structural lesions which might suggest that high ASOT levels might be indicators or risk factors for the development of structural affection. Diagnosis of RHD was related to a lower platelet count, while high IL-6 levels were related to functional lesions. Importantly, structural, functional and RHD features were related to high TNF-α levels while a lower WBC was an indicator for reversibility of the functional affection.

All cytokines were present in the majority of the valves, however, the presence and grade of staining was increased for IL-10 and IL-12. IL-4 was found in the majority of the valves, however, it showed the lowest grade of staining. The presented results suggest that an increase in IL-10 and IL-12 are the major regulatory cytokines in mediating valvular damage, while an increase of IL-2, IL-6 and IL-8 might also contribute to the pathogenesis. Importantly, till date IL-6 and IL-12 had not been reported to be elevated in RF and RHD. IL-1α was found only in 75% of the valves, however, it showed an increase grade in staining, suggesting its contribution to valvular lesions. Importantly, IL-4 was present in the majority of the valves, however, in a lower amount compared to the other cytokines, suggesting that a decrease in IL-4 contributed to the valvular affection. An increase in serum IL-10 and a decrease in IL-1b and IL-8 seem to be indicators for the development of valvular lesions, as they seem to be related to an increase of valvular IL-12 and decrease of IL-4. Moreover, high lymphocyte counts and ASOT indicate an increase of IL-8 and IL-1α, respectively.

Variables that seem to correlate with valvular lesions include older age at the time of the RF attack and female gender which seem be related to an increase in valvular IL-6 and decrease of IL-4. However, younger age at the time of screening, which is related to a younger age at the time of the first RF attack was related to a decrease of haemal IL-1b, leading to decrease of valvular IL-4, which might be a negative prognostic indicator.

7.6 Conclusions

In Egypt, lower ASOT and WBC levels seem to suggest a lower level of infection while high TNF-α and IL-6 levels seem to be an indicator of disease. Furthermore, an increase in serum IL-10 and decrease in IL-1b and IL-8 seem to be indicators for the development of valvular lesions.

In our series, CD4+, CD8+ and CD45+ were found in the majority of valves, which seems to be associated with the progression of the disease (Guilherme 2001, Figueroa 2002) with IL-10 and IL-12 being the major regulatory cytokines in mediating valvular damage, while IL-2, IL-6 and IL-8 might also contribute to the pathogenesis. Therefore, it is believed that T lymphocytes are the major effectors of heart lesions and display a degenerate pattern of antigen recognition (Guilherme 2004[2]). Nevertheless, the presence of activated inflammatory cells indicates ongoing inflammatory reaction suggesting that an ongoing immunological reaction is still present in the apparently quiescent phase of the disease, which might mediate the valvular affection seen in RHD.

$CD4^+$ T-cells are suggested the major effectors of the heart tissue, leading to RHD lesions in Egypt as Streptococcal and host antigen cross-reactive antibodies facilitate heart tissue infiltration by T-lymphocytes. Therefore, inflammatory cytokines such as, TNF-α and others seem to be mediators of valve lesions and the decrease of IL-4, a regulatory cytokine, seems to be related to the pathogenesis valve lesions in RHD patients.

The presented results illustrate earlier observations describing how the predominant Th1-type cytokine produced mainly by $CD4^+$ T-cells infiltrating valve tissue could mediate the severe RHD valve lesions, with the pattern of cytokine production in the heart lesions favouring a Th1-mediated disease.

The raised WBC and lymphocyte count found in our study, nevertheless the use of penicillin prophylaxis, are signs of ongoing infection, common seen in ARF. Furthermore, elevated ESR

and CRP levels suggest maintenance of acute phase inflammation. However, the elevated inflammatory parameters may be related to the short interval since the RF attack or the presence of recurrences. In Egypt, a low platelet count and high ASOT seem to be risk factors for cardiac affection while reversibility of functional lesions is related to a lower WBC. Importantly, penicillin prophylaxis results in a lower WBC and CRP and a higher platelet count, showing the importance of use and compliance to the regimen.

Based on the presented results that show immune activation there is evidence of the presence of an ongoing immunological reaction in an apparently quiescent phase of the disease in Egypt. The presence of several proinflammatory cytokines in peripheral blood as well as valvular tissue of RF and RHD patients enforces the hypothesis of a major role of inflammatory cytokines in mediating heart lesions earlier suggested. Importantly, disease seems more severe in females and in those who acquire RF at an older age and is related to non-compliance to the penicillin prophylaxis.

CHAPTER 8

STRUCTURAL CHARACTERISTICS OF RHD

8.1 Background

RHD valves have shown altered architecture and histology **(**Sampaio 2007), which seem to lead to distortion of valve function and mechanics. We hypothesized that the inflammation process seen in RHD changes the valvular histology and architecture, affecting the location, features and characteristics of the different areas of the valve. The aim was to analyze the structural characteristiscs of RHD valves from Egypt to determine the changes caused by the disease and the related determinants of RHD.

8.2 Patients and methods

Individuals admitted at the Cardio-thoracic department of the NHI, Imbaba, Cairo and scheduled for valve replacement due to RHD were recruited as described in chapter 3, pg. 75.

Sample collection

Excised valves from the individuals recruited were collected during the procedures. The valvular tissue was stored in formalin directly after collection and then embedded in paraffin as described in chapter 3, pg. 89. The samples were transported to the HSC for histology and immunohistochemistry analysis.

The condition of the normal valves is expected to be the same and there was no significant difference in the results found. We have therefore reported the combined results for an easier overview and comparison of the results. The reason that we have only used 2 control valves is due the fact that it is very difficult to collect healthy human valvular tissue.

Histology Analysis

Staining was graded as mild, moderate and intense depending on intensity of the staining.

Grading:

Mild (1+) - Weak staining.

Moderate (2+) – Moderate expression and staining of the fragment

Intense (3+) – Intense staining of the fragment

Van Gieson staining: Sections were dewaxed in xylene and immersed in 100% alcohol and stained with Miller's Elastin (VWR) for 2 hours. This was followed by differentiation in 90% methanol and washing in tap water and distilled water. The sections were incubated with Van Gieson for 5 minutes and then rehydrated rapidly and mounted with DPX.

Alcian Blue/ Sirius red staining: Sections were dewaxed and rehydrated in water and then the nuclei were stained with Weigests Haematoxylin A + B for 10 minutes. This was followed by differentiation in Acid Alcohol, washing in distilled water, staining with Alcian Blue 0.5% for 15-20 minutes then washing with distilled water. The sections were then stained with phosphomolybdic acid 1% acid for 20 minutes, washed with distilled water, then stained with Sirius Red for 1 hour. The sections were dehydrated with alcohol and mounted with DPX.

Statistical analysis

The statistical analysis was performed as described in chapter 3, pg 100.

8.3 Results

Human Rheumatic Valves and Control Valves

Valve tissues were collected at the time of surgery. We obtained 24 human rheumatic valves from patients (n=24, mean age, 35.42 years, range, 14 to 63 years, male, 13, female, 11) at the time of surgical valve replacement. We compared them to 2 control valve tissue (n=2, gender: male, age:, 56 and 62 years) obtained by autopsy.

Calcification

Macroscopic inspection of the valve fragments after collection revealed calcification in 80.2% (n=19) of the valves.

Neovascularisation

Haematoxylin stained sections showed neovascularisation in 71.4% (n=17) of the RHD valves as VWF stains endothelial cells in the blood vessels showing vascularisation in valves.

Histology staining of the Valves for ECM proteins

Elastin fibres and sulphated GAG distribution

A total of 19 valves were stained for ECM proteins. The Elastin Van Gieson staining showed that there was an increase of elastic fibres in the RHD valves. Furthermore, elastic fibres appeared to be fragmented and were distributed throughout the leaflet (84.2%, n=16). In the normal leaflet elastin fibres was localized mostly in the ventricularis (Figure 8.1). In the RHD valves, elastin fibres was also mostly found in the ventricularis in 14 of the 19 valves studied, however was present in all areas (ventricularis, spongiosa and fibrosa) in one of the valves.

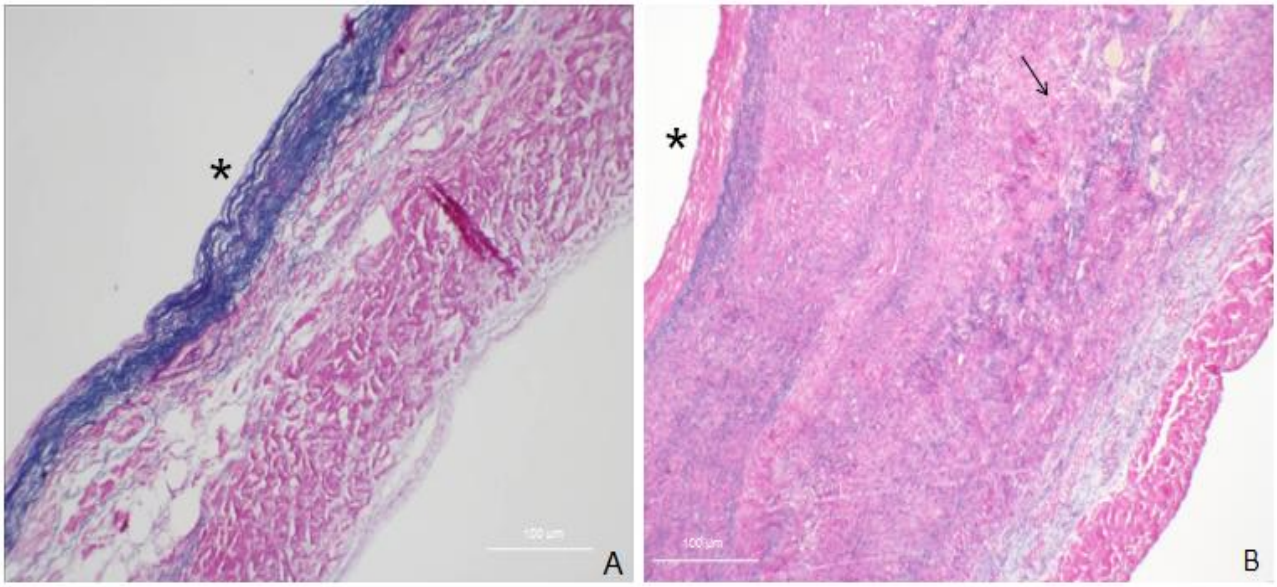


Figure 8.1 Elastin van Gieson staining in control valve (A) and RHD valve (B), respectively. (dark purple= elastin fibres, pink= collagen) The elastin fibres seem to be fragmented and distributed throughout the whole leaflet in the RHD valve while it is localized mostly in ventricularis in the control valve. * Ventricularis, arrow = fragmented elastin

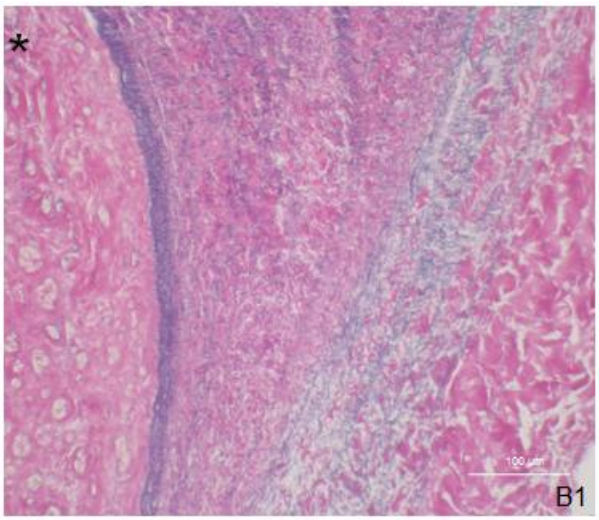


Figure 8.2 Magnification of elastin staining in RHD valve (B1)

Table 8.1 Strength and distribution of Elastin Van Gieson staining

	%	N
Score for staining strength		
Weak	5.3%	1
Intermediate	15.8%	3
Intense	78.9%	15
Distribution		
Ventricularis	73.7%	14
Spongiosa	63.2%	12
Fibrosa	10.5%	2
Fragmented	84.2%	16

Table 8.2 Localisation of elastin fibres

Localisation		**N**
Ventricularis (only)	31.6%	6
Spongiosa (only)	26.3%	5
Fibrosa (only)	0%	6
Ventricularis + spongiosa	31.6%	1
Ventricularis + fibrosa	5.3%	1
Ventricularis + spongiosa + fibrosa	5.3%	1
Spongiosa + fibrosa	0%	0

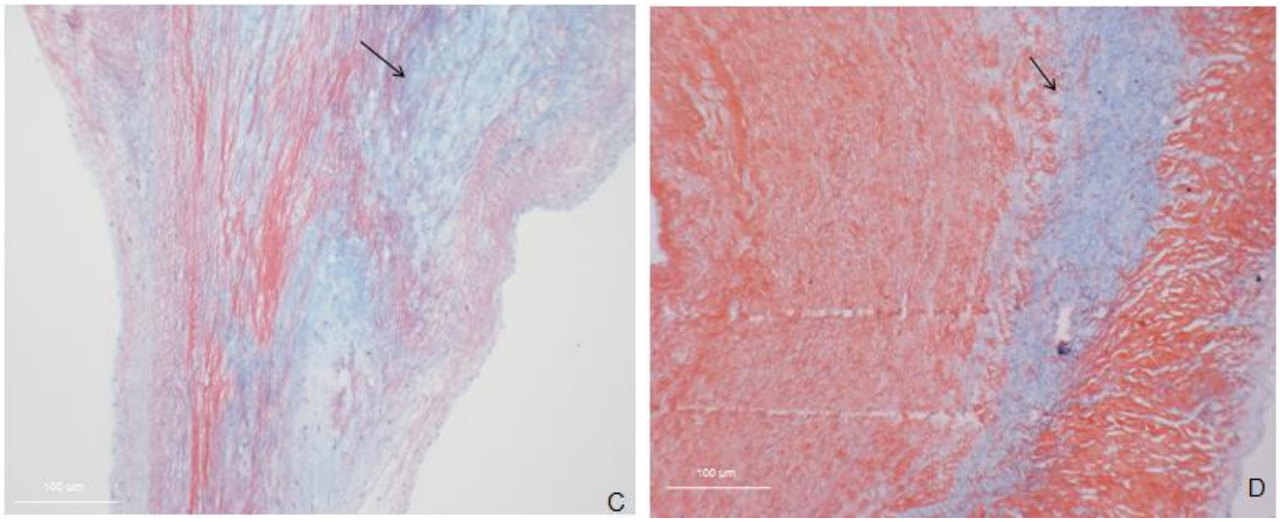


Figure 8.3 Alcian Blue staining in control (C) and RHD valve (D), respectively.

GAGs

Alcian blue staining in a total of 19 valves demonstrated that in the RHD valves leaflets sulphated GAG distribution and the amount was comparable to normal valves. GAGs were the most prominent the spongiosa (84.2%, n=16), followed by the fibrosa of 10.5% (n=2) valves and the least in the ventricularis (5.3%, n=1).

Table 8.3 Strength and distribution of GAGs

	%	N
Score for staining strength		
Weak	26.3%	5
Intermediate	26.3%	5
Intense	47.4%	9
Distribution		
Ventricularis	5.3%	1
Spongiosa	84.2%	16
Fibrosa	10.5%	2

VWF

A total of 7 valves were stained for VWF which was present in all (n=7) RHD valves with a total density of 3.01±3.77 cells/mm^2 among the valves. VWF was the most prominent in the spongiosa (100%, n=7) (Table 8.4).

Table 8.4 Strength and distribution of VWF

	%	N
Ventricularis	85.7%	6
Spongiosa	100%	7
Fibrosa	42.9%	3
Localisation		
Ventricularis (only)	0%	0
Spongiosa (only)	0%	0
Fibrosa (only)	0%	0
Ventricularis + spongiosa	57.1%	4
Ventricularis + fibrosa	0%	0
Ventricularis + spongiosa + fibrosa	28.6%	2
Spongiosa + fibrosa	14.3%	1

Staining of the Valves for Collagen

Collagen types I, III

Immunohistochemistry studies on Collagen types I and III show that collagen I was increased in the coapting edge and predominantly found in the spongiosa. Collagen III was mostly found in the spongiosa and ventricularis of the RHD valves.

Collagen I

Collagen I was present in the spongiosa of all valves stained (n=20), and in the ventricularis of 63.2% (n=12) and the fibrosa of 57.9% (n=11) of the valves. Most of the collagen I was fragmented (94.7%, n=18) and shifted from its natural location (89.5%, n=17). Furthermore, the total amount of collagen I was decreased in 84.2% (n=16) of the valves compared to normal valves.

Table 8.5 Strength and distribution of collagen I

	% (N)
Score of staining strength	
weak	47.4% (n=9)
Intermediate	26.3% (n=5)
Distribution	
Ventricularis	63.2% (n=12)
Spongiosa	100% (n=19)
Fibrosa	57.9% (n=11)
Fragmented	94.7% (n=18)
Distribution	
Normal	10.5% (n=2)
Shifted	89.5% (n=17)
Amount	
Normal	15.8% (n=3)
Increased	0% (n=0)
Decreased	84.2% (n=16)

Table 8.6 Localization of collagen I

Localization	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	26.3% (n=5)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	15.8% (n=3)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	47.4% (n=9)
Spongiosa + fibrosa	10.5% (n=2)

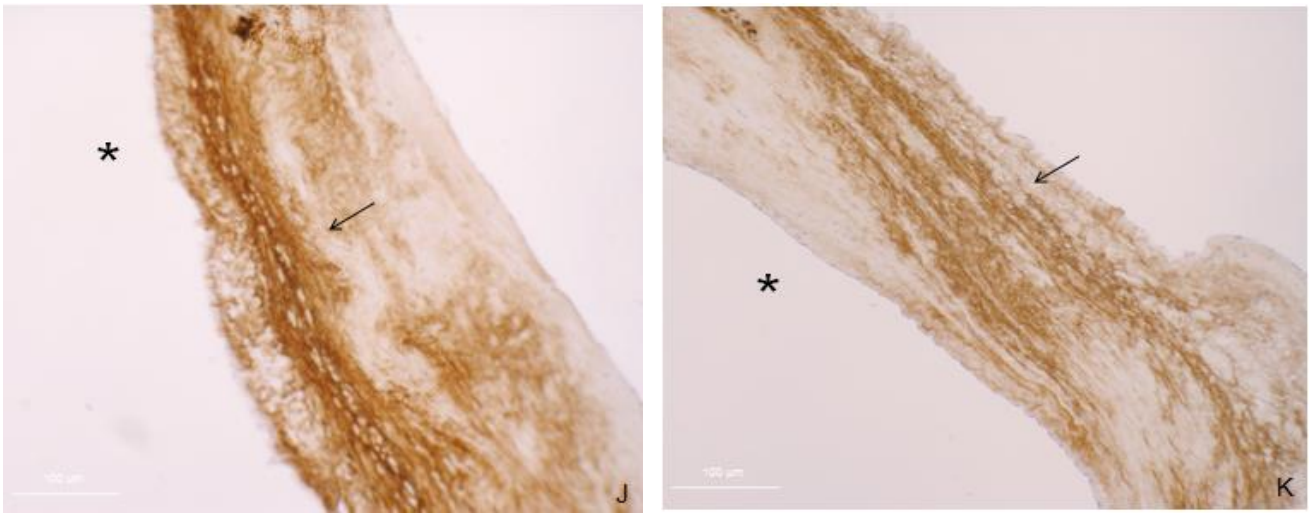


Figure 8.4 Collagen I staining in control valve (J) and RHD valve (K), respectively. * Ventricularis, arrow = collagen I

Collagen III

Collagen III was present in the spongiosa and ventricularis of all valves stained (n=20), and the fibrosa of almost all valves (94.7%, n=18). Collagen III was present in the ventricularis, spongiosa and fibrosa in 94.7% (n=18) of the valves. All collagen III present in the valves was fragmented (100%, n=19) and most was shifted from its natural location (84.2%, n=16). The amount of collagen III was normal in most valves (63.2%, n=12) compared to normal valves.

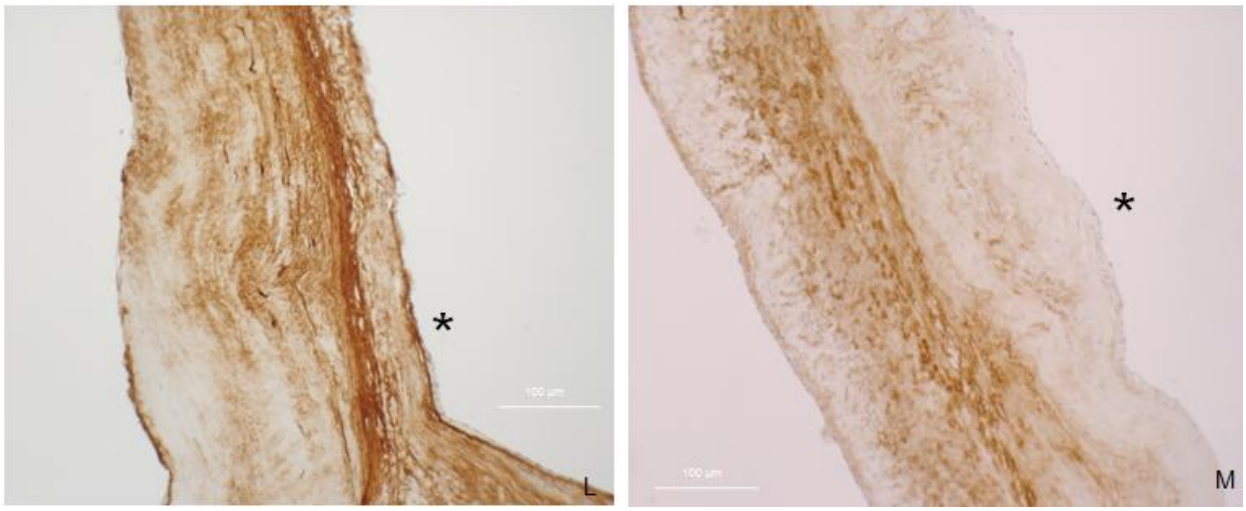


Figure 8.5 Collagen III staining in control valve (L) with collagen mainly in the fibrosa and RHD valve (M) where the staining seems to be shifted to the middle, respectively. * fibrosa

Table 8.7 Strength and distribution of collagen III

	% (N)
Score	
weak	0% (n=0)
Intermediate	15.8 (n=3)
intense	84.2% (n=16)
Distribution	
Ventricularis	100% (n=19)
Spongiosa	100% (n=19)
Fibrosa	94.7% (n=18)
Fragmented	100% (n=19)
Distribution	
Normal	15.8% (n=3)
Shifted	84.2% (n=16)
Amount	
Normal	63.2% (n=12)
Increased	31.6% (n=6)
Decreased	5.3% (n=1)

Table 8.8 Localization of collagen III

Localization	
Ventricularis (alone)	0% (n=0)
Spongiosa (alone)	0% (n=0)
Fibrosa (alone)	0% (n=0)
Ventricularis + spongiosa	5.3% (n=1)
Ventricularis + fibrosa	0% (n=0)
Ventricularis + spongiosa + fibrosa	94.7% (n=18)
Spongiosa + fibrosa	0% (n=0)

8.4 Discussion

In our series the stained sections of the diseased valves showed distortion of normal architecture. However, this was not surprising as RHD valves have shown altered architecture and histology, including fibrosis, calcification and neovascularization **(**Sampaio 2007) as well as changes in the collagen, elastin and GAGs distribution and localization, which seem to lead to distortion of valve function and mechanics.

The amount of elastic fibres found was increased and predominantly fragmented, and could be found throughout the whole leaflet while in normal valves it was found in the ventricularis (Scott 1995). However, elastic fibers have been reported to be increased with age (Gallegos 2005, Bashey 1967) and in pathological valves (Imayama 1989). Therefore, the found results might suggest that the pathology RHD affects the elastic fibres in the valve, accelerating the mechanism of aging in the valve. VWF was found in all RHD valves, but predominantly in the spongiosa and not lining the ventricularis as expected.

Only the GAG distribution and amount was comparable to normal valves, which together with water form the main components of the spongiosa (Scott 1995). This is not as we had expected as the composition and amount of GAGs is known to vary based on the type of mechanical loading (Grande-Allen 2004) as seen in valve affection, and the fact that altered GAG composition has been shown to play an important role in several heart valve diseases. However, our findings might be due the fact that GAG composition and amount might not be affected by inflammatory mechanisms associated with RHD.

Collagen I was found mainly fragmented and shifted to the spongiosa, although present in all areas of the leaflets and Collagen III was present in normal amounts but was fragmented and shifted predominantly to the spongiosa of the RHD valves. This is surprising as collagen with only a small amount of elastin is primarily found in fibrosa (Clark 1974). Importantly, the collagen in the fibrosa is more organized than in the ventricularis. However, damage to the elastin network alters the mechanics and accelerates mechanical degeneration, enforced by the

additional effects of fragmentation of elastin and reconformation and slippage of collagen fibers (Viidik 1982) which might explain the distorted location of the collagen fibers.

The presented results showing changes in elastin and collagen amount, localization and distribution suggest that changes in valve mechanics in RHD might be due to the changes in the elastin and elastic fibres in combination with changes in valvular collagen as the valve function and mechanics rely on an interaction between elastin and collagen conditions and changes of which the folded shape of the valve is dependent. Importantly, the function of elastin is highly dependent on its configuration and relative content in the valve and specially its organization relative to collagen. Furthermore, valvular elastin seems to act as a 'housekeeper' that restores the collagen fibre geometry back to its original conformation between successive loading cycles, and is therefore critical to proper valve function (Vesely 1998) which shows how changes in elastin can affect the collagen fibres and therefore the valvular function, leading to damage of the valve.

Collagen I has been found to be decreased in aortic stenosis compared to normal aortic valves, despite a two- to three-fold increase in type I procollagen synthesis indicating an increased turnover of type I collagen in aortic stenosis, with degradation exceeding the synthesis. Importantly, this finding seemed to be more predominant around calcified nodules. However, the amount of collagen III did not show changes and the proportions of type I and type III collagens remained preserved. These results could implicate that RHD influences the decrease in collagen I as found in our series leading to the calcification and the resulting valvular damage (Eriksen 2006).

Features like fibrosis and neovascularization have been observed in most of RHD valves, indicating that the valve has gone through previous episodes of rheumatic activity and it is believed that the degree of fibrosis is also proportional to the number of acute episodes (Sampaio 2007). In our series, neovascularization was seen in 71.4% of the RHD valves, confirming the earlier suspicions. Calcification is a feature that suggests chronic valvular involvement (Sampaio 2007). Furthermore, calcification in diseased/distorted valves in RHD as recent studies indicate

that calcification is not merely an inactive, "dystrophic" process but involves a regulated inflammatory process associated with expression of osteoblast markers and neoangiogenesis (Chopra 2007).

The composition of the mitral and aortic valves is very similar, being composed mostly of collagen and GAGs with elastin constituting a much smaller fraction of valve substance (Gross 1931, McMillan 1964, Sell 1965). However, alterations in valve tissue composition start to differ between the mitral and aortic valves as the valves age. The proportion of constituent GAGs and elastin increases significantly in the mitral valve between the third and sixth decades of life, while there is a concomitant decrease in the proportion of collagen. Surprisingly, the GAG content in aortic valves does not change significantly with advancing patient age.

Collagen fibers appear thicker and more disoriented with time in the mitral valve while in the aortic valve aging shows that modest decrease in relative collagen content is accompanied by a dramatic increase in elastin (McDonald 2002). The similarity between the mitral and aortic valves suggests that the mechanisms affecting the valves in RHD might be equal, leading to similar pathology.

8.5 Conclusion

Distortion of normal architecture was found in the majority of the studied valves as expected in RHD which is characterized by altered architecture and histology as well as composition. Features like fibrosis and neovascularization have been suggested to be the result of inflammatory process and rheumatic activity. The increase of elastic fibres suggests that the pathology RHD affects the elastic fibres in the valve, accelerating the mechanism of aging in the valve. Nevertheless, changes in the collagen, elastin and GAGs distribution and localization, seem to lead to distortion of valve function and mechanics as these features rely on an interaction between elastin and collagen conditions. Moreover, the function of elastin is highly dependent on its configuration and relative content in the valve and specially its organization relative to collagen. Therefore, fragmentation and dislocation of the majority of valvular components alters the valve mechanics and accelerates mechanical degeneration. The presented results also show that the mechanisms related to RHD differ from degenerative lesions, being more dependent of collagen conditions and minerals rather than cholesterol.

CHAPTER 9

STREPTOCOCCAL SENSITIVITY TO ANTIBIOTICS

9.1 Background

Penicillin remains the drug of choice for treatment of streptococcal infections, and ever since penicillin was first introduced all strains have remained exquisitely sensitive to this antibiotic. (Betriu 1989, Brook 1984, Kim 1985) However, in recent years, increasing rates of macrolide resistance have been described in various regions of the world (Michos 2009, Bingen 2004, Green 2006, Perez-Trallero 2007) and it is believed that changes in the prescription pattern of antibiotics or macrolide consumption could theoretically have an impact on the macrolide resistance rates (Bergman 2004, Fujita 1994).

The resistance for Erythromycin, which is currently recommended as an alternative antibiotic for treatment of GAS infections in patients allergic to B-lactams or in cases of penicillin failure (Lowbury 1959, Bisno 1996, Maruyama 1979, Zackrisson 1988, Trallero 1989, Linares 1992, Hsueh 1995, Wu 1997, Cornaglia 1998) has been reported to reach up to 100% (Liu 2009). Furthermore, resistance to clarithromycin, azithromycin, and clindamycin has been detected with resistance rates ranging up to 44.8%, 19.2% and 23.2%, respectively (Kim 1985). Therefore, the increase in macrolide resistance among streptococci is a concern, as they are the common regimen for the treatment of streptococcal infections.

The aim of this study was to determine the antimicrobial susceptibilities of B-hemolytic strains cultured from throat swabs of individuals with history of RF or RHD and evaluate the effectiveness of penicillin prophylaxis in these cases. It is also important to identify the current antibiotic sensitivity and resistance rate in our region for the best treatment regimen for streptococcal infections.

9.2 Patients and methods

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI were recruited as described in chapter 3, pg. 75.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement

-Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD

- Group II: Patients admitted for RHD and scheduled for valve replacement.

Sample collection

A throat swab was taken and streaked immediately across a blood agar plate for culturing of the organism as described in chapter 3, pg. 90. The B-hemolytic strains cultured from the throat swabs were analyzed for the antibiotic sensitivity.

Culturing

- The swab was streaked immediately after collection across a blood agar plate
- The blood agar plate was allowed to incubate at (35°–37°C) for 24–48 hours to allow the growth of bacteria
- After incubation the colonies on the agar plates were analyzed
- Hemolytic colonies were identified

Sensitivity testing: Susceptibility testing is used to determine which antimicrobial inhibits the growth of the bacteria causing the infection. If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition). The size of the zone and the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. The results from this test helps determine which antibiotic is the most effective in treating the infection.

- Antibiotics tested:
  - Levofloxacin 5 mcg (BioAnalyse)
  - Erythromycin 15 mcg (BioAnalyse)
  - Vancomycin 30 mcg (BioAnalyse)
  - Ciprofloxacin 5 mcg (BioAnalyse)
  - Gentamycin 10 mcg (BioAnalyse)
  - Doxycicline 30 mcg (BioAnalyse)

- Penicillin 10U (BioAnalyse)
- Bacitracin 10U (BioAnalyse)
- Taxo- differentiation discs (Benex Limited)

Detailed steps:

- Samples of the identified colonies were re-streaked on antibiotic agar plates
- Antibiotic discs were placed on the agar plate
- After incubation the colonies on the agar plates were analyzed for the growth pattern
- The antibiotic sensitivity was analyzed

Statistical analysis

The statistical analysis was performed as described in chapter 3, pg 95.

9.3 Results

From the total of 309 throat swabs collected, 16.18% (n=50) showed growth of B-hemolytic organisms. All cultures were sensitive to Ciprofloxacin and Levofloxacin. Three (6%) cultures were intermediately sensitive while 11 (22%) were resistant to Vancomycin. One (2%) culture was intermediately sensitive while 2 (4%) were resistant to Penicillin. Five (10%) cultures were intermediately sensitive while 14 (28%) were resistant to Gentamycin. Two (4%) cultures were intermediately sensitive while 4 (8%) were resistant to Erythromycin. One (2%) culture was intermediately sensitive while 1 (2%) were resistant to Doxycycline.

One (2%) culture was intermediately sensitive for Erythromycin and resistant to Gentamycin, while 1 (2%) culture was additionally resistant to Vancomycin. Two (4%) cultures were resistant to Vancomycin and Gentamycin. Resistance to Gentamycin and Erythromycin was seen in 1 (2%) culture, while another culture was intermediately sensitive to Vancomycin. One (2%) culture was resistant to Vancomycin, Penicillin and Gentamycin.

Determinants of antibiotic sensitivity

Females tended to show higher resistance rates to Vancomycin, Gentamycin and Doxycycline, while males showed higher resistance to erythromycin (Table 9.1). Younger patients tended to show more often resistance to erythromycin (p=0.045), and although not significantly correlated, older patients tended to show higher rates of resistance to Vancomycin (p=0.080).

History of RF and the presence of recurrences was related to higher resistance rates for all antibiotics and individuals on penicillin prophylaxis showed more often resistance to the tested antibiotics (Figure 9.2). A shorter time since the first RF attack was related to an increase in resistance to Gentamycin. No relation was found between the penicillin schedule and compliance and the resistance profile for the tested antibiotics. However, no significant correlation was found between the gender, history RF, recurrence, penicillin prophylaxis and the resistance to antibiotic.

Table 9.1 (1) Distribution of antibiotic resistance

	Vancomycin		**Penicillin**		**Gentamycin**	
	Intermediate	Resistant	Intermediate	Resistant	Intermediate	Resistant
Male	2% (n=1)	8% (n=4)	0% (n=0)	2% (n=1)	4% (n=2)	14% (n=7)
Female	2% (n=1)	14% (n=7)	2% (n=1)	2% (n=1)	4% (n=2)	14% (n=7)
History of RF	4% (n=2)	20% (n=10)	2% (n=1)	2% (n=1)	6% (n=3)	24%(n=12)
No history RF	0% (n=0)	2% (n=1)	0% (n=0)	2% (n=1)	2% (n=1)	4% (n=2)
Recurrences	4.9% (n=2)	14.6% (n=6)	2.4% (n=1)	2.4% (n=1)	7.3% (n=3)	26.8% (n=11)
No	0% (n=0)	9.8% (n=4)	0% (n=0)	0% (n=0)	0% (n=0)	2.4% (n=1)
Penicillin	4.0% (n=2)	20% (n=10)	2% (n=1)	4.0% (n=2)	8.0% (n=4)	24.0% (n=12)
No penicillin	0% (n=0)	2% (n=1)	0% (n=0)	0% (n=0)	0% (n=0)	4.0% (n=2)

Table 9.1 (2) Distribution of antibiotic resistance

	Erythromycin		Doxycycline	
	Intermediate	Resistant	Intermediate	Resistant
Male	0% (n=0)	6% (n=3)	0% (n=0)	0% (n=0)
Female	4% (n=2)	2% (n=1)	2% (n=1)	2% (n=1)
History of RF	4% (n=2)	8% (n=4)	2% (n=1)	2% (n=1)
No history RF	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)
Recurrences	4.9% (n=2)	7.3% (n=3)	2.4% (n=1)	2.4% (n=1)
No	0% (n=0)	2.4% (n=1)	0% (n=0)	0% (n=0)
Penicillin	4.0% (n=2)	8.0% (n=4)	2% (n=1)	2% (n=1)
No penicillin	0% (n=0)	0% (n=0)	0% (n=0)	0% (n=0)

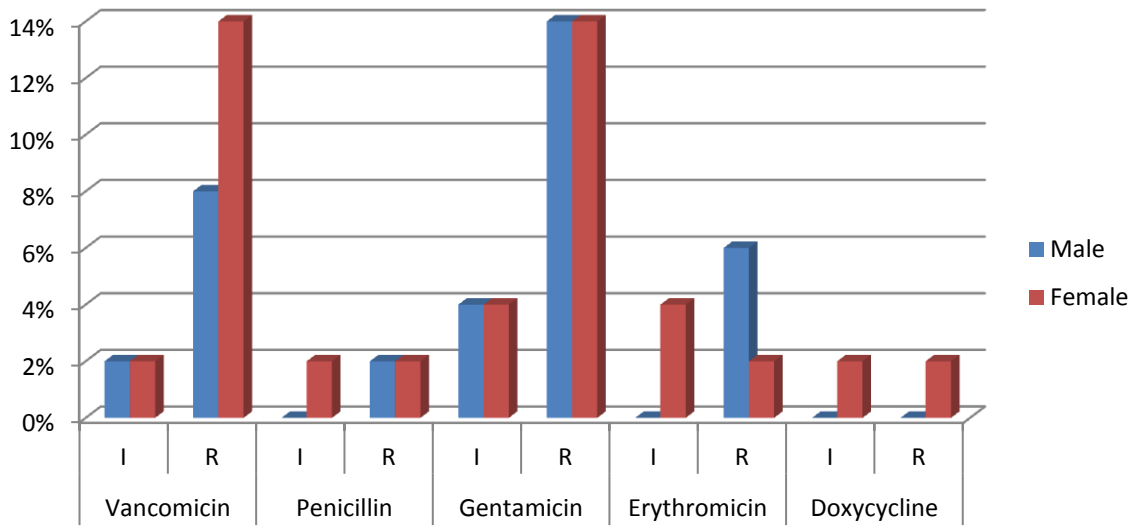


Figure 9.1 Distribution of antibiotic resistance by gender

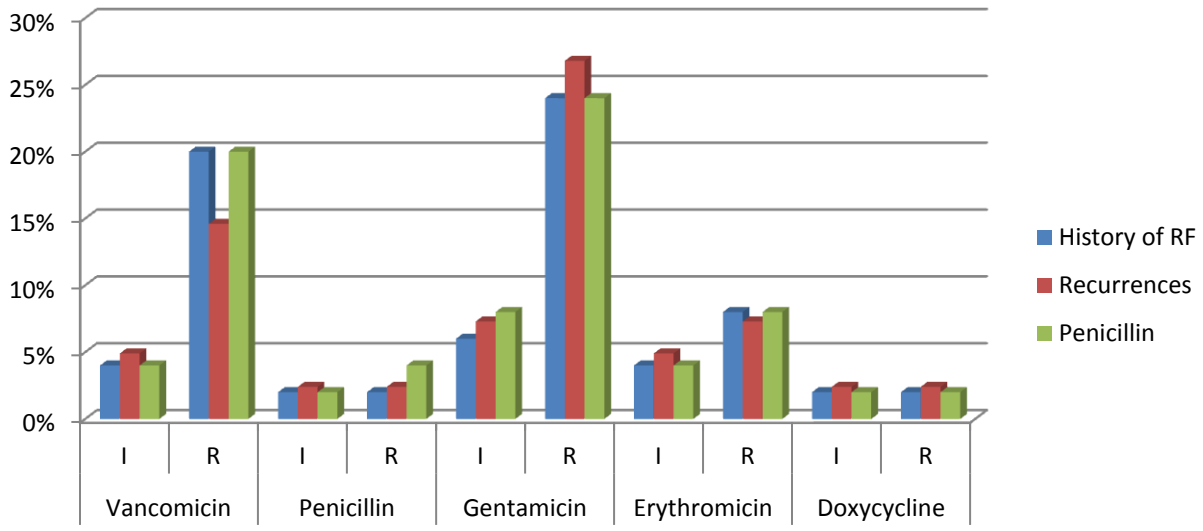


Figure 9.2 Distribution of antibiotic resistance by history of RF, recurrences and penicillin use

9.4 Discussion

The resistance for penicillin found in our series was surprising as all streptococcal strains have been reported to remain exquisitely sensitive and susceptible to Vancomycin (Ciftci 2003, Betriu 1993) and penicillin till date (Horn 1998, Betriu 1993), which is interesting, although the reason for this unique lack of development of resistance to penicillin is still unknown (Horn 1998). However, penicillin failure has also been reported in up to 10- 25% for streptococcal disease, (Stillerman 1986, Holm 1995) especially due to penicillin tolerance (Grahn 1987, Kim 1985[2]). Importantly, the presented results show for the first time resistance to Vancomycin in 22% of the cases, with intermediate sensitivity in 6% of the cases.

Resistance to erythromycin has been reported in 5% to 44.8% of the strains (Ciftci 2003, Koh 2010, Kim 1985) with a significant prevalence of Erythromycin resistant streptococci around the world (Maruyama 1979, Zackrisson 1988, Trallero 1989, Linares 1992, Hsueh 1995, Wu 1997, Cornaglia 1998). However, in our series the resistance for Erythromycin was 8%, with intermediate sensitivity in 4%. These results do not support the theory of the substantial increase in erythromycin resistance which has been associated with the increase in the consumption of

macrolide antibiotics (Ciftci 2003), however, these findings might be dependent on epidemiological and geographic factors.

Our series shows cross resistance of several strains for different combinations of antibiotics, especially Gentamycin, Erythromicin and Vancomycin and indicate a concern regarding tolerance and increased resistance rates for Vancomycin and Gentamycin, with also a slight tolerance or resistance to Penicillin. This is of high importance when selecting the best treatment regimen for streptococcal disease and indicates new public health concerns. The resistance rates and profiles found, which have been related to increase or excessive use of macrolide antibiotics show the importance to determine the geographic prevalence of resistant strains and resistance profiles to facilitate and improve clinical care.

The gender differences in resistance rates could not be explained in our study. However, resistance to erythromycin seems related to the male gender and younger age, while older patients tend to show resistance to Vancomycin.

The fact that history of RF and the presence of recurrences were related to higher resistance rates for all antibiotics might be due the fact that this resistance results in development and recurrences of RF due to antibiotic failure. Interestingly, individuals on Penicillin prophylaxis showed more often resistance to the tested antibiotics, which might be related to excessive and prolonged consumption of antibiotics. These results should be considered when prescribing antibiotic treatment and prophilaxis regimens and might have huge impact on clinical care.

9.5 Conclusions

Our series shows significant resistance for Penicillin and Vancomycin in Egypt, while the resistance to erythromycin was not shown to be as common as expected. Importantly, cross resistance for different combinations of antibiotics was found, indicating new public health concerns when treating streptococcocal infection. The presented results suggest that the resistance to antibiotic regimens might be the cause of development and recurrences of RF, being therefore a determinant factor for the development of RHD in Egypt. This study underlines the importance to determine the geographic prevalence of resistant strains and resistance rates and profiles for an improvement in clinical care. It also points to the importance of limiting antibiotic consumption to prevent decreased sensitivity and cross resistance to antibiotics.

CHAPTER 10

ANTIGENIC TARGETS FOR EVALUATION AND DIAGNOSIS OF STREPTOCOCCAL INFECTION

10.1 Streptococcal strain responsible for RF in Egypt

RF is believed to be caused by repeated infections with group A (GAS), and recently group C and G streptococci have been identified as possible causative pathogens. Furthermore, the variety in M-types of GAS caused by variations in *emm* gene sequences leads to different characteristics and rheumatogenic potential of the strains adding to the complexity of the pathogenesis of RF.

The variety in causative strains, their rheumatogenicity and geographic variety have led to difficulties in the understanding of the epidemiology of streptococcal disease, while RF and RHD remain major health care concerns. Importantly, the geographical variety in types of streptococcal strains and their rheumatogenicity has not been clarified till date. This study was designed to identify the particular strain of Streptococci responsible for RF/RHD in Egypt and its rheumatogenicity through serotyping for the particular type of M protein as well as DNA studies of the genes which encode these virulence factors (*emm*).

10.1.1 Patients and Methods

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI were recruited as described in chapter 3, pg. 75.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

Sample collection

A throat swab was taken and streaked immediately across a blood agar plate for culturing of the organism as described in chapter 3, pg. 90.

Culturing

- The swab was streaked immediately after collection across a blood agar plate

- The blood agar plate was allowed to incubate at (35°–37°C) for 24–48 hours to allow the growth of bacteria
- After incubation the colonies on the agar plates were analyzed
- Hemolytic colonies were identified

The cultured organisms were stored in THB as follows:

- Samples of the identified colonies were collected with a sterile loop
- Sterile loop were stabbed in THB agar tubes
- The tubes was allowed to incubate at (35°–37°C) for 24–48 hours
- The agar tubes were stored at 4°C for a maximum of 3 weeks
- The tubes were transported to the Helmholtz Centre at room temperature

10.1.2 Results and discussion

The cultured organisms were stored in THB prior to their transport to the Helmholtz Centre for analysis. Unfortunately, upon arrival, none of the organisms was retrieved.

To rule out technical failure, the protocol for culturing and storage of the organisms was revised and a pilot of 25 new samples was collected from the same patient group and in the same conditions. However, this has not led to retrieval of organisms for serotyping for the particular type of M protein as well as DNA studies of the genes for profiling of the *emm* gene sequences and determining the geographical variety of the strains and their rheumatogenic potential.

We have therefore, been unable to identify the particular strain of Streptococci responsible for RF/RHD in Egypt and its rheumatogenicity.

10.2 The potential of PARF as a diagnostic marker

It has been shown that rheumatogenic streptococcal strains are able to bind and aggregate human collagen and that the development of RF and RHD is related to the formation of a streptococcal autoantigenic complex with human collagen IV with binding and aggregation to collagen through an octapeptide in M proteins of rheumatogenic strains, PARF (peptide associated with rheumatic fever). Furthermore, a collagen autoimmune response accompanied by specific reactivity against the collagen-binding proteins has been shown in sera of RF patients, linking the observed effect to clinical cases. PARF is therefore believed to play a crucial role in the pathogenesis of RF through its action on collagen, making it a promising candidate for diagnosis of rheumatogenic streptococcal strains. This study aims at shedding more light on regional characteristics of RF pathogenesis trough identification of the causative bacteria and the value of PARF as a diagnostic marker for the early detection of rheumatogenic strains (Dinkla 2007). This included Collagen-binding assays were performed for the evaluation and analysis of the binding between the octapeptide PARF and collagen IV and the potential of PARF as a diagnostic marker for the early detection of rheumatogenic strains and the diagnosis of RHD.

10.2.1 Patients and Methods

Individuals visiting the outpatient clinic or admitted at the Cardio-thoracic department of the NHI were recruited as described in chapter 3, pg. 75.

The study population consisted of:

- Group I: -Individuals with a positive history of RF visiting the centre for penicillin prophylaxis with or without cardiac involvement
  -Individuals visiting the NHI outpatient clinic for suspicion of RF or RHD
- Group II: Patients admitted for RHD and scheduled for valve replacement.

Sample collection

Blood was collected by venipuncture into 1 non-additive tube as described in chapter 3, pg. 88. The serum samples were analyzed by collagen-binding assays for the evaluation and analysis of the collagen IV binding and the potential of PARF as a diagnostic marker.

Detection of collagen-reactive antibodies in patient sera

To determine anti-collagen antibody titers, plates (Greiner, Frickenhausen, Germany) were coated overnight at 4°C with anti–human CIV rabbit serum (Progen Biotechnik GmbH) diluted 1:100 in coating buffer, blocked with 2% BSA in PBS, and incubated with CIV (2 µg/ml in PBS) for 1 hour at room temperature.

Patient sera (diluted 1:50 to 1:50,000 in serial dilutions in PBS) were added to wells and incubated for 1 hour at 4°C. After washing, a 1:5,000 dilution of HRP-conjugated rabbit anti-human IgG (Sigma-Aldrich) was added and incubated for 1 hour at 37°C.

Antibody binding was detected using 2,2-azino-di-[3-ethylbenzthiazoline sulfonate] diammonium salt (ABTS) tablets (Boehringer Ingelheim GmbH, Mannheim, Germany) as substrate. The absorbance was determined at 405 nm in triplicates.

The cutoff titer for CIV was defined as 165 calculated from sera from healthy controls. Serum titers were obtained in independent assays and normalized against a rheumatic fever serum included in all tests for standardization.

10.2.2 Results and discussion

Testing of the protocol for detection of collagen-reactive antibodies in test sera (Rheumatic Fever serum) prior to the analysis of the collected patient samples resulted in insufficient levels of collagen and failed to show collagen reaction. This has led to revision and evaluation of the protocol.

The protocol was revised and adapted as follows:

-The dilution of CIV was decreased to 1:20 and 1:2

-The 1 hour incubation with CIV was was replaced by overnight incubaction.

-The dilution of the sera was decreased up to 1:1 and a finaly, undiluted serum was used.

-The 1 hour incubation of the sera was replaced by overnight incubaction.

-The dilution of HRP-conjugated rabbit anti-human IgG was decreased from 1:5,000 to 1:3,000 and 1:2,000

-The 1 hour incubation of the HRP-conjugated rabbit anti-human IgG was replaced by overnight incubaction.

Finally, newly acquired and prepared CIV, CIV rabbit serum, BSA, HRP-conjugated rabbit anti-human IgG and ABTS were tested. However, the anti-collagen antibody titers remained bellow significance threshold to allow analysis and comparison and the tests were therefore labeled negative for the collagen reaction.

As we were unable to show any collagen reaction in the test sera, which was positive for Rheumatic Fever we have therefore been also unable to test the collagen reaction in our collected samples, analyze the aggregation between rheumatogenic streptococcal strains and human collagen and evaluate the formation of a streptococcal autoantigenic complex with human collagen IV through PARF. This has also led to the fact that we were also unable to test the role of PARF in the pathogenesis of RF and its potential of PARF as a diagnostic marker for the early detection of rheumatogenic strains and the diagnosis of RHD.

10.3 Conclusions

We have failed in the aims of this study and in shedding more light on regional characteristics of RF pathogenesis trough identification of the causative bacteria and the value of PARF as a diagnostic marker for the early detection of rheumatogenic strains.

Unfortunately, the methods used today are still insufficient and need revision and evolution in order to satisfy the aims of this chapter. This shows the need and importance of more research focused on the antigenic features of the disease to define the mechanisms related to the pathogenesis of RF and evaluate the proposed formation of a streptococcal autoantigenic complex with human collagen IV through PARF and its potential as a diagnostic marker for RF and RHD.

CHAPTER 11

CONCLUSION AND FUTURE DIRECTIONS

11.1 Conclusions

Our project describes a series of studies on the epidemiology, clinical and laboratory profile of RF and RHD, a major health challenge and one of the leading neglected diseases worldwide. This condition affects several million people throughout the world, especially children and young adults. However, the true disease burden has been underestimated with many gaps in our knowledge of its pathogenesis and mechanisms, owing to the almost total lack of echocardiographic screening for this condition and little attention from the scientific community in the last decades leading to few important initiatives and little research.

Echocardiography is a successful screening tool to diagnose subclinical cardiac affection which benefits from identification and thence secondary prevention. However, there has been debate concerning the echocardiographic signs of early RHD and the diagnostic criteria to be used.

Our study has attempted at using modern tools and innovative approaches to investigate RF and RHD, and integrates large-scale epidemiological studies with clinical research and laboratory investigations aiming at unveiling the pathogenesis of the disease. Our main results concern epidemiology, diagnosis and pathogenesis of RF and RHD and have raised new interrogations regarding the mechanisms involved.

EPIDEMIOLOGY

Echocardiographic screening has shown that RHD is common in Egypt, affecting up to 69.2% of the individuals with history of RF and around 23 in 1,000 school children in Aswan. This leads to the calculated estimates that around 16% of the Egyptian population might have signs of the disease.

CLINICAL MANIFESTATION

RF

In Egypt, RF presents predominantly in children and young adults, however, it can affect adults as well. There are considerable variations in the prevalence of the symptoms and clinical manifestation of RF in different areas and age groups showing the need for a validation of the clinical diagnostic criteria. Recurrences are very common, and may contribute to the rapid progression to advanced valvular RHD. The disease awareness affects the use and compliance to

the penicillin prophylaxis and the success with secondary prophylaxis is critically dependent on patient education.

RHD

Our series confirms the predominance of mitral valve affection, with regurgitation being the most common feature in individuals with history of RF in Egypt. However, in overt RHD, combination of lesions is more predominant. Nevertheless, the amount of mitral stenosis was comparable to earlier studies.

Over 90% of the RHD cases found in school children in Aswan were subclinical, presenting predominantly by mitral regurgitation. Aortic or combined valvular lesions are less common and no stenosis was found in this young population.

Diagnosis

Clinical diagnosis has shown insufficient, resulting in under-diagnosis of cardiac affection in RF and RHD in Egypt. Echocardiography enables detection of cardiac affection in early phases of the disease in individuals who might benefit from secondary prevention with penicillin prophylaxis, resulting in major potential public health benefits.

Since echocardiography uncovers the presence of asymptomatic disease we conclude that it is the most powerful tool for screening of subclinical RHD, however, there are no universally agreed criteria for the echocardiographic diagnosis of RHD. As the most characteristic echocardiographic features of RHD are composed of a combination of structural and functional affection, we propose the use of diagnostic criteria which combines the functional and structural characteristics of the disease.

Determinants

Variables that correlate with severity of valve disease in Egypt include age, gender, age at the time of the first RF attack, family history for RF, recurrences of RF attacks, poor disease awareness and non-compliance to penicillin prophylaxis.

Younger patients are more at risk for the development of structural, functional and RHD features while older patients are more at risk of sustaining the found RHD features, with RF attacks at a

younger age being a risk factor for the development of RHD. However, mitral stenosis is more common in individuals reporting an older age at the time of the first RF attack. Cardiac affection seems more severe in males with a higher incidence and sustainability of functional lesions and RHD as well as increase in the amount of mixed lesions. Diagnosis by cardiac symptoms, recurrences and a longer time since the first RF attack are indicators for the presence and sustenance of structural and structural lesions.

A lower disease awareness and non-compliance to penicillin prophylaxis or a regimen with an interval longer than 15-days result in the development of structural as well as functional valvular lesions and sustenance of RHD features. Furthermore, a penicillin regimen with an interval longer than 15-days between the injections is a risk factor for the development of structural and RHD features. Furthermore, resistance to the antibiotic regimens seems to lead to development and recurrences of RF, being therefore a risk factor for the development and severity of RHD.

Antibiotic regimens

There is a significant resistance for Penicillin and Vancomicin in Egypt, with cross resistance for different combinations of antibiotics indicating new obstacles in the treatment of RF and prevention of RHD.

Prognosis

The outcome of RF is not well established, however, development, sustainance as well as deterioration of rheumatic valvular lesions are common in RHD while reversibility is related to adherence to antibiotic prophylaxis. However, in Egypt, structural or functional affection alone are more often sustained than the combination of both and functional affection are more prone to develop and less prone to resolve than the structural affection.

Echocardiographic follow-up should be encouraged to assess the progression and long term outcome of the valvular affection as well as the response to medical treatment and prophylaxis.

MECHANISMS OF THE DISEASE

Cellular features in RF and RHD

The presented results suggest the presence of immune activation and an ongoing immunological reaction in an apparently quiescent phase of the disease with a major role attributed to several proinflammatory cytokines in peripheral blood as well as valvular tissue in mediating heart lesions in RF and RHD patients. The presented results illustrate how the predominant Th1-type cytokines produced mainly by CD4+ T cells infiltrating valve tissue could mediate the severe RHD valve lesions as streptococcal and host antigen cross-reactive antibodies facilitate heart tissue infiltration by T lymphocytes. Importantly, valvular lesions seem more severe in females and in those who acquire RF at an older age and is related to non-compliance to the penicillin prophylaxis.

Structural features in RF and RHD

Our series shows distortion of normal architecture, histology and composition in RHD valves. Fibrosis, calcification and neovascularization as well as changes in the collagen, elastin and GAGs distribution and localization are common features in RHD, are the result of inflammatory process and rheumatic activity and lead to distortion of valve function and mechanics.

The pathology of RHD affects the elastic fibres in the valve leading to acceleration of the mechanism of aging in the valve. The changes in the collagen, elastin and GAGs distribution, localization and configuration, lead to distortion of valve function and mechanics as these features are dependent on an interaction between elastin and collagen conditions, which is highly dependent on its configuration and relative content in the valve and specially its organization relative to collagen.

Antigenic targets for evaluation and diagnosis of streptococcal infection

Unfortunately, the mechanisms known today are still not sufficient to explain the pathogenesis and mechanisms of RF or determine the antigenic targets for the evaluation and diagnosis of streptococcal infection. More epidemiological and laboratory work is needed to analyze and evaluate the role of PARF in the formation of the autoantigenic complex that seems to be the key

mechanism for the development of RF and RHD and analyze and evaluate the potential of PARF as a diagnostic marker for RF and RHD.

11.2 Future directions

This study shows high rates of RHD in Aswan, however, the prevalence in other parts of Egypt remains unknown.Current accurate data from Egypt remains lacking, while a high prevalence of RHD is expected around the country, showing the urgent need for more systematic echocardiography screenings to identify individuals at risk and determine the true disease burden. RF is a notifiable condition in Egypt, however, no measure has been taken till date to ensure that cases are enrolled in registers and are offered secondary prophylaxis with regular penicillin injections, leading therefore to insufficient compliance to the penicillin regimen. Registration of RF and RHD cases in high burden countries is crucial, as a measure to ensure control of the disease through the implementation of registry based secondary prophylaxis programs to improve the compliance to the penicillin prophylaxis.

The epidemiological research has established cohorts of individuals who are well phenotyped by echocardiographic examination, and whose follow up will give new insights into the mechanisms and determinants of this condition as well as the long term prognosis. Future research should include prospective case-control studies to identify the mechanisms of the disease, and define the determinants involved in the pathogenesis. The cohort of school children established in this study will be followed up regurlarly, with collection of samples for determination of the cellular and humoral mechanisms of the disease for the determinants of the disease and the long term prognosis.

We have confirmed that echocardiographic diagnosis of subclinical RHD is superior and more specific than clinical diagnosis and detection of a murmur. Therefore, there is the need to define a new classification of characteristics of valvular affection and lesions in RHD for evaluation and validation of the echocardiographic diagnostic criteria to be used in screening purposes as well as clinical diagnosis and management of patients. For this purpose, development of universally agreed criteria for the echocardiographic diagnosis of RHD is essential, as well as the most appropriate screening approach to be used, as early detection of subclinical RHD and application

of and adherence to secondary prevention lead to major public health benefits by decrease of the morbidity and mortality. However, this will require broader epidemiological research, with larger cohort and evaluation of the criteria used. Importantly, standardization of the echocardiographic criteria will allow comparison of prevalence in different areas and improve case detection.

Apart from its importance for indication of the true disease burden and detect those in danger of developing or progressing into valvular disease and application of secondary prevention, screening programs in different parts of the world where the disease is common, are essential for understanding the pathogenesis of the disease, developing new specific diagnostic methods and importantly helping the development of effective vaccines.

Long-term follow-up is necessary, nonetheless, to define the clinical significance of subclinical rheumatic valve lesions and determine the outcome of subclinical echocardiographic evidence of valvar disease and the effect of secondary prophylaxis. The serial use of echocardiography in patients with RHD is helpful in finding the progression or regression of the disease and will help determine the development, characteristics, progression and reversibility of valvular lesions. On the other hand research should concentrate on the prognostic value of the different structural and functional features found by echocardiography.

Although we have succeeded in providing new insighs in different aspects of RF and RHD in Egypt, the presented results are irrefutable proof of the need for more laboratory, epidemiological, and clinical research.

Routine laboratory assessment of the major inflammatory parameters and acute phase indicators such as ESR and CRP correlates with the magnitude and severity of inflammation as well as disease activity (Lane et al 2002, Wagner-Weiner 2002), however, the levels and distribution of these infection parameters in different areas, as a response to RF, especially in a quiescent phase of the disease is unknown. Additional research is needed to evaluate the value of inflammatory parameters in the diagnosis of RF and their role as prognostics indicators of disease.

A major role is suggested for inflammatory cytokines in mediating RF and RHD (Guilherme 2009) as they appear to play a crucial role in triggering immunologic and inflammatory

reactions in RF. Their presence in peripheral blood of acute RF and active RHD patients (Yegin 1997, Miller 1989, Morris 1993, Narin 1995, Samsonov 1995) as well as valvular tissue, leads to the suggestion of their contribution to the mediation of heart lesions (Guilherme 2009). Furthermore, cellular infiltration with a predominance of $CD4^+$ T-cells (Guilherme 1995) and some $CD8^+$ T-cells is found in patients that present rheumatic activity (Guilherme 2001). However, the immune response and signs of immune activation vary regarding the characteristics of the cellular as well as the humoral response.

The low cytokine levels found in our studies are surprising as their role in the pathogenesis of RF and RHD has been suggested to be determinant. Several cytokines have been reported to increase in RF and RHD due to their activity as stimulators of T-cells and for being in combination with other cytokines important mediators of inflammatory reactions (Miller 1989) (Morris 1993) and playing a role in anti-inflammatory reactions (Yegin 1997). Furthermore, IL4 is of clinical importance in inflammatory diseases and autoimmune diseases as it inhibits the production of inflammatory cytokines such as IL1, IL6 and TNF-alpha (Guilherme 2005[3]). TNF-a mediates part of the cell mediated immunity against obligate and facultative bacteria and parasites and is required for normal immune responses (Narin 1995, Samsonov 1995, Yegin 1997, Miller 1989, Morris 1993, Narin 1995, Yegin 1997) and is suggested to play an important role in the initiation of multisystemic inflammatory response in RF and in the pathogenesis of RHD (Yegin 1997, Settin 2006). The presented results confirm the presence of an ongoing immunological reaction in apparently quiescent phase of the disease with sustenance of raised inflammatory parameters in RF and RHD. Nevertheless, further studies are needed to validate the analysis of the inflammatory parameters and pro- and anti-inflammatory cytokines for the evaluation of the signs of immune activation in RF and RHD for the recognition of potential inflammatory parameters to monitor RF activity and determine the role of inflammatory cells and cytokines in mediating RF and RHD. Furthermore, the definition of the biological profile of RF and RHD is of paramount importance, particularly the clarification of the role of the immunological reaction and immune activation as well as inflammatory parameters and cytokines in mediating RF and RHD.

The altered architecture and histology shown in RHD valves **(**Sampaio 2007), which seems to lead to distortion of valve function and mechanics should be investigated in greater depth. The inflammation process related to RHD seems to lead to changes in the valvular histology and architecture, affecting the location, features and characteristics of the different areas of the valve, however, the precise mechanisms remain unclear.

Research focusing on all these issues should be performed using valvular tissue and blood obtained from participants in prospective studies, since it could be of value for unveiling the sequence of events that lead to cardiac lesions. On the other hand, better understanding of the development and progression of the lesions and identification of specific markers of injury is mandatory.

Another area of future research will be the genetics aiming at clarification of the role of heredity in determining familial predisposition and the immunogenetic susceptibility found in earlier studies. This could be done through genetic epidemiological studies in endemic and non-endemic areas, looking for candidate genes known to have a role in RF and RHD, as well as searching for genetic polymorphisms to biological markers with relevance to the mechanisms of the immune activation and immunological reaction related to the disease.

We aimed to analyze and evaluate the role of PARF in the formation of the autoantigenic complex that seems to be the key mechanism for the development of RF and RHD. It is crucial to evaluate the potential of PARF as a diagnostic marker for RF and RHD in Egypt for early detection of rheumatogenic strains and recognition of patients at risk of developing RF and RHD and in need for clinical management and prophylaxis. Unfortunately, the mechanisms known today are still not sufficient to fully explain the pathogenesis of RF and re-evaluations of data and epidemiological studies are needed to shed more light on regional characteristics of RF pathogenesis and evaluate the potential of PARF as a diagnostic marker for RF and RHD. This demonstrates the importance of continuous monitoring of the molecular epidemiology of GAS strains and comparison of these strains on a large scale for the appropriate selection of treatment agents and efforts for infection control.

We have planned a new trial, with collection of new samples and revision of the protocols and methods to satisfy these aims and to contribute to elucidation of the mechanisms and the antigenic targets of the disease, as well as the development of new diagnostic markers for RF and RHD.

Our study shows worrying resistance to different antibiotics, which might lead to an increase in development and recurrences of RF and subsequently an increase in incidence and severity of RHD. This shows the need to determine the geographic prevalence of resistant strains and resistance rates and evaluate the regimens used in the treatment of streptococcal disease. Furthermore, there is an urgent need for limiting antibiotic consumption to prevent decreased sensitivity and cross resistance to antibiotics.

Geographic variation in prevalence and characteristics of RF and RHD has been recognized within countries. We aknowledge that the results presented in this thesis reflect the situation in one specific region with high prevalence of RF and RHD. However, replication of similar programs of translational research in endemic countries will allow indepth analysis of the epidemiological and clinical characteristics of RF and RHD, the mechanisms involved in its pathogenesis and determining factors as well as the validation of the echocardiographic criteria proposed by us for diagnosis of early disease. Standardized national registries should be implemented for the encouragement of penicillin prophylaxis and follow up of the cardiac involvement in order to prevent the development and deterioration of the cardiac affection and the determination of the long term outcome.

We believe that this thesis addresses critical issues regarding the epidemiological and clinical characteristics including improved diagnostic criteria as well as the cellular and structural features of RF and RHD, contributing to better understanding of the disease in Egypt, and will hopefully stimulate further research looking at elucidation of the pathogenesis and new therapeutic targets for the disease.

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Yacoub S, Kotit S, Mocumbi AO, Yacoub MH (2008). Neglected diseases in cardiology: a call for urgent action. Nature Clin Prac Cardiovasc Med 5(4):178-9

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Appendices

Appendix I: Population characteristics

Appendix II: Schools per area (Arabic)

Appendix III: Calculations of number needed to screen

Appendix IV: List of the schools randomly selected

Appendix V: Standardized questionnaire

Appendix VI: Ethical permission Imperial College, London

Appendix VII: Information sheet and informed consent

Appendix VIII: Consent from Ministry of Health and Governorate of Aswan (Arabic)

Appendix I

Population:	83,082,869 (July 2009 est.)
Age structure:	0-14 years: 31.4% (male 13,345,500/female 12,743,878) 15-64 years: 63.8% (male 26,823,127/female 26,169,421) 65 years and over: 4.8% (male 1,701,068/female 2,299,875) (2009 est.)
Median age:	total: 24.8 years male: 24.4 years female: 25.2 years (2008 est.)
Population growth rate:	1.642% (2009 est.)
Population density	84 persons per sq km (2009 est.)
Birth rate:	22.12 births/1,000 population (2008 est.)
Death rate:	5.09 deaths/1,000 population (2008 est.)
Sex ratio:	at birth: 1.05 male(s)/female under 15 years: 1.05 male(s)/female 15-64 years: 1.02 male(s)/female 65 years and over: 0.74 male(s)/female total population: 1.02 male(s)/female (2009 est.)
Life expectancy at birth:	total population: 72.12 years male: 69.56 years female: 74.81 years (2009 est.)
Urbanization	
Urban	42% (2005 est.)
Rural	58% (2005 est.)

Appendix II

م	اسم المدرسة	عنوان المدرسة	عدد الطلاب	اسم مدير المدرسة	رقم تليفون المدير	المنطقة	السن
1	عمر بن الخطاب بنات	شرق المحطة	575	محمد عبد الدايم	2250876	شرق محطة السكة الحديد (1)	13 y \ 15 y
	عمر بن الخطاب بنين	شرق المحطة	685	محمد الصغير	2261433		13 y \ 15 y
2	الامام علي (صباحي)	شرق المحطة	18	احمد فكري احمد	2257519		5 y
	الامام علي (مسائي)	شرق المحطة	775	عبد الشافي جاد الرب	016047361\2257518		6 y \ 12 y
3	عبد الرؤوف حسنين	شرق المحطة	213	فاطمة احمد عبد الغفور	2260522		6 y \ 12 y
4	مصطفي كامل	شرق المحطة	523	معزوزة عبد الرحيم	0112381320\2261672		6 y \ 12 y
5	فتحي منصور	شرق المحطة	339	ابو زيد توغان	0107642602\2261665		6 y \ 12 y
6	الامام صباحي	شرق المحطة	779	جمعة رمضان حسن	0125745628\2257518		6 y \ 12 y
7	عبد الخالق علام	بجوار المطحن	320	احلام هاشم حجاز	2258780		6 y \ 12 y
8	خالد بن الوليد	بجوار المطحن	251	محمد ابو المجد	2258780		6 y \ 12 y
9	الناصرية	الحكروب	568	حسين فتحي صادق	2258645		13 y \ 15 y
10	شوقي عابد	الحكروب	308	فاطمة احمد عثمان	2252319		13 y \ 15 y
11	محمود عسران	ش السماد	817	ابراهيم لطيف خلة	2258775		6 y \ 12 y
12	محمد عبد الوارث	ش الناصرية	1017	نجاح السيد	2254067		6 y \ 12 y
13	احمد ماهر	النفق	597	صلاح الدين ربيع	0103557814\225721		6 y \ 12 y
14	ملحقة دار المعلمين	خلف نادي الحصايا	703	محمد سلطان علي مدني	100700195\2257849		6 y \ 12 y
15	مصطفي محمد عثمان	غرب سهيل بحري	146	محمد ابرهيم الجدي	3450293	غرب اسوان (2)	6 y \ 12 y
16	الرائد يعقوب	غرب سهيل	257	بخيتة علي محمد	3450410		6 y \ 12 y
			183	بكري فتحي	3450345		13 y \ 15 y
17	جزيرة سهيل	قرية جزيرة سهيل	56	فاطمة امين عبد الفتاح	2326109		6 y \ 12 y
18	غرب اسوان	بحري	263	فكرية محمد احمد	2210265\2211312		6 y \ 12 y
19	احمد نصر سنجق	غرب اسوان	216	محمد محمد توفيق	2210007		13 y \ 15 y
20	السدر	غرب اسوان	180	عبد الرؤوف مالك	2210506		13 y \ 15 y
21	احمد صابر	جزيرة غرب اسوان	91	عبد الله رشدي	2309656		13 y \ 15 y
22	ش عبد القادر	جزيرة غرب اسوان	201	امنة عبد الوارث	2302583		6 y \ 12 y
23	البسيون	نجع البسيون	180	سعيدة محمد دهب	2211322		6 y \ 12 y
24	عبد العظيم فضل	نجع القبة القديمة	269	نادية السيد بشير	2211325		6 y \ 12 y
25	القبة الجديدة	نجع القبة	12	نصر الدين عبد الحميد	2211596		5 y
			123	فاطمة محمد احمد	2211324		6 y \ 12 y
26	طه حسين	اطلس	349	تهاني محمد السيد	0161672109\2327512		6 y \ 12 y
27	ابطال التحرير	اطلس	370	ماجدة رسمي حسن	0108820864\2327526		6 y \ 12 y
28	علي ناصر	الرمكة	155	عبد الحميد محمد شحات	01130446462302945		5 y

6 y \ 12 y	وسط البلد (3)	0115044040\2302945	[illegible]	962	[illegible]	[illegible]	28
13 y \ 15 y		2309937	فاتن محمد	574	البركة	سوزان مبارك بنات	29
13 y \ 15 y		2303020	مديحة سعد	581	البركة	عبد الحميد عبد الغفار	30
13 y \ 15 y		2305160	رفاعي محمد	680	البركة	الدكتور طه حسين	31
5 y		0129370293\2327511	جمال عمر عباس	35	ش هميمي الجبلاوي	مجمع هميمي الجبلاوي	32
6 y \ 12 y				792			
12 y \ 15 y		2308102	رجاء عبد الحي	61	ش هميمي الجبلاوي	المهنية بنات	33
13 y \ 15 y		2309943	رجاء انور	634	بجوار عمر افندي	الفصول الاعدادية الملحقة	34
5 y		2301766	ماري بشارة	78	ش سعد زغلول	سانت تريزا	35
6 y \ 12 y		2301766	فايزة جرجس مليك	810			
6 y \ 12 y		0182381598\2302786	نادية طانيوس	230	ش المطار	باحثة البادية	36
12 y \ 15 y		---	احمد عزب	376	ش المطار	نفسية حسين	37
13 y \ 15 y		2303169	عاطف دمرداش محمود	825	ش المطار	المواساة	38
13 y \ 15 y		2315689	جمال سعد علي	624	ش المدارس	كمال عباس ناصر	39
5 y		0102906477\2327523	شادية عبد المتعال	108	ش المدارس	السيدة نفيسة	40
6 y \ 12 y				935			
6 y \ 12 y		0121017943\2302492	ثريا فخري علي	502	ش المدارس	اسوان بنات	41
6 y \ 12 y		2302987	صلاح عبد الوهاب	506	ش المدارس	عبد المحسن طاهر رزق	42
6 y \ 12 y		0182038834\2314657	ناهد عبد المجيد	216	ش الطابية	السلطان ابو العلا	43
6 y \ 12 y		010824424\2305656	مصطفي عطيتو	529	ش الطابية	سنية طه	44
5 y		2305144	نعمة محمد	191	ش كورنيش النيل	احمد ابا زيد (انجليزي)	45
13 y \ 15 y		2323038	سناء لمعي	278	بجوار معهد الخدمة الاجتماعية	الاقباط	46
13 y \ 15 y		2302059	ياسين طه محمد	512	بجوار معهد الخدمة الاجتماعية	عبد الله حسين	47
6 y \ 12 y		011244922\2327520	محمد راغب	919	بجوار معهد الخدمة الاجتماعية	مجمع عبد المجيد حسين	48
6 y \ 12 y		012965198\2327509	ابو القاسم حسن محمد	863	بجوار معهد الخدمة الاجتماعية	ابطال اكتوبر	49
5 y		2303795	محمد عوض عواض	59	ش عباس فريد	محمد منير	50
6 y \ 12 y		2303795	محمد عوض	620			
6 y \ 12 y	المحمودية (4)	0115431031\2309938	عاطف علي حافظ	679	المحمودية	ش عبد الحميد جاد الرب	51
5 y		0182381598\2302581	اوعاد احمد هارون	47	المحمودية	مجمع المحمودية	52
6 y \ 12 y				769			
13 y \ 15 y		2307468	هدي محمد محمود	648	المحمودية	عبد الصبور الجبلاوي	53
6 y \ 12 y		2263237	فتحي احمد نوبي	1181	كيما (عمارات السد)	العروة الوثقي	54
13 y \ 15 y			عبد التواب بشير	639			
6 y \ 12 y		2263349	شادية محمد عبد السلام	796	كيما (شرق)	كيما الصباحية	55

56	كيما الجديدة	كيما (شرق)	642	فاطمة حسن سعد الله	2250529	6 y \ 12 y	
57	مجمع عزبة النهضة	كيما (عزبة النهضة)	38	محمد علي عبد الدايم	2250083	5 y	
			623			6 y \ 12 y	
			198	محمد نعماني	---	13 y \ 15 y	
58	رمضان ارباب	كيما (الحدود)	15	ثريا محمد علي	---	5 y	
			185			6 y \ 12 y	
			282	عبد القادر شحات	---	13 y \ 15 y	
59	كيما بنات	كيما	394	فداء صادق بياوي	2330328	13 y \ 15 y	
60	ش ممدوح عثمان	السيل (محطة الكهرباء)	332	رمضان محمد ابو الحمد	0107262341\2268176	6 y \ 12 y	
61	ش عبد المالك عوضون	السيل	38	اسمهان مجمد بسيوني	2267813	5 y	
			588			6 y \ 12 y	
62	مجمع عزيز ابراهيم	السيل	519	هدية يوسف حسن	2268003	6 y \ 12 y	
			379	محمد احمد محمود	2268193	13 y \ 15 y	
			274	امنة محمد خالد	2268479	13 y \ 15 y	
63	ممدوح عثمان	السيل	22	رمضان محمد	---	5 y	
64	شوقي عابد	خور عواضة	679	عنتر عبد اللطيف حسين	2266109	13 y \ 15 y	
65	محمد حسني مبارك	خور عواضة	456	مكرم مجلع غبريال	2268167	13 y \ 15 y	
66	عبد الله محمود شحات	خور عواضة	97	حسنية حسن متولي	2268172	5 y	
			873			6 y \ 12 y	
67	نوبي حسن سعيد	خور عواضة	918	جلال سيد فرغلي	2268235	6 y \ 12 y	
68	خور عواضة الجديد	خور عواضة	615	سنية جلال احمد محمد	---	6 y \ 12 y	
69	رجب حسنين	السيل	1071	اعتدال عبد الله	2263267	6 y \ 12 y	
70	ابطال بدر	الشيخ هارون	1119	حسين محمد محمد	0120519614\2306889	6 y \ 12 y	
71	مبارك	الصداقة (الجديدة)	231	مصطفي محمد بكري	14407588	6 y \ 12 y	
72	تقسيم الصداقة	الصداقة (القديمة)	338	رمضان محمد السنوسي	0107548071\3440189	6 y \ 12 y	
73	الصداقة بالسد العالي	السد العالي (شرق)	20	فوزي عبيد	---	5 y	
			610	محمد سيد علي	3481667	6 y \ 12 y	
74	ابو الحسن الشاذلي	السد العالي (شرق)	373	اماني يوسف صالح	2348574	13 y \ 15 y	
75	اللواء امين الشريف	السد العالي (شرق)	350.00	عبد ربه احمد	480882	6 y \ 12 y	
76	السادات	حي العقاد	80	وصفي سعد الدين	2327510	5 y	
			843			6 y \ 12 y	
77	الجمهورية	نجع المحطة	71	محمد عبد الله	2304074	5 y	
			499			6 y \ 12 y	
			526	ليلي عبد الحافظ	2323803	13 y \ 15 y	

حي شرق (5)

78	السد العالي غرب	صحاري	363	عبير ابراهيم محمد	3841846	طريق السادات (الخزان) (6)	6 y \ 12 y
79	جمال عبد الناصر	صحاري	218	سيدة عيد حمدي	3481371		6 y \ 12 y
			329	فاطمة محمد احمد	3480971		13 y \ 15 y
80	الشهيد مجدي كامل	الخزان غرب	224	نوال سيد	3452573		13 y \ 15 y
81	وادي النيل	الخزان غرب	184	مصطفي احمد مصطفي	3451661		6 y \ 12 y
82	النهضة	الخزان غرب	197	حورية فوزي يوسف	3452353		6 y \ 12 y
83	نجع المنشية	طريق الخزان	230	اوجنيت فهمي ناشد	2303941		13 y \ 15 y
84	عبد الجليل حسنين	الكرور	429	عفاف شاكر محمد	2305517		13 y \ 15 y
85	عادل مرسي	الخزان شرق - الكرور	216	عنايات شاكر محمد	2303987		6 y \ 12 y
86	محمد عبد المعطي	الخزان شرق - الكرور	393	نرجس سعيد منقاروس	2302254		6 y \ 12 y
87	عبد العزيز مركب	عزبة الدابودية - الكرور	29	عبد ربه احمد	2327530		5 y
			274	عبد الصبور محمد	2312574		6 y \ 12 y
88	شجرة الدر	ش السادات ش البساتين	481	سيادات حنفي	2307508		6 y \ 12 y
89	الغات انجليزي	ش السادات	1367	وفاء عبد الحميد	0111169718		6 y \ 12 y
			371				13 y \ 15 y
90	اللغات فرنسي	مدينة نصر	43	انعام سعيد	0122501560\2327589		5 y
			220				6 y \ 12 y
			75				13 y \ 15 y
91	السلام اكاديمي	خلف التأمين الصحي	93	محمد نجيب	2306666		5 y
			343	مصطفي احمد مصطفي	010222278		6 y \ 12 y
92	محمود ابراهيم فضل	الجزيرة - نجع الجامع	234	منى امين ابراهيم	0180930177\2262937	شمال اسوان (7)	6 y \ 12 y
93	عبد المنعم ابراهيم	الجزيرة - نجع الجامع	249	محمود حسن	0183465396\2262937		6 y \ 12 y
94	الحجلاب	الجزيرة - الحجلاب	257	حجاج سيد	2268625		6 y \ 12 y
95	عبد الخالق علام بدر	الجزيرة	487	جانيت لبيب	2268547		13 y \ 15 y

Total

All	51402	% of total
5 y	1249	2.42
6 y - 12 y	14457	28.12
13 y - 15 y	35696	69.45

Appendix III

Area	Age group	N children in area	Representation of area	N needed to screen in area	Representation of age groups	N needed to screen in age group
1	**5 y**	18			0.21%	1
	6 y \ 12 y	6334			74.60%	370
	13 y \ 15 y	2136			25.17%	125
	Total	**8488**	**16.50%**	**495**		
2	**5 y**	12			0.55%	12
	6 y \ 12 y	1495			68.70%	87
	13 y \ 15 y	670			30.77%	39
	Total	**2177**	**4.20%**	**127**		
3	**5 y**	626			4.35%	37
	6 y \ 12 y	8603			60.00%	502
	13 y \ 15 y	5145			35.80%	300
	Total	**14374**	**27.90%**	**839**		
4	**5 y**	47			2.10%	3
	6 y \ 12 y	1448			67.50%	85
	13 y \ 15 y	648			30.20%	38
	Total	**2143**	**4.10%**	**125**		
5	**5 y**	230			1.54%	14
	6 y \ 12 y	10991			73.80%	641
	13 y \ 15 y	3674			24.70%	214
	Total	**14895**	**28.98%**	**869**		
6	**5 y**	316			4.00%	18
	6 y \ 12 y	5598			69.00%	327
	13 y \ 15 y	2184			27.00%	128
	Total	**8098**	**15.75%**	**473**		
7	**5 y**	0			0.00%	0
	6 y \ 12 y	740			39.70%	30
	13 y \ 15 y	487			60.30%	43
	Total	**1227**	**2.38%**	**72**	2.38%	

Appendix IV

Area	Name of school	Age group	Amount of children	Number of children screened
1	Mostafa Kamel	6 y \ 12 y	523	**370**
	Shawky Abed	13 y \ 15 y	308	**125**
2	AlKoba AlGedieda	5 y	12	**12**
		6 y \ 12 y	123	**87**
	Ahmed Saber	13 y \ 15 y	91	**39**
3	Ali Nasser	5 y	155	**37**
		6 y \ 12 y	962	**502**
	AlMo2assa2	13 y \ 15 y	825	**300**
4	Mogame3 ElMahmoudeya	5 y	47	**3**
		6 y \ 12 y	769	**85**
	Abdel Sabour AlGuilawy	13 y \ 15 y	648	**38**
5		5 y	22	**14**
	AlArwa Alwasska	6 y \ 12 y	1181	**641**
		13 y \ 15 y	639	**214**
6	El Gomhoreya	5 y	71	**18**
		6 y \ 12 y	499	**327**
		13 y \ 15 y	526	**128**
7	Mahmoud Ibrahim	6 y \ 12 y	234	**30**
	Abdelkhalek Allam	13 y \ 15 y	487	**43**

* In area 1 and 7 there are no schools in the cathegory of 5 years of age

Appendix V

Aswan Heart Centre
Magdi Yacoub Heart Foundation

Screening of school children

Patient ID	
Name	
Date of birth	
Gender	○ Male ○ Female
Address	
Phone number	
Name of school	
Area	
Date of assessment	
Disease awareness	○ Excellent ○ Poor ○ Fair

Family environment

Father: active job	○ Yes ○ No
Father: Job description	
Father: literacy	○ Yes ○ No
Mother: active job	○ Yes ○ No
Mother: job description	
Mother: literacy	○ Yes ○ No
Number of siblings	
Sibling medical history	☐ Rheumatic fever ☐ Congenital cardiac ☐ Endomyocardial fibrosis ☐ Other ☐ Hypertrophic cardiomyopathy
Details of other sibling medical history	
Notes	

1

Risk factors

Family history	☐ CAD ☐ DCM ☐ EMF ☐ Rheumatic disease ☐ HOCM ☐ Rheumatic heart disease
Pharyngitis	○ Yes ○ No
Skin infection	○ Yes ○ No
Dental care	○ Good ○ Poor ○ Fair
History of previous medical interventions	○ Yes ○ No
Specify	
History of previous cardiac interventions	○ Yes ○ No
Specify	

History of rheumatic fever

Timing of first attack	○ <6 months previously ○ 1-5 years ago ○ 6-12 months previously ○ >5 years ago
Angina	○ Yes ○ No
Cutaneous infection	○ Yes ○ No
Rheumatic Fever	☐ Arthralgia ☐ Fever ☐ Poly arthritis ☐ Sydenham's chorea ☐ Carditis ☐ Erythema marginatum ☐ Cutaneous nodules
Recurrences	○ Yes ○ No
Medication	☐ Salicylate ☐ β-blocker ☐ Corticosteroids ☐ Digoxin ☐ Penecillin V ☐ Diuretics ☐ Benzathine penicillin G ☐ Calcium channel blocker ☐ Erythromycin ☐ Anticoagulation
Antibiotic prophylaxis	○ Yes ○ No
Schedule for antibiotic prophylaxis	○ 15 days ○ 3 weeks
Prophylaxis score	○ Excellent (>12 shots per year) ○ Fair (9-12 shots per year) ○ Poor (<9 shots per year)

Physical examination

Height	cm
Weight	kg
Murmurs	☐ AV systolic ☐ AV diastolic ☐ MV systolic ☐ MV diastolic ☐ TV systolic ☐ TV diastolic ☐ PV systolic ☐ PV diastolic
Peripheral oedema	○ No ○ Yes
Splenomegaly	○ No ○ Yes
Hepatomegaly	○ No ○ Yes
Pulmonary rales	○ Minor ○ Major

echocardiogram

Rhythm	○ Sinus ○ Atrial flutter ○ Atrial fibrillation ○ Pacemaker
Valve disease	○ Degenerative ○ Rheumatic ○ Congenital
Morphological features	○ Thickening of valve cusps ○ Commisures fusion ○ Doming of valve cups ○ Calcification ○ Sub-valvular apparatus affection ○ Fixed leaflets

Functional features	○ Aortic	○ Mitral	○ Tricuspid	○ Pulmonary
	☐ Regurgitation ☐ Stenosis	☐ Regurgitation ☐ Stenosis	☐ Regurgitation ☐ Stenosis	☐ Regurgitation ☐ Stenosis
	☐ Trivial ☐ Mild ☐ Moderate ☐ Severe	☐ Trivial ☐ Mild ☐ Moderate ☐ Severe	☐ Trivial ☐ Mild ☐ Moderate ☐ Severe	☐ Trivial ☐ Mild ☐ Moderate ☐ Severe

Conclusion	○ Normal	○ RHD	○ Other
Plan	○ None	○ Follow-up	○ Consultation

Appendix VI

Imperial College London

Imperial College Research Ethics Committee

Imperial College London
G02 Sir Alexander Fleming Building
South Kensington Campus
SW7 2AZ
Tel: +44 (0)207 594 1770 Fax: +44 (0) 207 594 1792

researchethicscommittee@imperial.ac.uk

Thursday 17 September 2009

Professor Julia Buckingham
Room 4.09
Level 4 Faculty Building
Imperial College London
South Kensington Campus
London
SW7 2AZ

Dear Professor Buckingham

Study Title: Admissions test pilot study

ICREC reference: ICREC_8_5_2

CI: Professor J Buckingham (PI)

A request for an amendment to the above study was reviewed by members of the Imperial College Research Ethics Committee on 17 September 2009.

Ethical Opinion

The members of the Committee present gave full approval of the study on the basis described in the cover email and the revised supporting documents.

Documents

The documents reviewed were:

- Cover email explaining the amendments and their justifications
- Amended Participant Information Sheet
- Amended Consent Form

Membership of the Committee

The members of the Ethics Committee who reviewed the study were:

Mr Michael Dixon (Chair)
Professor Chris Hankin (Deputy-Chair)
Professor Andrew George
Mr David Wilbraham

Appendix VII

Title of the project: **Rheumatic Heart Disease in Egypt**

We invite you to consider taking part in a research project.
It is important that you read and understand some general principles that apply to all those who take part in clinical research:

A) Your participation in this study is entirely voluntary
B) Personal benefit may or may not result from taking part in the study, although it is hoped there will be personal benefit to you. Knowledge may also be gained which could benefit other people.
C) You may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled.
D) If you choose not to enter this study, this will not affect you in any way.

Explanation of the project:
There is a large group of individuals suffering from Rheumatic Fever which without proper treatment and other medical health measures to prevent disease or health problems, may lead to damage of the heart. We are therefore examining individuals for the presence of heart damage due to this infection. We are approaching you because you have or are suspected of a history of Rheumatic Fever or Rheumatic Heart Disease or have a child with this medical history.
The goal of our study is to detect how many individuals are affected with the disease, how severe it is, how it presents itself, the factors that are involved in getting the disease and how we can prevent and treat it the best way. This screening will help us obtain important information on the disease which will be used for future disease prevention and treatment.
We will be asking you and/or child a number of questions regarding the general health condition and medical history. This will be followed by a non-painful imaging of the heart called echocardiogram, which gives an image of the heart and its function. The test is painless and takes about 15 minutes. The study also involves taking a swab, done by rubbing a cotton bud on the inside of the throat, and the collection of 10 ml of blood (about 1 large spoonful) from the arm at the end of the examination.
This analysis will be strictly for the detection of factors that could make you or your child more susceptible to Rheumatic Fever and Rheumatic Heart Disease and will not be used for any other purpose now nor in the future. We have no reason to think that you or your child have the disease. You and your child have the ample opportunity to decline to take part and this will not affect you nor your child's education or health services in any way.

Confidentiality – who will have access to the information?
All information that is collected about you or your child during the course of the study will be kept strictly confidential. Any information about you or any results of the tests will have your name and address removed so that you cannot be identified. Only authorized staff and representatives from the funding organisation or regulatory bodies will be permitted to review your medical records.

What will happen to the study results?
A report will be produced for scientific papers for publication in medical journals. Your child's name will not appear in any of these reports. You will be notified about the results of the tests and eventual need of any further treatment or follow-up.

Request
You are expected to attend the echocardiographic examination, answer the questionnaire, undergo the collection of a throat swab and blood sample and receive information regarding the procedures as well as the results.

INFORMED CONSENT

I,…….....

(agree to / give my consent for my child to) undergo the research procedures described overleaf and take part in this project. The nature, purpose and possible consequences have been fully explained to me.

I have received information on the reasons for the tests and I understand that the tests will contain non-invasive imaging and that the results of the tests will be made known to me and any suggested form of treatment will be explained to me before any sort of treatment is instituted.

I understand that if I refuse participation in this project, this will not in any way jeopardize me nor my child's treatment neither now nor in the future nor his school attendance and results.

Date

Signature

Appendix VIII

بسم الله الرحمن الرحيم

محافظة أسوان
مكتب المحافظ

١٣ + ٢٢٨٤
٢٠١٠/٩/٢

الســـيد الدكتـــور/ محمد عبدالمحسن
المدير الإدارى لمركز أبحاث القلب للدكتور/ مجدى يعقوب

تحية طيبة وبعد

نتشرف أن نرفق طيه كتاب السيد الأستاذ الدكتور/وزير الصحه بشأن قيام مركز أبحاث القلب لمؤسسه الدكتور/مجدى يعقوب لفحص عينه من طلبه المدارس لتقيم الإصابه بمرض الحمى الروماتيزميه القلبيه بالمحافظة أبتداءاً من العام الدراسى ٢٠١٠/٢٠١١ .

برجاء التكرم بموافاتنا بصوره من المشروع البحثى الذى يتم من خلاله المسح والفحوص الطبيه والخطه الزمنيه والماليه لتنفيذ أنشطه المشروع البحثى ونتائج البحث وبيان بالحالات المرضية التى سيتم فحصها مع توفير المتابعة والعلاج الطبى الجراحى للحالات المرضية مع مراعاه عدم نشر نتائج الأبحاث المتعلقة بهذا المشروع الا بعد موافقة وزارة الصحه .

برجـــاء التنبيـــة بإتخـــاذ الـــلازم والإفـــادة بالمطلـوب فـــى هـــذا الشـــــأن

وتفضلوا بقبول وافر الإحترام

التوقيع « »
مهندس/ جابر طه محمد عامر
مدير عام شئون مكتب المحافظ