Original Research Article

Role of serum interleukin-6 in heart failure

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ABSTRACT

Background: A growing body of evidence suggests that inflammation plays the key role in different cardiovascular diseases. But very study has been done so far in relation to serum interleukin-6 in heart failure patients. The aim of the study was to measure serum interleukin-6 in heart failure patients.
Methods: Total 22 heart failure patients and 22 age and sex matched controls were included in this study from August 2015 to June 2016 for serum analysis of interleukin-6.
Results: serum interleukin-6 was significantly [median(IQR) 14.3(26.2) pg/mL] increased in heart failure patients compared to age and sex matched controls [median(IQR) 0(2.4) pg/mL].
Conclusions: Even though little is known about function of interleukin-6 in heart failure patients, this study shows that increased level of IL-6 in heart failure patients plays an important role as a pro-inflammatory marker in development of cardiovascular disease i.e. heart failure.

Keywords: Cardiovascular disease, Enzyme-linked imunosorbent assay, Heart failure, Inflammatory markers

INTRODUCTION

Globally, coronary artery disease is proven to be main cause of death with increase in prevalence in Western world.[1] Once it was a considered as a disease of rich people is widely spreading among low and middle-income countries contributing three-quarters of all CVD deaths in global.[2] HF incidence and prevalence are unreliable in India due to lack of surveillance system to capture the data properly and lack of standard definition. In India prevalence of HF has raised mainly due to traditional risk factor CVD, RHD, anaemia etc. As HF is predominant disease of elderly population risk increases along with the age. In India alone in 2000 there were 30 million people suffered with CHD with 3% prevalence and incidence of HF with CHD is 0.4% to 2.3% per year.[3] Several epidemiological studies have concluded that dislipidemia like increased total cholesterol, decreased high density lipoproteins are linked with Coronary heart disease mortality and incidence.[4] Now a day's atherosclerosis is extensively accepted as a chronic inflammatory disease. It is due to initiators like vascular or extra vascular sources.[5] Complex network of mediators and signaling pathways are important components of inflammatory reactions. Interleukins are responsible for communication between white blood cells, chemokines that promote chemotaxis and interferons which have antiviral effects which are further involved in innate and adaptive immunity, playing a noteworthy role in lymphoid tissue oncogenesis, vasculogenesis and tissuue repairing.[6] Chronic alteration of expression of these

inflammatory markers alter, diseases often occur. Thus, inflammation is the important process takes place in heart failure.[7]

Pro-inflammatory marker like interleukin-6 is involved in the systemic inflammatory response, but also engages in local tissue inflammation35 and promotes differentiation of naive T-helper cells into Th17 cells, released from macrophages and T-lymphocytes.[8] In liver this pro-inflammatory cytokine further stimulates release of acute phase reactants like C-reactive protein (CRP).[9] Increased level of interleukin-6 was seen in acute myocardial infarction, coronary syndrome, unstable angina patients, acute and in advanced heart failure patients.[4,5,9-18] Until now C reactive protein is the only established inflammatory marker by World Health Organization standard for coronary risk.[19] Therefore, authors hypothesized that this interleukin-6 can not only serve as new marker for diagnosis of heart failure but also can be used for identification of high risk heart failure patients. To address this hypothesis, we examined pro-inflammatory cytokine interleukin-6 level in heart failure patients.

METHODS

This was a case control study conducted at Mumbai city from August 2015 to June 2016. In this study 22 cases of heart failure and 22 age and sex matched controls were enrolled after written and informed consent. Cases were from Cardiology and Medicine Department from Lokamanya Tilak Municipal General Hospital, Sion, Mumbai. Age and sex matched controls were health care workers from Hinduridaysamrat Balasaheb Thackeray Municipal Medical College and Dr. R. N. Cooper General Hospital, Juhu, Mumbai. Patients with chronic illnesses such as malignancies, infections, rheumatoid arthritis, and liver cirrhosis were excluded, where the inflammatory markers are presumed to be raised. Venous blood was drawn in a plain tube without anticoagulant and centrifuged for 10 minutes at 2000 rpm. Serum was separated in the screw type vials and stored at -40oC (B.D. Instruments). The serum IL-1β was measured by human IL-1β Enzyme-Linked Imunosorbent Assay (ELISA) kits (Affymetrix, eBiosciences, San Diego, CA, USA) at Hinduridaysamrat Balasaheb Thackeray Municipal Medical College and Dr. R. N. Cooper General Hospital. This ELISA set is specifically engineered for accurate and precise measurement protein levels from serum samples which recognizes the cleaved mature form and uncleaved pro-form of Human interleukin-6.

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 is summarized

as frequencies and percentages. The normality of the data was tested by Shapiro-Wilk test. For interleukin-6, Mann-Whitney U test was applied. p values <0.05 (2-tailed) was used to identify statistical significance.

RESULTS

The Inflammatory marker serum interleukin-6 was significantly raised in cases as compared to controls (p < 0.0005). The median (IQR) levels of interleukin-6 was 14.3(26.2) pg/mL in cases compared to healthy age and sex matched controls 0(2.4) pg/mL.

Table 1: Comparison of baseline characteristics and serum level of interleukin-6 between two groups.

	Case (n=22)	Control (n=22)	p-value
Age (years)	54.49±11.71	54.03±12.35	0.636
Male	14 (63.6%)	12 (54.54%)	-
Female	8 (36.4%)	10 (45.45%)	-
Body Mass Index (kg/m^2)	24.43 (3.61)	25.74 (3.19)	<0.0005
Blood pressure			
Systolic (mmHg)	122.97 (18.98)	–	–
Diastolic (mmHg)	77.32 (11.32)	-	-
Interleukin-6 pg/mL	Median (IQR) 14.3(26.2)	Median (IQR) 0 (2.4)	<0.0005*

DISCUSSION

Heart failure is the condition with topical symptoms and signs like dyspnea, leg swelling, paroxysmal nocturnal dyspnea, and orthopnea which is the cause of any structural or functional impairment of ventricular filling or ejection of blood. This leads to decrease in oxygen delivering capacity to the metabolizing tissues like kidney, bone marrow and liver.[20] Traditional cardiovascular disease and persistent- pretransitional diseases like rheumatic heart diseases (RHD), anemia has increasing the prevalence of heart failure in India.[3] Pro-inflammatory marker interleukin-6 plays an important role in immune cell activation, influences lipid metabolism and it also destabilizes atherosclerotic plaque.[4,21,22] The present study indicates that significantly increased serum interleukin-6 level works as a risk factor in heart failure patients. This preliminary study, to our knowledge is the first study to detect increased serum interleukin-6 levels in heart failure patients from Mumbai city.

Wainstein et al observed raised interleukin-6 level in coronary artery disease and suggested to be useful marker for prediction of coronary artery disease.[1] Volpato et al showed association between serum interleukin-6 levels with mortality in 620 elderly women with 3 years follow-up.[23] They found significantly increased levels of

interleukin-6 in cardiovascular disease patients have 4-fold increase in death than the patients who had less interleukin- 6 levels. Sendesni et al found significant increase in serum interleukin-6 levels in coronary patients compared to controls.[13] In a case control study, Nijm et al evaluated significantly raised serum interleukin-6 in acute coronary syndrome and stable angina pectoris than the controls.[24] Min et al found significantly raised serum IL-6 levels in coronary artery patients compared to healthy controls.[25] Ikonomidis et al observed raised serum IL-6 levels in a stable angina patients had more than twice median IL-6 (3.9 versus 1.7 pg/mL) compared to controls and found significant reduction in circulating IL-6 levels after aspirin supplementation in a randomized placebo-controlled trial.[26]

Wainstein et al observed that serum IL-6 level above 1 pg/mL is predictive of significant CAD in 27% out of 48 patients with a mean 10-year and found to had 100% CAD.[1] In a cross sectional study done by Alwi.[9] found out raised serum IL-6 levels in acute coronary syndrome than in coronary heart disease (mean 40.85pg/mL verses mean 4.58 pg/mL) and concluded the role of IL-6 in the pathophysiology of ACS. Testa et al reported elevated serum levels of IL-6 in functional class IV of New York Heart association(NYHA) than in NYHA class I, II and III, suggested the role of immune activation in congestive heart failure patients.[27] Torre-Amione et al observed increased levels of serum IL-6 in heart failure patients in deteriorating functional classes I to III (3.3±0.55, 6.2±1.1, 5.22±0.9 pg/ml, respectively) compared with age-matched control subjects (1.8±0.5 pg/ml) and suggested to serve as biochemical markers for the progression from asymptomatic to symptomatic left ventricular dysfunction dut to activation of neurohormonal system.[28] Rauchhaus et al revealed that increased plasma concentrations of cytokines (IL-6) and soluble cytokine receptors significantly predict impaired median to longer-term survival in patients with CHF.[29] IL-6 were significantly greater in the patients with NYHA class IV heart failure when compared with NYHA class III heart failure patients, a study done by Deswal et al 1200 consecutive advanced heart failure patients.[30] They also found that level of serum IL-6 were significantly higher in the nonsurvivors than in the survivors. Tsutamoto et al indicated IL-6 spillover in the peripheral circulation increases with the severity of CHF and suggested that high plasma levels of IL-6 can provide prognostic information in patients with CHF.[31] Thus, present study goes hand in hand with above studies.

IL-6 is a 26 kDa cytokine, produced by many different cells in the body, including lymphocytes, monocytes, fibroblasts, vascular smooth muscle cells, and endothelial cells which is involved in local and circulating marker of coronary plaque inflammation where it is responsible for platelate aggregation, production of acute phase proteins like CRP and expression of IL-1β, TNF-α. Thus IL-6 is directly or indirectly associated with unfavorable short- and long-term prognoses in patients with coronary artery disease.[32] IL-6 is sometimes discussed as a "remodeling" biomarker because alterations in IL-6 concentrations are associated with cardiac dysfunction and alteration of the cardiac extracellular matrix where IL-6 directly affects cell-to-cell communications between cardiac myocytes and fibroblasts. In heart failure the predictive value of IL-6 for adverse outcome in HF may be independent of other inflammatory biomarkers.[7] Most probably, in the heart or in periphery or in both, foci of injury must have activated immune system as heart itself is the producer of cytokines in congestive heart failure.[27]

Origin of these pro-inflammatory cytokines in heart failure patients is unknown and has been the subject to controversy. Two mechanisms for this have been proposed. One is endotoxin-induced immune activation which may be due to bowel oedema and another may be due to myocardial cytokine production because of hemodynamic overload. It may be due therapeutic implications such as sterilization of gut with antibiotics or it may be due to inhibition of endotoxin-induced cytokine expression with amiodarone or digitalis. Thus, these raised pro-inflammatory cytokines in heart failure are more because of extra myocardial production which is the results of altered tissue perfusion and tissue hypoxia possibly modulated by bacterial endotoxin release from the gut.[33] Myocardial ischemia is a known trigger for myocardial biosynthesis of IL-6 and that might have contributed to the total cytokine burden in heart failure patients where their over expression induced by a variety of cardiac stressors plays an important, permissive role in heart failure progression and thus contribute to disease progression and worsening outcomes in patients with heart failure indicating generalized inflammatory process.[30,34] Atheroma itself is the source of cytokines reflecting atherosclerotic burden or the degree of inflammatory activity within these lesions or any non-vascular sources and reflects inflammatory states such as chronic infections that may accelerate atherogenesis and its manifestations.[19]

CONCLUSION

Thus, the results of the present study suggest that IL-6 may correspond to an important risk factor along with traditional proven markers and therapeutic target in patients with heart failure. These findings will be confirmed by future longitudinal prospective studies and could be concluded with long term clinical trials with large sample size in Indian populations.

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