# STUDY OF NOVEL MARKERS FOR RHEUMATIC FEVER

Thesis

Submitted By

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# *Abstract*

Rheumatic fever is a chronic inflammatory multifactorial disease which may lead to rheumatic carditis and permanent valve damage.Our study included 50 patients with acute rheumatic fever and 50 patients with rheumatic heart disease; they were 58 females and 42 males ranging in age from 5 to 18 years. 50 age matched healthy children were includedas controls. Using ELISA technique, we studied the changes in the serum level of collagen I, collagen III, laminin and hyaluronic acid as new rheumatic fever markers more prominent and relevant forearly detection and diagnosis. The results were analyzed statistically and revealed that there is a highly significant increase in the serum level of collagen I, collagen III, laminin and hyaluronic acid in patients with acute rheumatic fever and rheumatic heart disease. Moreover, we found a highly significant statistical correlation between these biochemical markers and the clinical severity of the disease.

**Key words:**

Acute rheumatic fever- rheumatic heart disease- collagen type I- collagen type III – laminin- hyaluronic acid.

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| | |
|---|---|
| **AR** | Aortic Regurgitation |
| **ARF** | Acute Rheumatic Fever |
| **ASOT** | Anti Streptolysin O Titre |
| **BM** | Basement Membrane |
| **CBC** | Complete Blood Count |
| **CFBs** | Cardiac Fibroblasts |
| **CICP** | *C- Terminal Propeptide Of Type I Collagen* |
| **CITP** | Carboxy-Terminal Telopeptide Of Collagen Type I |
| **CIIITP** | Carboxy-Terminal Telopeptide Of Collagen Type III |
| **COL I** | Collagen Type I |
| **COL III** | Collagen Type III |
| **CRP** | C- Reactive Protein |
| **ECG** | Electrocardiogram |
| **ECM** | Extracellular Matrix |
| **ELISA** | Enzyme Linked Immunosorbant Assay |
| **EM** | Erythema Marginatum |
| **ER** | Endoplasmic Reticulum |
| **ESR** | Erythrocyte Sedimentation Rate |
| **FACITs** | Fibril-Associated Collagens With Interrupted Triple Helices |
| **FS** | Fractional Shortening |
| **GAGs** | Glycosaminoglycans |
| **GAS** | Group A Streptococci |
| **GlcNAc** | N-Acetyl-Beta-D-Glucosamine |
| **HA** | Hyaluronic Acid |
| **HABP** | Hyaluronic Acid Binding Protein |
| **HF** | Heart Failure |
| **HLA** | Human Leukocyte Antigen |
| **HS** | Highly Significant |
| **IFN-g** | Interferon Gamma |
| **IL** | Interleukin |

| | |
|---|---|
| **IQR** | Interquartile Range |
| **LN** | Laminin |
| **MACITs** | Membrane-Associated Collagens With Interrupted Triple Helices |
| **MBL** | Mannose Binding Lectin |
| **MMPs** | Matrix Metalloproteinases |
| **MR** | Mitral Regurgitation |
| **MS** | Mitral Stenosis |
| **MULTIPLEXINs** | Multiple Triple-Helix Domains And Interruptions |
| **NITP** | Amino-Terminal Telopeptide Of Collagen Type I |
| **NIIITP** | Amino-Terminal Telopeptide Of Collagen Type III |
| **NS** | Non Significant |
| **NYHA** | New York Heart Association |
| **PARF** | Peptide Associated With Rheumatic Fever |
| **PGs** | Proteoglycans |
| **PICP** | C-Terminus Of Procollagen I |
| **PINP** | N-Terminus Of Procollagen I |
| **PIIIP** | Pro Collagen III *Peptide* |
| **PIIINP** | N-Terminus Of Procollagen III |
| **RER** | Rough Endoplasmic Reticulum |
| **RHD** | Rheumatic Heart Disease |
| **S** | Significant |
| **SD** | Standard Deviation |
| **SST** | Serum Separator Tube |
| **Th** | T Helper |
| **TIMPs** | Tissue Inhibitors Of Metalloproteinases |
| **TNF-α** | Tumor Necrosis Factor Alpha |
| **WHO** | World Health Organization |

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# Introduction

Liver cirrhosis [LC] is the final evolutive stage of any chronic liver disease and it is a condition prone to multiple complications because of portal hypertension. Development of esophageal varices [EV] is a major complication that may occur in up to 90% of cirrhotic patients ***(Jensen, 2002).***

Variceal bleeding is a life-threatening event that has an incidence of 5% in patients with small EV and up to 15% in those with large esophageal varices [LEV]. Mortality per bleeding episode is around 10-20% ***(Carbonell et al., 2004)***, and one year survival is only 63%. Therefore, screening for EV in LC patients is a strong recommendation in all consensus statements ***(de Franchis, 2005).***

The current screening method is endoscopy at 2-3 years in patients without EV, and at 1-2 years in those with small EV. This approach is, however, invasive, poorly accepted by patients and expensive. This is why the selection of patients with LEV at high risk for bleeding has become an issue of growing importance. In this respect, several clinical, biological, ultrasonographic and scores methods have been proposed [and some of them validated] as noninvasive alternatives to endoscopy ***(de Franchis., 2008).***

Based on the concept that the development of portal hypertension is due to liver fibrosis, as the most important factor contributing to the increased hepatic resistance, noninvasive serum markers of liver fbrosis have been tested as predictors of EV in cirrhotic patients with promising results. The most validated is the FibroTest, which is not currently widely available because of its cost and complexity ***(Stefanescu et al., 2011).***

# Aim of the Work

The hypothesis behind our study is that common tests previously validated as predictors of liver fibrosis, such as APRI, Fib-4, Forns Index and Lok Score can also be used to predict the presence of esophageal varices. Our aim was to evaluate the performance of the above mentioned fibrosis scores and the fibroscan in diagnosing and grading of esophageal varices.

# Pathophysiology of Portal Hypertension

## ***Anatomical Background:***

### The Liver Vasculature:

The liver is a richly perfused organ receiving approximately 25% of the cardiac output. About 75% of hepatic blood flow (rich in nutrients but poorly oxygenated) is supplied by the portal vein. The remainder of the blood supply (oxygen rich) is provided by the hepatic artery. The intrahepatic vasculature is composed of portal venules, hepatic arterioles, lymphatics, hepatic sinusoids, and central venules ***(Mc Cuskey, 2000).***

The hepatic sinusoid, which is the principal site of blood flow regulation, is the narrowest vascular structure within the liver; the highest vascular resistance occurs in the sinusoids ***(Bhunchet and Wake, 1998).*** The hepatic sinusoid is the vital site for transvascular exchange between blood and hepatocytes. The sinusoidal surface of the hepatocytes is separated from blood by fenestrated sinusoidal endothelial cells lining the sinusoid, Kuppfer cells (liver macrophages) protruding into the lumen of the sinusoid, Pit cells (liver specific natural killer cells) and hepatic stellate cells (HSC) ***(Burt et al.,1993).*** HSC may play a role in blood flow regulation in the normal liver. The first direct evidence was provided by in vivo microscopy in normal rat liver ***(Bauer et al., 2000).***

### Anatomy of the Portal Venous System:

The portal vein begins at the junction of the splenic vein (SV) and the superior mesenteric vein (SMV), immediately posterior to the pancreatic neck at about the level of L2.From its origin, it courses superiorly and toward the right passing behind the first part of the duodenum and anterior to the inferior vena cava (IVC). After it enters the liver, the PV divides into right and left branches that re-divide into

smaller branches to terminate in the sinusoidal network of the liver ***(Fruechte and Zwiebel, 1992).***

The ligamentum teres joins the left branch of the portal vein containing the umbilical vein within it. The left gastric vein (coronary vein) joins the portal vein near its origin (or the splenic vein in 16%). The inferior mesenteric vein draining the left colon and rectum joins the main splenic vein usually in its medial third. The main splenic vein is formed at the splenic hilum by the convergence of the splenic veins (5-15), joined by the short gastric veins ***(Fruechte and Zwiebel, 1992).***

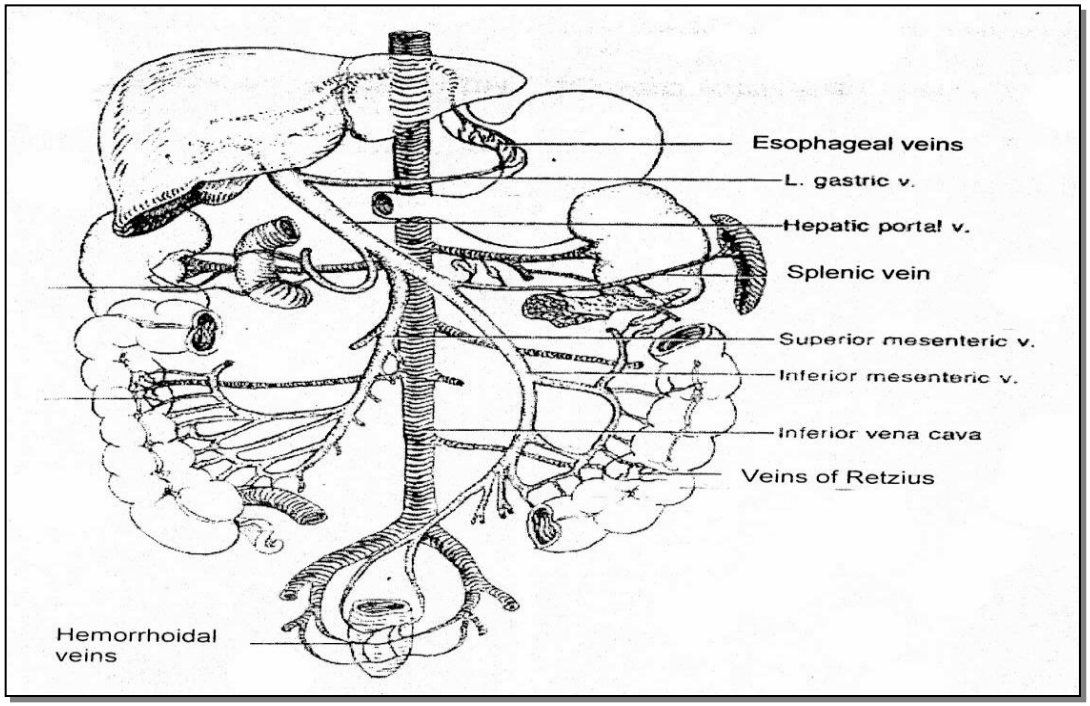


**Figure (1-1):** The anatomy of the portal venous system ***(Sherlock & Dooley, 2002)***

## Portosystemic Collaterals:

It is necessary that the portal pressure gradient reaches a value of 12 mm Hg for the formation of collateral channels to the vena cava system. Closure of the collaterals can occur gradually when its resistance exceeds that of the porto-hepatic bed as after porto-caval shunt surgery ***(Garcia-Tsao, 1997).*** Normally 100% of the portal blood flow can be