Serum levels of IL-8 in chronic RHD

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Abstract
Rheumatic heart disease (RHD) remains a serious public health problem in developing countries. The form of immune response after revelation to streptococcus is one of the main factors of the rheumatic inflammatory process, making it essential to identify the patients who present a higher risk of disease progression. Inflammation plays an important role in rheumatic heart disease. Very few studies are done so far concerning serum interleukin-8 (IL-8) levels in chronic rheumatic heart disease (RHD) patients. This study aimed to determine if serum IL-8 can be used as a biomarker of RHD. The study employed 20 chronic RHD patients and 20 healthy age and sex-matched controls for serum analysis of IL-8 on Enzyme-Linked Immunosorbent Assay (ELISA). Study results show that there is a significant difference ($p<0.0005$) in the IL-8 levels of chronic RHD patients [median (IQR) 104.3(121.3) pg/mL] from controls [median (IQR) 18.6 (72.0) pg/mL]. At present very less is known about the role of IL-8 in chronic RHD, the study findings show that IL-8 which a pro-inflammatory cytokine can be used as a biomarker in chronic RHD patients.

Keywords: *Interleukin-8, cardiovascular disease, ELISA, proinflammatory cytokine, rheumatic fever, rheumatic heart disease.*

I. Introduction

Regardless of advancement in diagnosis and treatment, Cardiovascular disease (CVD) is the leading cause of death worldwide which has become a global public health concern [1] including India [2]. In India, the prevalence of Heart Failure (HF) has raised mainly due to traditional risk factors like CVD, RHD, anemia, etc [3]. Thus CVD is a wide term that describes a cluster of disorders of the heart and blood vessels with CHD, cerebrovascular diseases, peripheral arterial disease, congenital heart disease, RHD, deep vein thrombosis, and pulmonary embolisms [1]. Infection by group A streptococcus (Streptococcus pyogenes) is responsible for Rheumatic fever (RF), which is a rheumatic and inflammatory disease of autoimmune origin, which has become a serious public health problem. On a worldwide measure, this agent is responsible for roughly 15.6 million annual cases of RHD, with an incidence of 282,000 cases and mortality of 233,000 each year [4]. Thus, the pathogenesis of RF in vulnerable individuals is related to autoimmune humoral and cellular responses directed toward human tissues, generated by the response to β-hemolytic group A streptococci. About 30 to 45% of RF patients progress towards RHD where cardiac involvement induces pericardium, myocardium, and endocardium inflammation, which permanently damages heart valves[5]. Many researchers have investigated the pathogenesis of RHD, yet there are many gaps to understand the basic mechanism, whereas many genes have been studied so far to understand the basic pathogenesis of RHD. After so much researches, it has been shown that the inflammatory cytokines may contribute to the pathogenesis of RHD. Thus, the cytokines are hormones like (because their properties are similar to those of the classic hormones of the endocrine system) diverse group of proteins generated by body's immune system which are small molecules secreted by various cells, turn as a signal between cells which regulate immune response to infection and injury. They are usually regulated by cascade where induction of the early cytokines serves to increase the production of later cytokines. Thus, these cytokines are further divided into pro and anti-inflammatory cytokines where interleukin-8 works as a pro-inflammatory cytokine[6].

IL-8 is synthesized by mononuclear cells, fibroblasts, endothelial cells, keratinocytes, and thrombocytes. Synthesis of IL-8 is stimulated by IL-1 and TNF-α where chemotactic features of IL-1 and TNF-a are mediated by IL-8 which is a decisive chemokine recognized to attract and activate neutrophils as well as T lymphocytes and to regulator their interaction[7]. IL-8 a chemokine has been implicated in a wide range of acute and chronic inflammatory pathologies, which circulating in picomolar concentrations. It is synthesized by various immunologic and nonimmunologic cells and exerts

pleiotropic effects[1]. However, their role in the development of RHD has not been clearly defined. Very few studies have been done so far regarding the role of IL-8 in RHD. Therefore, it seemed of interest to study the role of serum IL-8 in RHD patients.

II. Materials and methods

This was a case-control study conducted at the Cardiology and Medicine Department from a tertiary care Sion hospital of Mumbai metro city of India. In this study, 20 cases of RHD were enrolled in Cardiology and Medicine Department. Age and sex-matched controls were health care workers from HBTMC and Dr. R. N. Cooper General Hospital, Juhu, Mumbai. All the cases were diagnosed by cardiologists and final patient selection was done. The diagnostic test included 2D Echo along with an electrocardiogram.

The inclusion criteria were RHD patients undergoing hospitalization in Cardiology and Medicine ward, who were willing to participate in the study and signed the informed consent. Patients with chronic illnesses such as malignancies, infections (where the inflammatory markers are presumed to be raised) were excluded, the study protocol was approved by the institutional ethics committee. Informed consent was taken from all study subjects, both cases and controls after explaining the purpose of the study.

Venous blood was drawn in a plain tube without anticoagulant and centrifuged for 10 minutes at 2000 rpm at room temperature. Serum was separated in the screw-type vials and stored at -40°C (BD Instruments). The serum IL-8 was measured by human IL-8 Enzyme-Linked Immunosorbent. Assay (ELISA) kits (Affymetrix, eBiosciences, San Diego, CA, USA) at Hinduridaysamrat Balasaheb Thackeray Municipal Medical College and Dr. RN Cooper General Hospital. This ELISA set is specifically engineered for accurate and precise measurement of protein levels from serum samples which recognize the cleaved mature form and uncleaved pro-form of Human IL-8.

III. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 16.0 and Microsoft Excel 2007. Continuous data has been expressed as mean (Standard deviation) and median (Interquartile range) and categorical data are summarized as frequencies and percentages. The normality of the data was tested by the Shapiro-Wilk test. For interleukin-8, Mann- Whitney U test was applied. p values <0.05 (2-tailed) was used to identify statistical significance.

IV. Results

Table 1: Comparison of baseline characteristics and serum level of interleukin-8 between two groups.

	Case (n=20)	**Control (n=20)**	***p-value***
Age (years)	42.41 (16.92)	42.00 (17.05)	0.958
Male	8	8	-
Female	12	12	-
Body Mass Index (kg/m2)	24.43 (3.61)	25.74 (3.19)	<0.0005
Blood pressure			
Systolic (mmHg)	122.97 (18.98)	-	-
Diastolic (mmHg)	77.32 (11.32)	-	-
IL-8 pg/mL median (IQR)	104.3 (121.3)	18.6 (72.0)	<0.0005*

*Using Mann-Whitney U test, significant at 0.05 level of significance

V. Discussion

The main finding in this study is that significantly raised concentrations of serum inflammatory marker IL-8 were seen in the chronic RHD group compared to controls ($p<0.0005$). The median (IQR) levels of interleukin-8 were 104.3(121.3) pg/mL in cases compared to healthy age and sex-matched controls 18.6 (72.0) pg/mL.

A single severe episode or multiple recurrent episodes of RF leads to RHD which further leads to long-term cardiac damage. Not all patients who develop chronic RHD have a clinical history of RF. Most patients with mild to moderate valvular connection will remain asymptomatic for years. RHD remains a significant worldwide cause of morbidity and mortality, particularly in a resource-poor setting, mainly affect those in low- and middle-income nations, as well as in home-grown populations in wealthy nations where initial S. pyogenes infections may not be treated [8].

IL-8 circulates in two major isoforms: a 72-amino acid monocyte-derived form ([Ser-IL-8]72) and a 77-amino acid ([Ala-IL-8]77), mainly produced by endothelial cells, has been shown to increase production and surface expression of tissue factor, an important inducer of blood coagulation in monocytes providing a potential link between inflammation and thrombosis (thrombogenesis). IL-8 binds to its

endothelial receptors IL-8R (CXCR1 or CXCR2) and acts as a chemoattractant for monocytes and neutrophils into the vascular wall, a key step in atherogenesis. Thus, it is involved in the transition from acute to chronic inflammation. IL-8 has shown to increase production and surface expression of tissue factor, an important inducer of blood coagulation in monocytes providing a potential link between inflammation and thrombosis [1].

Synovial cells too secret IL-8 may contribute to cellular accumulation in inflamed joints. Thus, in RF inflamed joints and tissues, which may boost IL-8 production and may cause cellular infiltration in the synovial membranes and heart valves, as IL-8 has a major activity of chemotactic attraction of neutrophils and T cells[9].

Up till now, very few studies have been carried out. Our study goes hand in hand with Davutoglu V. et al (2005) who had shown significantly raised plasma IL-8 concentrations in rheumatic valve disease patients compared to controls.

VI. Conclusion

The inflammatory marker, serum IL-8 (measured by ELISA which is relatively cost-effective assay) is a potentially useful biomarker for evaluation of chronic RHD and can be included as a diagnostic marker along with other proven markers. As per existing knowledge, ours is the first study in Western parts of Maharashtra, in Mumbai metro city, India. These findings will be confirmed by future longitudinal prospective studies and could be determined with long term clinical trials with large sample size in Indian populations. Thus, future research in this area should focus on whether anti-inflammatory drugs might reduce progression, morbidity, and mortality in patients with chronic RHD.

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