SERUM LAMININ IN RHEUMATIC FEVER AND RHEUMATIC HEART DISEASES

Thesis
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INTRODUCTION

Acute rheumatic fever (ARF) is an inflammatory disease occurring during recovery from infection with group A streptococci (GAS), having an onset marked by fever and joint pain ***(Carapetis et al., 2007).***

World wide there are 470,000 new cases of RF and 233,000 deaths, attributable to RF (rheumatic fever) or RHD (rheumatic heart disease) each year, most occur in developing countries ***(Carapetis et al., 2007).***

Acute rheumatic fever is believed to be caused by antibody cross reactivity with group A streptococci and can involve the heart, joint, skin, and brain ***(Kumar et al., 2007).***

The GAS is a unicellular bacterium composed of a core of cytoplasm enclosed in a thin cytoplasmic membrane which in turn surrounded by a rigid cell wall of 3 layers: - an inner mucopeptide layer, a middle layer of group specific carbohydrate, and an outer protein layer, the chief component of which is M protein, the major antigen implicated in pathogenesis ***(Martin et al., 2006).***

Mimicry between streptococcal M protein and cardiac myosin is important in the pathogenesis of RHD(rheumatic heart disease), M protein specific T cells

clones derived from rheumatic carditis were cross reactive with human cardiac myosin, and laminin (a valve protein) ***(Ellis et al., 2005).*** Laminins are major proteins in the basal lamina also called the basement membrane, which is a protein network foundation for most cells and organs ***(Timpl et al., 2007).*** Laminin has many functions most important of which is its role in cross linking and anchoring extra cellular structures to the cell surface ***(Mochizuki et al., 2007).*** Laminin form network of web like structure that resist tensile forces in basal lamina. They also assist in cell adhesion. Laminin binds other extra cellular membrane components such as collagens, nidogens and entactins ***(Plopper et al., 2007).***

Anti streptococcal antibodies cross reactive with laminin are present in sera of patients with RF (rheumatic fever). The cross reactivity of those antibodies with human heart valvular endothelium and underlying basement membrane has been suggested to be to be possible cause of immune mediated valve lesion ***(Rajendranath et al., 2008).***

Extracellular matrix components were found to be increased in patients with dilated cardiomyopathy **(*Figalla et al., 1995)*,** to reflect its clinical staging and to correlate with level of laminin in those patients ***(Schaper et al., 1995).***

Serum levels of laminin or its fragments could reflect the changes observed in the basement membrane of the patients with acute rheumatic fever ***(Segarra et al., 2000).***

Serum laminin level was found to be different among patients with acute rheumatic fever, as, the highest level was noticed in patients with carditis, followed by those with arthritis, whereas the lowest level was observed in patients with chorea ***(Abdel Aal et al., 2008).***

Studies in failing human myocardium have shown that there is a quantitative increase in laminin ***(Schaper et al., 2001).***

Rheumatic fever has many possible complications as arrhythmias, damage of heart valves (in particular M.S (mitral stenosis)and AS(aortic stenosis)), endocarditis, heart failure, pericarditis and sydenhams chorea) ***(Gerber, 2007).***

AIM OF THE WORK

The aim of this study is to estimate the level of serum laminin in patients with rheumatic fever and rheumatic heart disease and to detect its utility to be used as a marker of activity, severity, or rheumatic affection in those patients.

ACUTE RHEUMATIC FEVER

Definition

Rheumatic fever (ARF) is multisystem inflammatory disease that occurs in genetically susceptible host after a pharyngeal infection with group A Streptococci (GAS) ***(Fort et al., 2012).*** As shown in figure (1).

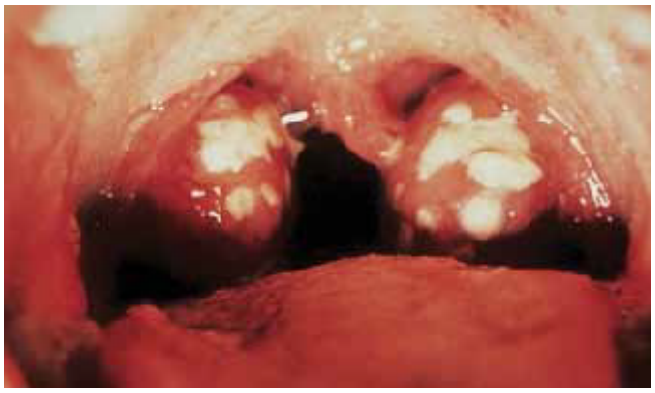

Fig. (1): Acute streptococcal pharyngitis ***(Goldman et al., 2012).***

Epidemiology

The prevalence of ARF appears to be much higher in less developed countries, particularly in indigenous and less rich areas, and varies significantly from one region to the other. In 2005, it was estimated that the incidence of ARF was more than 471,000 cases per year, with 336,000 cases in those 5–14 years of age ***(Carapetis et al., 2005).***

In a recent study from Australia, there were 203 notifications of ARF in 194 Indigenous people in north Queensland from mid 2004 to mid 2009, and this was a 23% increase in the average annual incidence compared with that in the preceding 5 years ***(Hanna and Clark, 2010).***

In a recent study in Saudi Arabia, it was found that, the frequency rate of ARF admissions was 12 cases per 100.000 hospitalizations ***(Abdul-Mohsen and Lardhi, 2011).***

In Egypt, one study aimed to determine the epidemiologic features of Rheumatic Heart Disease (RHD) among basic education students in Alexandria, including the demographic and clinical profiles as well as risk factors for RHD development. A stratified random sampling technique was used to allocate 5465 school children in the six educational zones of Alexandria. Clinical RHD cases were confirmed by using chest X-ray, ECG and echocardiography. Thirty four RHD cases (with an estimated prevalence of 6.2/1000 students) were diagnosed. Low socioeconomic status, chronic tonsillitis, positive family history of acute rheumatic fever/RHD and paternal consanguinity were significantly associated with the occurrence of RHD ***(Abdel-Moula et al., 1998).***

The prevalence of RHD is estimated to range from 15.6 to 19.6 million cases worldwide, with 282,000 newly diagnosed with over 233,000 deaths attributed to RHD each year. Unfortunately, given the disease's predilection for children, over 2.4 million of these cases were in patients aged 5–14 years old ***(Carapetis et al., 2005).***

In a recent study from Nicaragua, The overall prevalence of RHD in children was 48 in 1,000, a figure that exceeds the previously predicted rates ***(Paar et al., 2010).***

In a recent study to estimate the prevalence of the common self-reported chronic diseases among 1493 adolescent students in Mansoura, Egypt, an important finding is that 3.4% of students have rheumatic heart disease. This reflects the widespread of streptococcal infection and rheumatic fever during childhood with inadequate treatment among students belonging to families of low and very low socioeconomic status ***(El-Gilany, 2011).***

Pathogenesis

ARF is believed to be a consequence of molecular mimicry, an autoimmune phenomenon that occurs after host infection with GAS and involves both humoral and cellular immune responses. Antistreptococcal antibodies

are produced by B cell lymphocytes that cross-react with host tissue epitopes, causing inflammation in various organ systems. In addition, bacterial peptide fragments, many of which are similar to host proteins, are presented to T cell lymphocytes (with a prominent role by CD4+ T cells in rheumatic valvular lesions) via major histocompatibility complex (MHC) molecules, inducing an immune response ***(Guilherme et al., 2007).***

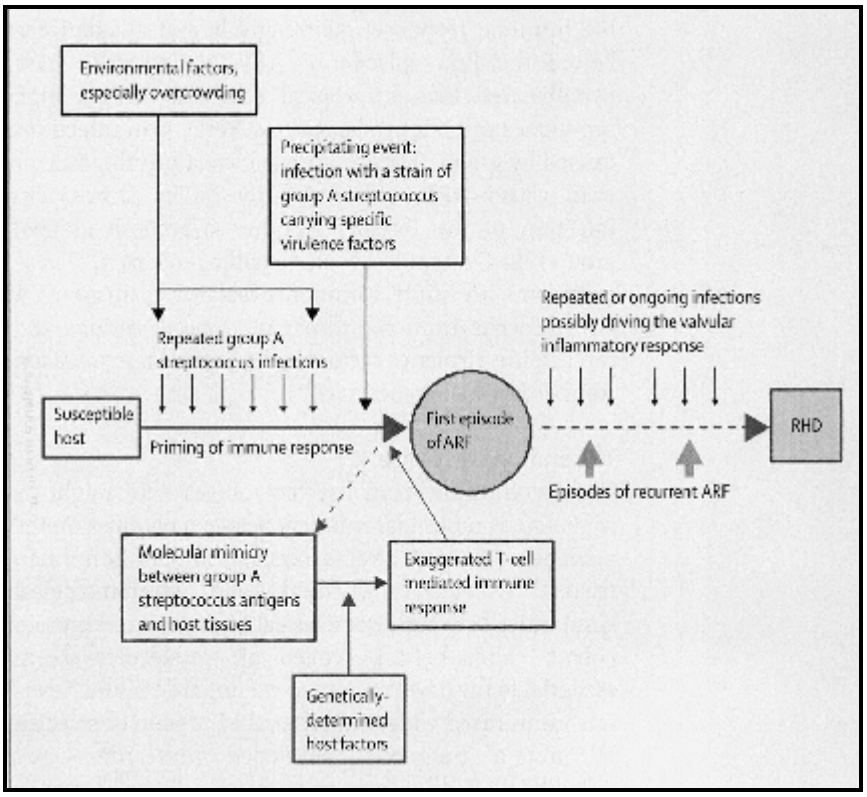

Fig. (2): Pathogenic pathway for ARF and RHD ***(Carapetis et al., 2005)***

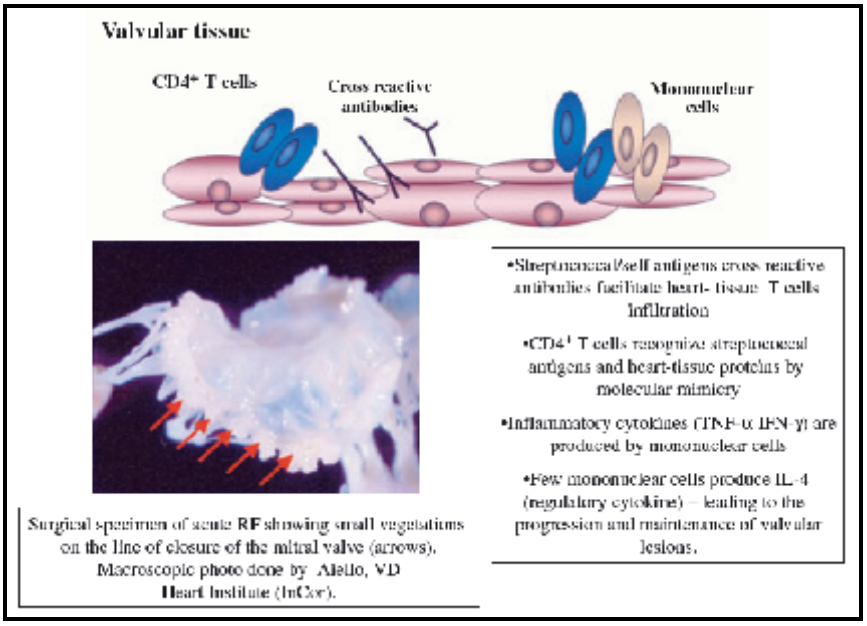

Fig. (3): Major events triggering rheumatic valvular lesions in RHD. Heart tissue cross-reactive antibodies bind to the surface of the valve endothelial and facilitate mainly CD4+ T-cell infiltration ***(Guilherme et al., 2007).***

- ***Genetic Susceptibility***

There are some genes that have been described as associated with the development of RF and RHD ***(Guilherme et al., 2007).*** The dominant contributors to the autoimmune reactions in RF and RHD are the susceptibility MHC class II alleles (DR and DQ), which are located on human chromosome 6. Several human leukocyte antigen (HLA) class II alleles have been found to be associated with RF/RHD; however, HLA-DR7 was the allele most frequently associated with the disease, and its combination with some DQ alleles seems to be associated

with the development of valvular lesions in RHD. The molecular mechanism by which MHC class II molecules confer susceptibility to autoimmune diseases is not clear. However, the role of HLA molecules is to present antigens to the T cell receptor (TCR), thus, triggering the activation of the adaptive immune response. Those associated alleles may facilitate the presentation of some streptococcal peptides that later will trigger autoimmune reactions ***(Stanevicha et al., 2003).***

In Egypt, in the study of HLA class I in patients with rheumatic fever, there was a statistically significant increase in the B5 allele in patients compared to controls, while B49 and B52 alleles were found in controls only. Also, a statistically significant increase in HLA DR* 04-02 and HLA DR *10-0101 in patients, meanwhile HLA DR*1309120 was found only in controls ***(El-Hagrassy et al., 2010).***

In another study, the molecular association between HLA and RHD was investigated in patients with defined clinical outcome. Significant increases in DRB1*0701 and DQA1*0201 alleles and DRB1*0701-DQA1*0201 haplotypes were found in patients. Removal of the MVL (multivalvular lesions) patients from analysis increased the strength of HLA associations among the MVD (mitral valve disease) sample. The frequency of DQA1*0103 allele was decreased and the DQB1*0603 allele was absent

from the patient group, suggesting that these alleles may confer protective effects against RHD. DQ alleles in linkage disequilibrium with DR alleles appear to influence risk/protection effect: whereas the DRB1*13-DQA1*0501-3-DQB1*0301 haplotype showed a trend toward risk, the DRB1*13-DQA1*0103-DQB1*0603 haplotype was absent in the RHD sample. Those data indicate that certain class II alleles/haplotypes are associated with risk or protection from RHD and that these associations appear to be stronger and more consistent when analyzed in patients with relatively more homogeneous clinical manifestations ***(Guedez et al., 1999).***

TNF-α gene, another gene associated with the disease, is also located on human chromosome 6 between MHC class I and class II. It has a role in the inflammatory response. The association between TNF-α alleles (-308A and -238A) and RF, and RHD was recently described. It is possible that this association could be due to linkage disequilibrium with MHC class II molecules and/or responsible for an exacerbation of the inflammatory response by high levels of TNF-alpha ***(Ramasawmy et al., 2007).***

In a recent Egyptian study, TGF-β-1 T869C TT genotype, 869T allele and 509T allele were reported as possible risk factor for RHD in Egypt ***(Kamal et al., 2010).***

Mannan-binding lectin (MBL) is an acute phase inflammatory protein that functions as a soluble pathogen recognition receptor. Mannan-binding lectin binds to a wide variety of sugars on the surface of pathogens and plays a major role in innate immunity due to its abilities to opsonize pathogens, enhancing their phagocytosis, and to activate the complement cascade via the lectin pathway ***(Jack et al., 2001).***

Different variants of promoter and exon 1 regions of the MBL2 gene, which codes for the production of MBL, have been reported in patients with RF/RHD. It is interesting to note that different alleles were found based on the clinical picture of the disease. Mitral stenosis in RHD patients has been associated with the "A" allele, which codes for high production of MBL; the patients studied presented with high levels of MBL in the sera ***(Schafranski et al., 2008).***

In contrast, aortic regurgitation in RHD patients seemed to be associated with the "O" allele. The "O" allele codes for low production of MBL, and the patients studied presented low levels of MBL in the sera ***(Ramasawmy et al., 2008).***

- ***Molecular Mimicry***

Molecular mimicry is defined as a sharing of epitopes between antigens of the host and the infectious

agent, which in the case of RF/RHD is S. pyogenes. Three types of mimicry mediated by antibodies against S. pyogenes have been described including the sharing of (1) identical amino acid sequences, (2) homologous but non identical sequences, and (3) epitopes of different molecules, such as carbohydrates, DNA, and gangliosides ***(Guilherme et al., 2006).***

Molecular mimicry for T cells is mediated by a different mechanism. The recognition of bacterial and/or self 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. The recognition of self 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 self-antigens that triggers an amplification and diversification of the autoimmune response ***(Guilherme and Kalil, 2010).***

Guilherme et al. (2007) identified several M protein epitopes recognized by peripheral T cells of RF/RHD patients and by heart tissue infiltrating T cell clones of severe RHD patients.

- ***Role of T Cells in the Pathogenesis of the Disease***

Studies performed in the last 25 years have showed that CD4+ cells are the major effectors of autoimmune reactions in the heart tissue in RHD patients ***(Guilherme and Kalil, 2010).*** Activation of CD4+ T cells is triggered by the presentation of streptococcal antigens, mainly, via HLA class II molecules. Pathogen epitopes that present structural or sequential similarity to self epitopes might activate autoreactive T lymphocytes that have escaped immune tolerance by the molecular mimicry mechanism. These autoreactive T cells can also activate B cells that will produce pathogen and self-antigen-specific antibodies. The interplay of humoral and cellular immune responses in RHD was only recently demonstrated; in rheumatic carditis, streptococcal, and human protein cross-reactive antibodies up-regulate the adhesion molecule VCAM-1 after binding to the endothelial surface ***(Galvin et al., 2000)***, leading to inflammation, cellular infiltration, and valve scarring ***(Roberts et al., 2001).***

These data established the role of the heart tissue cross-reactive antibodies (anticardiac myosin and laminin) in the early stages of inflammation and T cell infiltration in RHD lesions. The analysis of heart tissue infiltrating T cell clones strongly demonstrated the role of T cells in the pathogenesis of RF and RHD. Immunodominant peptides of the M5 protein displayed crossreactivity with valvular