Association of Interleukin-4 and IgE Levels with LDL Oxidation in Atherosclerosis

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ABSTRACT

Background: Atherosclerosis is an inflammatory and multifactorial disease, with a high prevalence rate in Pakistan. **Objective:** To find a relation between serum IL-4 and IgE levels with oxidized LDL in atherosclerosis. **Methods:** In this observational, cross sectional study 99 male patients, between forty and sixty years of age, with a history of ischemic heart disease (IHD) and established atherosclerotic plaques on angiography were recruited. The study was completed within three years (Jan 2007 to Jan 2009). One hundred and one age and gender matched healthy subjects with no known history of IHD were also recruited. All the study participants were non-diabetics. Serum IL-4, IgE and oxidized LDL (ox-LDL) levels were measured by quantitative ELISA technique. **Results:** Serum IL-4 levels were generally undetectable or very low, but were higher in the patient group compared to the control subjects. Similarly, oxidized LDL and serum IgE levels were also increased in the patient group compared to the control, but the differences were not statistically significant. **Conclusion:** Our study could not detect any relationship between IL-4 and IgE levels with LDL oxidation in atherosclerosis.

Keywords: Atherosclerosis, IgE, Interleukin-4, LDL, Oxidized

INTRODUCTION

Cardiovascular disease (CVD) is a leading health problem in the world. Atherosclerosis is a major contributor to the pathogenesis of CVD (1,2). Although the frequency of atherosclerosis is low in the East as compared to the West, it is predicted that it will be a leading cause of death in the developing world at the end of this century (3). In Pakistan around 1 in 4 middle-aged adults have evidence of heart disease. This is the highest prevalence of CVD reported from this region of the world (4). Atherosclerosis is a multifactorial chronic inflammatory disease involving a large number of cytokines and inflammatory cells. Interleukin-4 (IL-4) is an important inflammatory cytokine. It is a highly pleiotropic cytokine produced by the TH_2 subset of T cells and mast cells that are also present in the plaque. It contributes to plaque formation upon increasing low-density lipoprotein (LDL) oxidation (5-8).

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LDL in its native form is not harmful; however when it is oxidized, it becomes a real initiator of plaque formation (9-11). IL-4 causes LDL oxidation by a free radical dependent and by a free radical independent or enzyme dependant LDL oxidation. Radical dependant LDL oxidation occurs when macrophages and other cells are activated by IL-4 to undergo respiratory burst and generate free radicals (12,13). The non-radical dependant oxidation of LDL is mediated by arachidonate 15-lipoxygenase enzyme (15-LO) and IL-4 has a significant role in 15-LO induction (14). Basophils and mast cells are among inflammatory cells requiring immunoglobulin-E (IgE) for their activation and IL-4 is responsible for immunoglobulin class switching leading to an increased serum IgE levels (13,14). Thus, when there is an increased concentration of serum IgE, these inflammatory cells are maximally sensitized, degranulate, and release a variety of pharmacologically active molecules such as heparin, serotonin, bradykinin, free radicals, etc. They also synthesize a variety of new proteins, the most significant of which are lipoxygenases including 15-LO. Thus an increased IgE can contribute significantly to an increased LDL oxidation and propensity for atherosclerosis (15). Therefore, it is reasonable to assume that there would be an association between IL-4, IgE and oxidized LDL.

MATERIALS AND METHODS

Study Design. This study was approved by the Ethical Committee and Research Board of the University of Health Sciences (UHS), Lahore. Written informed consent was obtained from every study participant. A total of two hundred male subjects between forty and sixty years of age, divided into two groups were included in the study. The patient group consisted of 99 male subjects with a diagnosis of ischemic heart disease on angiography. Control group consisted of 101 subjects of the same age and gender with no known history of ischemic heart disease. All participants were asked to fill a questionnaire providing information on the history of hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, smoking and also conditions such as acute or recent infections, immunodeficiencies, immuno-proliferative and autoimmune diseases. Subjects with diabetes mellitus, immunodeficiency and immunoproliferative disorders, acute or recent infections, cardiogenic shock, recent history of immunosuppressive treatment and those taking statins were excluded.

Blood Sample Collection. 5 ml of venous blood was drawn from each fasting subject in a plain (red top) vacutainer (BD). Serum was separated by centrifugation at 1800 rpm for ten minutes. All samples were stored at -80^{o}C until analysed.

Serum oxidized LDL, IL-4 and IgE levels. Serum ox-LDL, IL-4 and IgE levels were determined by commercially available quantitative sandwich enzyme immuno-assay kits (DRG diagnostics, Marburg, Germany).

Statistical Analysis. Statistical analysis was done using SPSS version 12 (SPSS, Inc, Chicago, IL, U.S.A.). Categorical data is presented as percentages (frequencies), and quantitative data as medians and interquartile range (IQR) values. For categorical data, Pearson Chi-Square test was used to determine significant association between the groups for different variables. For quantitative data, Mann Whitney rank sum test was used to determine significant differences between the study groups. Spearman's correlation coefficient test was used to determine correlation among three predetermined variables that is ox-LDL, IL-4 and IgE. To see any association of these three variables with

the severity of the disease, Kruskal-Wallis test was applied. The pvalue ≤0.05 was considered statistically significant.

RESULTS

The baseline clinical characteristics of study subjects are summarized in Table 1. A significant difference in the frequency of smoking between control and the test groups was observed. Fasting blood sugar levels were also significantly higher in the test group as compared to the control group, although they remained within the expected normal range.

Serum Oxidized LDL. Table 2 shows the difference in serum ox-LDL levels between the control and patient groups. Ox-LDL levels were higher in the patient group as compared to the control group but the difference between the two groups was not statistically significant (p=0.114). The median values were 106.12 (IQR 79.30) and 112.63 (IQR 66.26) for the control and patient groups, respectively.

Serum Interleukin-4. Table 3 shows the differences in serum IL-4 levels between the two groups of the study population. Concentration of IL-4 was mostly below the detection levels. IL-4 measurement was done for 186 patients due to the limitations in funding. Only 15/186 (24.9%) samples had IL-4 concentration above detection limits of ELISA kits used and even, these were quite low. Analysis of positive samples showed that IL-4 levels were higher in the patient group as compared to the control group (p=0.051). Median values of the control and patient groups were 0.06 (IQR 0.00) and 0.06 (IQR 0.00), respectively.

Serum Immunoglobulin-E (IgE). Table 4 shows the comparison of serum IgE levels between the control and patient groups. Serum IgE levels were higher in the patient group as compared to the control population; however, this difference was not statistically significant (p=0.157). Median values of IgE in the control and patient groups were 204.77 (IQR 299.17) and 251.21 (IQR 318.06), respectively.

Correlation among ox LDL, IL-4 and IgE Levels. Upon applying Spearman's correlation coefficient test, a positive correlation was found among the variables i.e. oxidized LDL, Interleukin-4 and IgE with r-values of 1, 0.065 and 0.123, respectively. However these correlations among the variables were not statistically significant (ox-LDL vs. IL-4, p=0.402, ox-LDL vs. IgE, p=0.085 and IL-4 vs. IgE, p=0.946).

Association of Oxidized LDL, IL-4 and IgE and Severity of the Disease. Kruskal-Wallis test was applied to find out an association between severity of atherosclerosis i.e. involvement of single, double and triple vessels and the measured variables (ox-LDL, IL-4 and IgE). There was no significant (p=0.833) association between ox-LDL and severity of atherosclerosis. Ox-LDL median values of 121.34, 113.96 and 109.66 were obtained for single, double and triple vessel diseases, respectively. There was also no significant (p=0.223) association between IL-4 and the severity of atherosclerosis. IL-4 median values of 0.06, 0.07 and 0.07 were obtained for single, double and triple vessel diseases, respectively. The levels of IgE increased with disease severity but it was not statistically significant (p=0.439). Median values of IgE for single double and triple vessel diseases were 224.43, 259.16 and 262.56, respectively.

Table 1. Baseline clinical characteristics of the study groups.

Variables	Controls			Patients			
	Number	Frequency %	age	Number	Frequency %	age	p value[a]
Hypertension	101	24	12	99	20	10	0.543
Smoking[b]	101	31	15.5	99	54	27	0.003
Family H/O IHD[c]	101	29	14.5	99	41	20.5	0.060
Fasting Blood Sugar[d]	101	89.00[e]	16.50[f]	99	100.00[e]	16.00[f]	<0.0001
Cholesterol	100	185.00	43.00	99	165.00	58.50	0.002
Triglyceride	100	156.00	100.00	99	124.00	107.00	0.101

[a]P values were determined by Pearson Chi-Square test and for fasting blood sugar by Mann-Whitney rank sum test. [b]Smoking=A person is considered as an smoker if he is currently smoking or has quitted smoking within the last six months. [c]Family H/O IHD=was considered positive if the first degree relatives had ischemic heart disease. [d]Fasting blood sugar=using reference values of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[16]. [e]Median values. [f]Inter-quartile range values (IQR).

Table 2. Comparison of serum oxidized LDL levels between patients with atherosclerosis and the control group.

	Oxidized LDL mU/L	
	Control	**Patient**
Number	101	95
Median	106.12	112.63
IQR[a]	79.30	66.26
p value[b]	0.114	

[a]IQR = Inter-quartile range
[b]P value was determined by Mann Whitney rank sum test

Table 3. Comparison of serum IL-4 levels between atherosclerotic patients and control group.

	IL-4 pg/ml	
	Control	**Patient**
Number	83	83
Median	0.06	0.06
IQR[a]	0.00	0.00
p value[b]	0.051	

[a]IQR = Inter-quartile range
[b]P value was determined by Mann Whitney rank sum test

Table 4. Comparison of serum IgE levels between patients with atherosclerosis and control group.

	IgE IU/ml	
	Control	**Patient**
Number	101	99
Median	204.77	251.21
IQR[a]	299.17	318.06
p value[b]	0.157	

[a]IQR = Inter-quartile range
[b]p-Value was determined by Mann Whitney rank sum test

DISCUSSION

The present study was designed to investigate the relationship between the levels of oxidized LDL, IL-4 and IgE in angiographically proven patients with atherosclerosis and healthy control subjects. So far only a few studies have been performed to find out an association of IL-4 and IgE with atherosclerosis (5-7,14,17,18) and none documented significant positive correlations between these variables and atherosclerosis. Similarly, results of this study also indicated no overt positive relationship among these parameters and atherosclerosis.

This study showed an increased level of oxidized LDL in the test population compared to the control group but the difference was not statistically significant. These findings

could be due to the presence of very small quantity of ox-LDL in the serum, its heterogeneity and modification by various oxidants (19). In most of the studies, oxidative modification of LDL has been accepted as one of the crucial steps in the development of atherosclerosis (12,13,20-22). Tsimikas et al. reported an association of the circulating levels of oxidized LDL with the extent of coronary artery disease in a group of patients immediately before coronary angiography (9). However, some other studies showed that oxidized LDL may even be high in the controls compared to the test group. Fredrickson et al. compared patients of hypercholesterolemic patients (49-59 years) afflicted with myocardial infarction or CHD with hypercholesterolemic counterparts showing no disease. The controls had higher oxidized LDL than the test group (23). In our study both patients and controls had normal fasting lipid profile and this may be the reason for the lack of a significant difference in the ox-LDL levels of the two groups. In the present study although overall oxidized LDL was high in the patient group, it was not significantly different from the controls. It may be assumed that some of these control individuals may have sub-clinical atherosclerosis. Their inclusion in the control group may have contributed to a lack of significant correlation between oxidized LDL and atherosclerosis. This is consistent with and supported by the work of Hulthe et al. who reported that subjects with no plaques had significantly lower levels of ox-LDL compared with subjects having sub-clinical atherosclerotic plaques (24). Ox-LDL has a significant positive correlation with total cholesterol and LDL cholesterol. This finding is consistent with a study by Itabe et al. who also noted a correlation between ox-LDL and LDL cholesterol and suggested that one ox-LDL particle is present in every 10000 LDL particles (19). Therefore, the higher concentration of LDL, the greater will be the serum level of ox-LDL. Some cytokines (IL-4, IL-6, IL-7, IL-9 and IL-13) are considered as potentially potent contributors to atherogenesis, while others have anti-atherosclerotic properties (25,26). Various studies demonstrated athero-protective effect of Th_2 cells, but there are conflicting results about IL-4. Few studies were conducted in the past to see the effect of IL-4 on atherosclerosis in human beings. Most of these studies were on animal models, while others were in vitro studies. Therefore, it was worth demonstrating the difference in the levels of IL-4 in atherosclerotic patients and the control subjects. Previous studies indicated the potential contribution of IL-4 in the pathogenesis of atherosclerosis because it increased LDL oxidation through increased expression of 15-LO activity, increased cholesterol esterification, up-regulation of MCP-1 expression and decreased nitric oxide bioavailability (5,7,8,14). However a study using transgenic ApoE knock out mice to develop diet induced atherosclerosis did not show any role for IL-4 in the progression of atherosclerosis (27). In another study, T-cell clones from human aortic atherosclerotic plaques showed that 17% of the clones secreted higher levels of interferon-γ and lower levels of IL-4 while only 2% of the clones secreted lower levels of interferon-γ and higher levels of IL-49 (28).

In the present study, levels of serum IL-4, were either undetectable or very low. Only 4/83 (4.8%) of the control subjects and 11/83 (13.2%) of the test group had detectable serum IL-4 levels. Nonetheless, the level was higher in the patient group compared to the controls. This may be due to the fact that IL-4 acts locally and their levels in serum are not raised. According to another study, IL-4 was expressed locally at different stages of atherosclerotic plaque and had potential atherogenic effects (5). Moreover, like other cytokines, IL-4 is not in preformed state and it exists for a short time in their natural form after secretion. Therefore, IL-4 should be measured by more sensitive techniques such as measurement of IL-4 intracellular proteins in the atherosclerotic plaque cells or

as IL-4 mRNA expression. Different conclusions were drawn from previous studies that investigated the role of IL-4 in atherosclerosis. The controversy may have arisen from the inclusion criteria of different studies and also the detection method of serum IL-4. The effects of IL-4 on atherosclerosis are still controversial and further studies will be of value for its better understanding (29).

Inflammatory and immune mechanisms have been suggested to play an important role in the development of coronary atherosclerosis. Basophils and mast cells also belong to leukocyte populations that have significant roles in inflammation and atherosclerosis. Inflammatory functions of these cells are closely dependent on IL-4 and IgE. It is suggested that IgE mediated events stimulate mast cells and release chemical mediators that may lead to the enhanced uptake of LDL by macrophages and the formation of foam cells (15,30) A study by Criqui et al. also suggested an association of serum IgE levels with cardiovascular disease (31). Therefore, we hypothesize that there may be an increased concentration of IgE in atherosclerotic patients.

Expectedly, serum IgE levels were increased in the test population compared to the control group but the difference was not statistically significant. Langer et al. found raised serum levels of IgE associated with nonfatal myocardial infarction (MI) events, rather than chronic atherosclerosis by the release of vasoactive mediators responsible for coronary vasoconstriction, platelet activation and aggregation and arterial smooth muscle cell hyperplasia (32). According to Kovanen et al., levels of IgE, IgG and IgA were found to be elevated in dyslipidemic MI patients. However, in this study lipid profile of the study group was within normal range and perhaps this is the reason for the lack of significant results, because raised IgE may be associated with the hyperlipidemic condition (17). Another study showed an association of raised serum IgE levels with carotid atherosclerosis and intima media thickness of carotid and femoral arteries in patients with common allergic diseases (18). But in this study we were not focusing on allergy mediated increase in IgE and its association with atherosclerosis. According to a study IgE >200KU/L is a marker for increased MI risk. In our study, the patient group also had an IgE (median level of 251.21IU/ml) which confirms the previous findings (32).

In conclusion, our study shows a positive non-significant correlation of IL-4 and IgE with oxidized LDL. A larger sample size could be of value to clarify the possible statistical significance. Further in our study, the data is cross-sectional; thus, elevated IgE levels may follow rather than precede cardiovascular disease. It may be assumed that some of the control individuals had sub-clinical atherosclerosis. Their inclusion in the study may have contributed to a lack of significant correlation between oxidized LDL and atherosclerosis. However, in the control group the presence of atherosclerotic plaques could not be ruled out by angiography due to ethical reasons; there is a need for further studies using non-invasive techniques like vascular intima media thickness in both subsets of the population. Regarding IL-4, it should be measured by more sensitive techniques such as the measurement of IL-4 intracellular proteins in atherosclerotic plaque cells or as IL-4 mRNA expression.

ACKNOWLEDGEMENT

This work was supported by a grant (20-858/ R&D / 07) of Higher Education Commission of Pakistan and University of Health Sciences, Lahore, Pakistan.

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