

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقُلْ رَبِّ زِدْنِي عِلْمًا

صدق الله العظيم

**STUDY OF NOVEL
MARKERS FOR
RHEUMATIC FEVER**

❖ Great Thanks and reward to my prof.

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BRIEF HISTORICAL REVIEW OF RHEUMATIC FEVER

- ▶ **Bouillaud (1836)** was the first to describe the clinical picture of acute rheumatic carditis and chronic rheumatic heart disease.
- ▶
- ▶ The entire clinical spectrum of acute rheumatic fever (from tonsillitis to carditis) was first described by **Cheadle in 1889**.
- ▶ The diagnostic criteria for ARF were first developed by **Duckett Jones in 1944** and have since been modified in 1965, 1984, 1992 and 2002 (*Ferrieri, 2002*).

INTRODUCTION

Acute rheumatic fever (**ARF**) and rheumatic heart disease (**RHD**) are **significant public health** concerns around the world. Despite decreasing incidence, there is still a **significant disease burden**, especially in developing nations (*Seckeler and Hoke, 2011*).

INTRODUCTION

ARF is a sequel of pharyngeal infection with Group A β hemolytic *Streptococcus*. Of the associated symptoms, only damage to the valve tissue within the heart can become a chronic condition leading to congestive heart failure, strokes, endocarditis, and death (Seckeler and Hoke, 2011).

INTRODUCTION

It is estimated that there are over 15 million cases of rheumatic heart disease worldwide.

282,000 new cases and 233,000 deaths annually (Carapetis et al., 2005).

INTRODUCTION

- ▶ Who 2011 stated that in Asia (9.6 /1000 Live births)

INTRODUCTION

The evaluation of rheumatic carditis by ultrastructural and histopathological parameters revealed different patterns of loose and dense fibrosis with variable content of collagen type I, type III, type IV, procollagen, laminin, fibronectin and elastin (Nasr, Hegazi et al., 1996).

STUDY OBJECTIVE

to detect the changes in the serum level of collagen I, collagen III, laminin and hyaluronic acid as new rheumatic fever markers more prominent and relevant for early detection and diagnosis.

EPIDEMIOLOGY OF RHEUMATIC FEVER

The overall mean incidence rate of first attack of ARF was 5-51/100,000 population. A low incidence rate of $\leq 10/100,000$ per year was found in America and Western Europe, while a higher incidence ($>10/100,000$) was documented in Eastern Europe, Middle East (highest), Asia and Australia (Tibazarwa et al., 2008).

PATHOGENESIS OF RHEUMATIC HEART DISEASE

Acute rheumatic fever is a **multifactorial** disorder that is caused by an interaction between a **rheumatogenic strain** of group A streptococcus and a susceptible host who lives in poor social conditions (*Engel et al., 2011*).

PATHOGENESIS OF RHEUMATIC HEART DISEASE

The **M protein** is the most important antigenic structure of the *S. pyogenes* and shares structural homology with cardiac myosin, tropomyosin, laminin and several valvular proteins (*Guilherme and Kalil, 2010*).

PATHOGENESIS OF RHEUMATIC HEART DISEASE

Molecular mimicry between components of *B hemolyticus streptococci* and human heart tissues is the central problem in the pathogenesis of ARF and RHD (Guilherme et al., 2011).

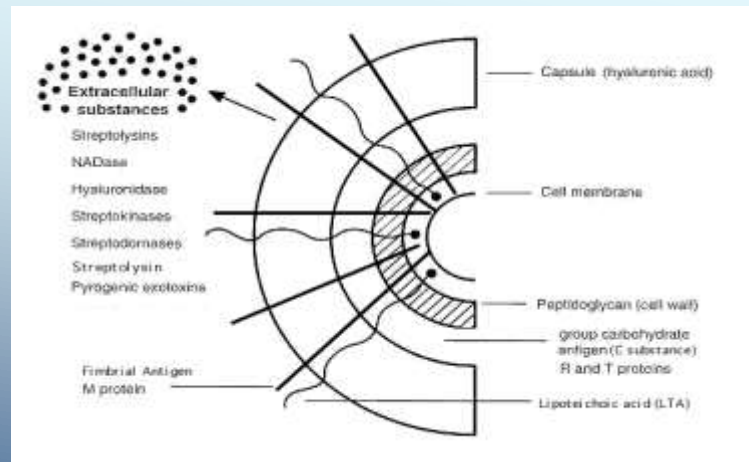
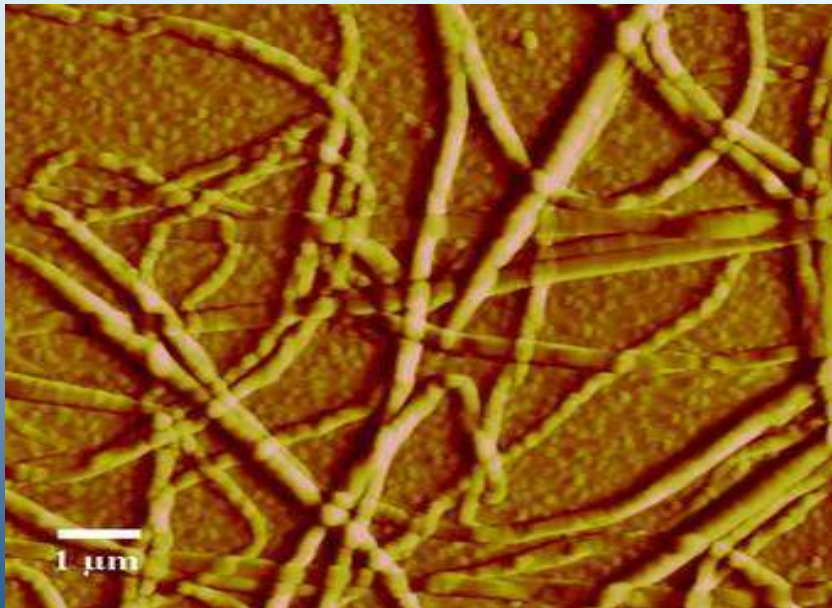
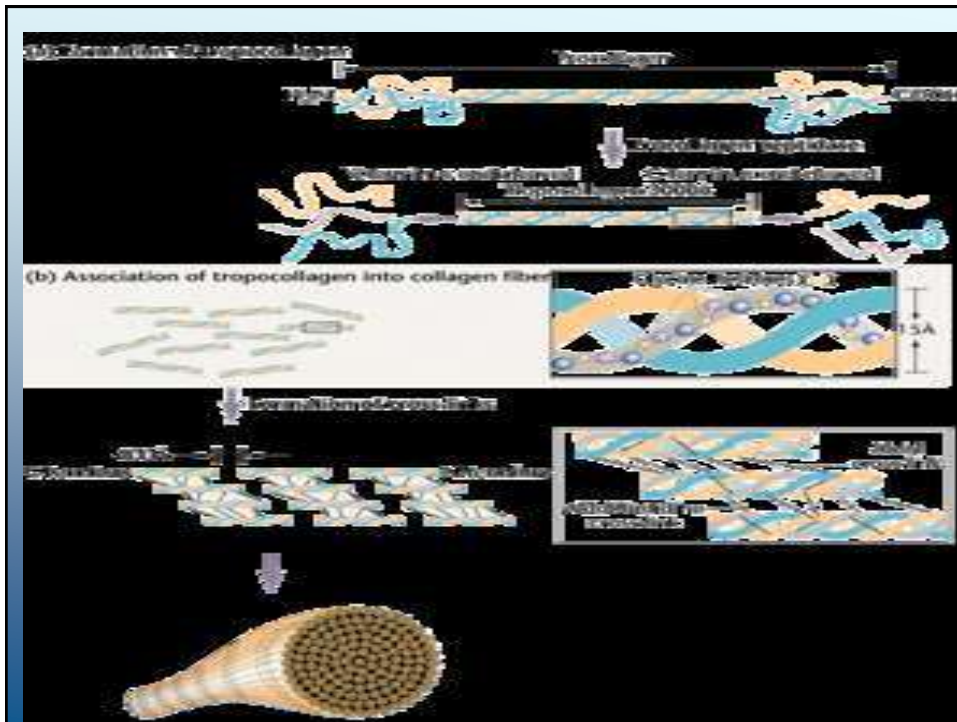


Figure : Cell surface structure of *Streptococcus pyogenes* and secreted products involved in virulence.

THE COLLAGEN

Collagens are the major proteins comprising the extracellular matrix. There are at least 30 different collagen genes that create over 28 different types of collagen fibrils. Types I, II and III are the most abundant (King, 2013).





THE COLLAGEN

The main collagen types in the aortic heart valve are collagen I (74% of total collagen) and collagen III (24% of total collagen) (Schornik et al., 2012).

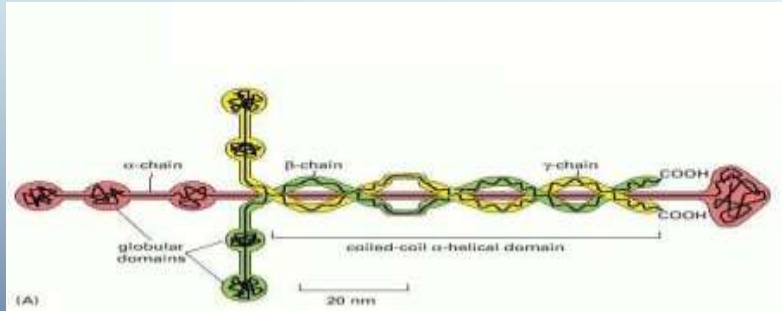
THE COLLAGEN

Collagen has been theorized to be an **autoimmune target** in the pathogenesis of ARF and RHD (Veasy and Hill, 1997).

THE COLLAGEN

Autoantibodies against the group A streptococcal carbohydrate and **cardiac myosin** appear during progression of RHD. However, autoantibodies against collagen that are not cross reactive may form because of **the release of collagen from damaged valve** (Cunningham, 2012).

LAMININ



Laminins are large heterotrimeric cross-shaped extracellular matrix glycoproteins (Santos-Valle *et al.*, 2012).

LAMININ

Laminin present in valves may cross-react with anti-myosin T cells and antibody that recognizes M protein, myosin and laminin (Ellis *et al.*, 2005).

HYALURONIC ACID

Hyaluronic acid is a major component of the valve ECM; it is found in the spongiosa layer of the valve leaflet and comprises 60% of the total content in the heart valve. (Stephens et al., 2008).

HYALURONIC ACID

Hyaluronic acid has been theorized to be an autoimmune target in the pathogenesis of ARF and RHD as they have structural similarities to components found in the capsule of Group A Streptococci (Dale et al., 1996).

PATIENTS AND METHODS

Our study is a cross sectional analytic study that included 50 patients with ARF and 50 patients with RHD ranging in age from 5 to 18 years. Age-matched group of 50 healthy children were included in our study as controls.

PATIENTS AND METHODS

All cases and controls were subjected to *thorough history taking, full clinical examination* and routine investigations of ARF which included: CBC, ESR, CRP, ASOT, chest x ray, ECG and echocardiography. In addition to evaluation of the serum level of collagen I, collagen III, laminin and hyaluronic acid using ELISA technique.

Results

TABLE (I): COMPARISON BETWEEN CASES OF ARF, RHD AND CONTROL SUBJECTS REGARDING THE SERUM LEVEL OF COLLAGEN I, COLLAGEN III, LAMININ AND HYALURONIC ACID.

	ARF	RHD	Controls	P value
Collagen I (ng/ml)	86.8 (58.9-180.4)	127.5 (84.3-233.8)	13.8 (7.3-23.3)	<0.001
Median (IQR)	B	C	A	HS
Collagen III (ng/ml)	131.0 (78.6-279.5)	109.5 (62.9-189.9)	17.3 (12.8-26.9)	<0.001
Median (IQR)	B	B	A	HS
Laminin (ng/ml)	59.0 (48.2-163.6)	92.5 (64.7-332.0)	30.3 (27.4-33.8)	<0.001
Median (IQR)	B	C	A	HS
HA (ng/ml)	216.2 (172.0-666.6)	283.5 (187.8-881.8)	57.4 (48.2-69.2)	<0.001
Median (IQR)	B	B	A	HS

RESULTS

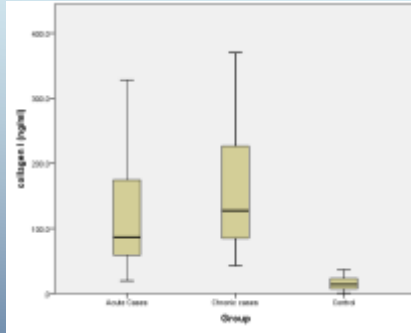


FIGURE (1): COMPARISON BETWEEN CASES OF ARF, RHD AND CONTROL SUBJECTS REGARDING THE MEDIAN AND THE INTERQUARTILE RANGE OF THE SERUM LEVEL OF COLLAGEN I

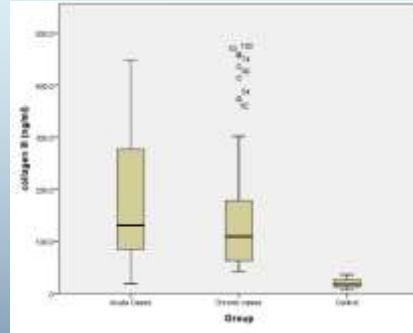


FIGURE (1): COMPARISON BETWEEN CASES OF ARF, RHD AND CONTROL SUBJECTS REGARDING THE MEDIAN AND THE INTERQUARTILE RANGE OF THE SERUM LEVEL OF COLLAGEN III

RESULTS

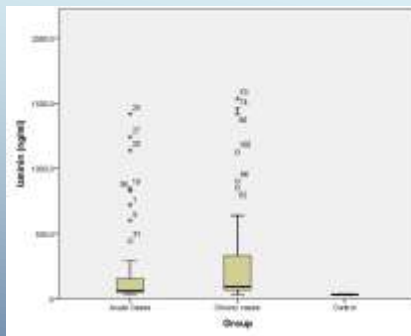


FIGURE (1): COMPARISON BETWEEN CASES OF ARF, RHD AND CONTROL SUBJECTS REGARDING THE MEDIAN AND THE INTERQUARTILE RANGE OF THE SERUM LEVEL OF LAMININ.

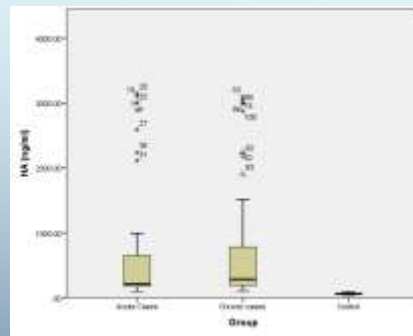


FIGURE (1): COMPARISON BETWEEN CASES OF ARF, RHD AND CONTROL SUBJECTS REGARDING THE MEDIAN AND THE INTERQUARTILE RANGE OF THE SERUM LEVEL OF HA.

Table (I): Relation between serum level of Collagen I and the clinical manifestations.

	Collagen I (ng/ml)	P value
LA. Penicillin		
Take	87.3 (70.5 – 116.5)	<0.001
Don't take	224.5 (139.9 – 298.6)	HS
H/O of Operation		
+VE	305.0 (287.4 – 335.5)	<0.001
-VE	64.0 (21.3 – 118.8)	HS
Clinical presentation		
Arthritis	69.5 (58.3-107.3)	A 0.4
Carditis	106.3 (62.3-211.9)	A NS
Combined arthritis/carditis	92.0 (59.0-229.0)	A
Congestive Heart Failure		
+VE	297.3 (250.4 – 324.0)	<0.001
-VE	63.5 (19.9 – 116.4)	HS

Table (I): Relation between the serum level of Collagen III and the clinical manifestations.

	Collagen III (ng/ml)	P value
LA. Penicillin		
Take	74.3 (55.4 – 110.9)	<0.001
Don't take	166.0 (109.9 – 355.8)	HS
H/O of Operation		
+VE	425.0 (281.0 – 457.8)	<0.001
-VE	60.5 (26.0 – 134.3)	HS
Clinical presentation		
Arthritis	103.0 (47.0-131.8)	A 0.007
Carditis	207.5 (50.6-335.6)	AB HS
Combined arthritis/carditis	232.5 (117.0-354.0)	B
Congestive HF		
+VE	381.0 (281.3 – 441.3)	<0.001
-VE	57.5 (25.3 – 127.3)	HS

RESULTS

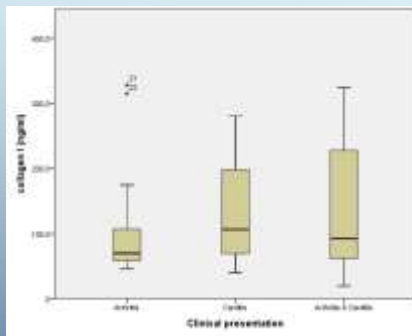


FIGURE (I): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN I AND THE CLINICAL PRESENTATION.

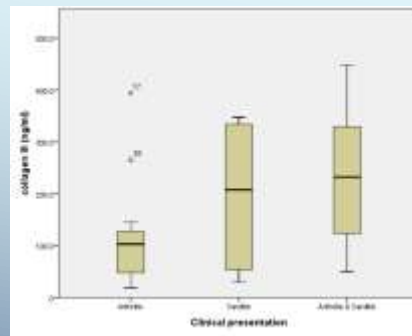


FIGURE (II): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN III AND THE CLINICAL PRESENTATION.

RESULTS

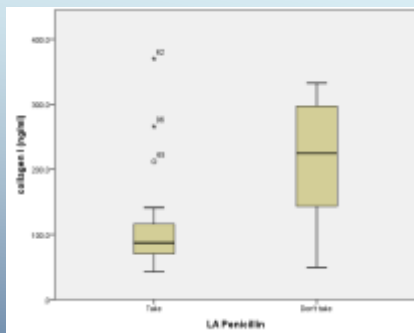


FIGURE : CORRELATION BETWEEN THE SERUM LEVEL OF **COLLAGEN I** AND LA PENICILLIN INTAKE.

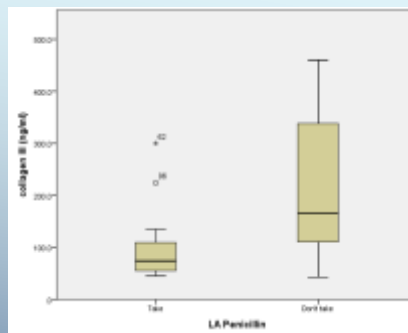


FIGURE : CORRELATION BETWEEN THE SERUM LEVEL OF **COLLAGEN III** AND LA PENICILLIN INTAKE.

Table (I): Relation between serum level of laminin and the clinical manifestations.

	Laminin (ng/ml)	P value
LA. Penicillin		
Take	72.0 (54.3 – 102.9)	0.001
Don't take	175.6 (87.0 – 447.3)	HS
H/O of Operation		
+VE	1271.9 (888.6 – 1476.5)	<0.001
-VE	52.1 (33.5 – 89.9)	HS
Clinical presentation		
Arthritis	48.8 (42.3-54.7)	A <0.001
Carditis	254.0 (49.2-734.8)	B HS
Combined arthritis/car ditis	97.6 (60.5-199.3)	B
Congestive HF		
+VE	1130.8 (817.8 – 1429.6)	<0.001
-VE	50.3 (33.2 – 85.0)	HS

Table (I): Relation between the serum level of hyaluronic acid (HA) and the clinical manifestations.

	Hyaluronic acid (ng/ml)	P value
LA. Penicillin		
Take	200.3 (157.4 – 248.6)	<0.001
Don't take	634.3 (319.6 – 1803.2)	HS
H/O of Operation		
+VE	2994.8 (2717.0 – 3062.8)	<0.001
-VE	172.5 (67.2 – 316.1)	HS
Clinical presentation		
Arthritis	194.3 (170.2- 223.7)	A 0.02
Carditis	857.4 (138.9- 2693.2)	AB S
Combined arthritis/car ditis	460.1 (190.1- 979.2)	B
Congestive HF		
+VE	2994.8 (2717.0 –	<0.001

RESULTS

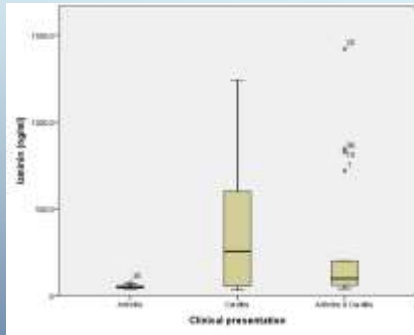


FIGURE (1): CORRELATION BETWEEN THE SERUM LEVEL OF LAMININ AND THE CLINICAL PRESENTATION.

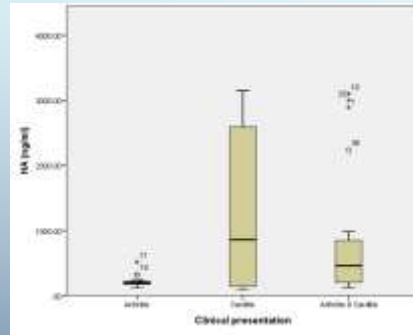


FIGURE (2): CORRELATION BETWEEN THE SERUM LEVEL OF HA AND THE CLINICAL PRESENTATION.

RESULTS

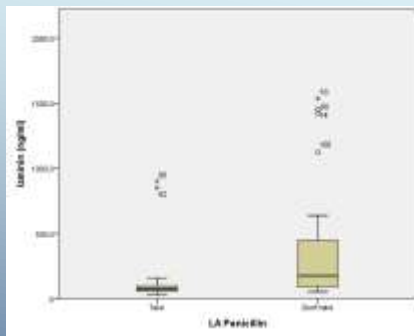


FIGURE (3): CORRELATION BETWEEN THE SERUM LEVEL OF LAMININ AND LA-PENICILLIN INTAKE.

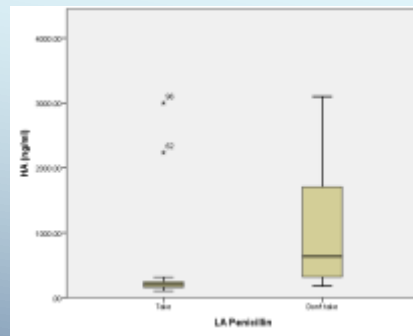


FIGURE (4): CORRELATION BETWEEN THE SERUM LEVEL OF HA AND LA-PENICILLIN INTAKE.

RESULTS

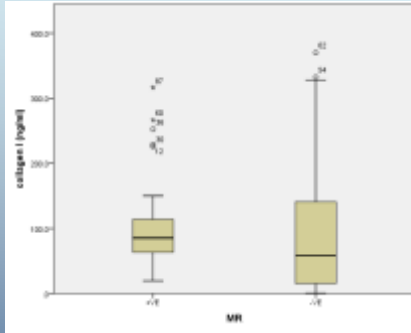


FIGURE (I): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN I AND MITRAL REGURGITATION.

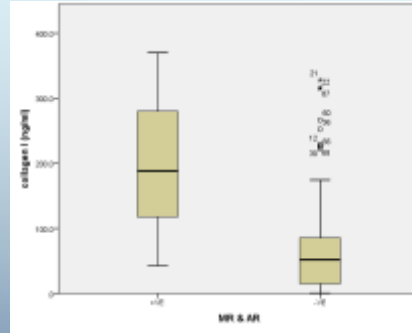


FIGURE (J): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN III WITH COMBINED MITRAL AND AORTIC REGURGITATION.

RESULTS

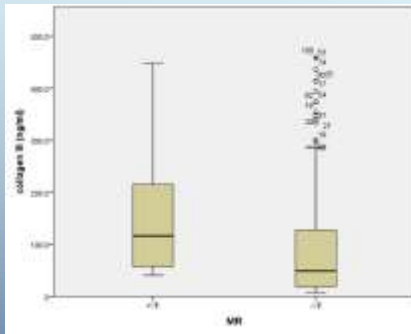


FIGURE (I): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN III AND MITRAL REGURGITATION.

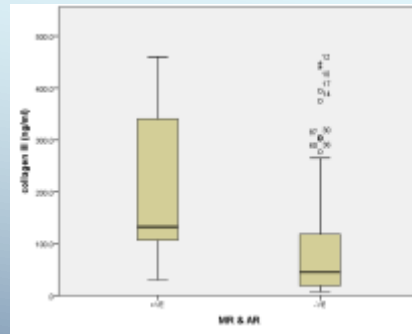


FIGURE (J): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN I WITH COMBINED MITRAL AND AORTIC REGURGITATION.

RESULTS

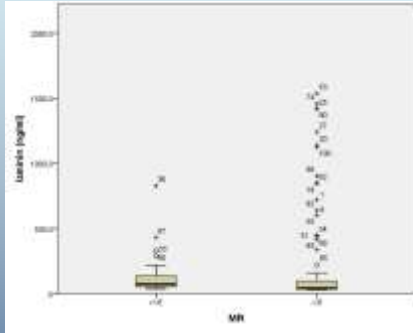


FIGURE (): CORRELATION BETWEEN THE SERUM LEVEL OF LAMININ AND MITRAL REGURGITATION.

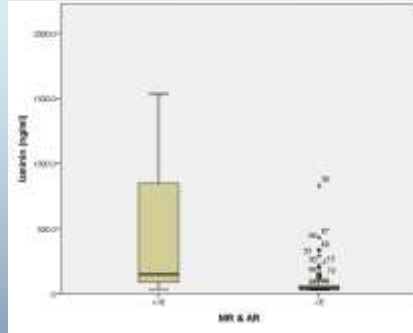


FIGURE (): CORRELATION BETWEEN THE SERUM LEVEL OF LAMININ WITH COMBINED MITRAL AND AORTIC REGURGITATION.

RESULTS

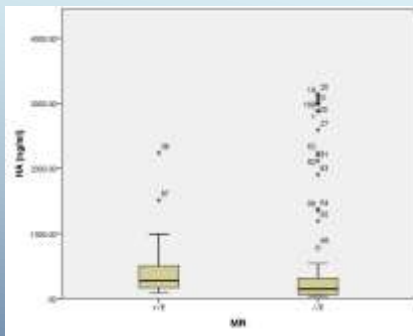


FIGURE (): CORRELATION BETWEEN THE SERUM LEVEL OF HA AND MITRAL REGURGITATION.

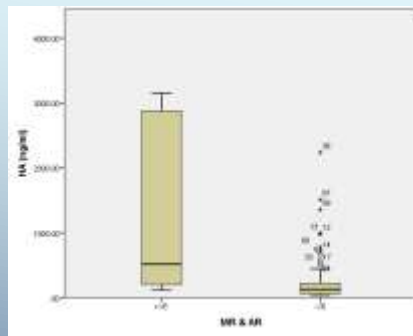


FIGURE (): CORRELATION BETWEEN THE SERUM LEVEL OF HA WITH COMBINED MITRAL AND AORTIC REGURGITATION.

TABLE (1): CORRELATION OF THE 4 BIOCHEMICAL MARKERS WITH NYHA CLASSIFICATION.

Within ARF group		
		NYHA
Collagen I	rho	0.74
	P	<0.001
Collagen III	rho	0.54
	P	<0.001
Laminin	rho	0.70
	P	<0.001
HA	rho	0.79
	P	<0.001
Within RHD group		
		NYHA
Collagen I	rho	0.85
	P	<0.001
Collagen III	rho	0.86
	P	<0.001
Laminin	rho	0.80
	P	<0.001
HA	rho	0.68
	P	<0.001

RESULTS

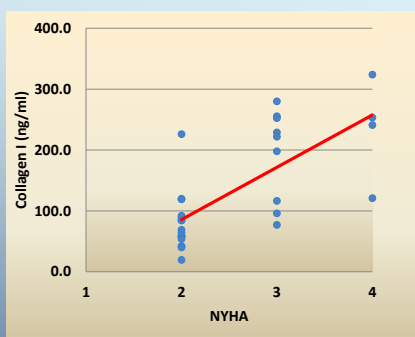


FIGURE (1): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN I AND NYHA CLASSIFICATION IN ARF.

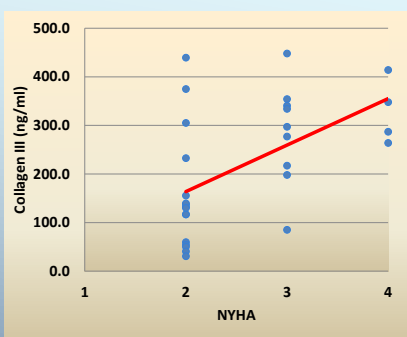


FIGURE (2): CORRELATION BETWEEN THE SERUM LEVEL OF COLLAGEN III AND NYHA CLASSIFICATION IN ARF.

RESULTS

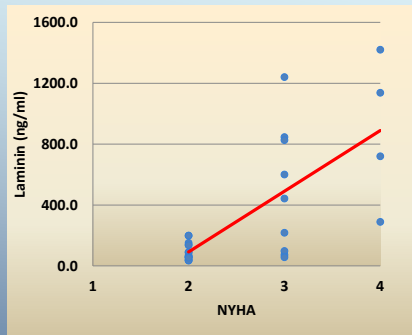


FIGURE (1): CORRELATION BETWEEN THE SERUM LEVEL OF LAMININ AND NYHA CLASSIFICATION IN ARF.

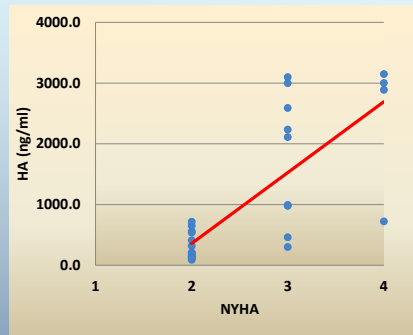


FIGURE (2): CORRELATION BETWEEN THE SERUM LEVEL OF HA AND NYHA CLASSIFICATION IN ARF.

CONCLUSION

- ▶ Until now rheumatic fever is considered **public health** problem all over the world.
- ▶ **Collagen I, collagen III, laminin and hyaluronic acid** proved to have a significant role in the biological process of ARF and RHD.

CONCLUSION

- Mitral valve is more affected by RHD than the aortic valve.

CONCLUSION

- In the sera of ARF and RHD patients, the extracellular matrix components as collagen I, collagen III, laminin and hyaluronic acid can be used as new markers for diagnosis and follow up the course of the disease as it correlates with the NYHA classification based grading.

RECOMMENDATIONS

It is important to measure the serum level of collagen I, collagen III, laminin and hyaluronic acid in the acute phase of rheumatic fever, a case of which will add new parameters for early diagnosis and enable the control and follow up of the disease.

RECOMMENDATIONS

- ▶ We draw the attention to the importance of the follow up of the RHD patients pre and/or post surgical treatment by the serum level of acute phase reactants as well as the new markers (collagen I, collagen III, laminin and hyaluronic acid) in correlation with the NYHA based grading.

RECOMMENDATIONS

The national authorities must raise the **socioeconomic and educational** status of the community especially in rural areas.

The **junior staff** must be trained well for the early detection of rheumatic fever. This will be of great value in lowering the complications, in management and eradication of RHD.

RECOMMENDATIONS

- Further **genetic**, molecular biology, **histobiochemical** and immunologic investigations are recommended to establish the exact pathogenesis of rheumatic fever.

RECOMMENDATIONS

- ▶ Further study can be repeated on a **large scale** to verify the exact role of serum level of collagen I, collagen III, laminin and hyaluronic acid in ARF and RHD.

TAKE HOME MESSAGE

RHEUMATIC FEVER IS A **MULTIFACTORIAL**, AND **MULTISYSTEMIC** DISEASE .

THE **CORNER STONE** OF ITS DIAGNOSIS TILL NOW IS DEPEND ON THE **CLINICAL SCALES** AS IT SHARE A LOT OF OTHER DISEASES .

THE **EARLY** DIAGNOSIS OF THE DISEASE IS THE **KEY** FOR ITS **ERADICATION**.

