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Investigation of the Effect of Serum IL-1β Levels on Atherosclerosis: A Turkish Population-Based Study

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ABSTRACT

Objective: Atherosclerosis, as a cardiovascular disease associated with chronic inflammatory conditions and involving a large number of risk factors, harbors a lot of unknowns. Evidence has emerged that almost all stages of the disease are intertwined with inflammation. It has not been fully elucidated in which stages and how interleukin-1 beta (IL-1β), which is associated with inflammations, plays a role in the chronic inflammatory process of the disease. Also, the effect on atherosclerosis progression and biomarker candidacy of IL-1β in the Turkish population is unknown. The aim of this study was to investigate the serum IL-1β expression levels and whether it is associated with changes in risk factors that develop due to the disease in the Turkish population.

Materials and Methods: ELISA method was used to analyze the serum IL-1β levels. Statistical data was obtained utilizing the SPSS v.23 software.

Results: No linkage evidence was found between the serum IL-1β and atherosclerosis ($p>0.05$). However, the serum IL-1β levels were significantly associated with gender, smoking status, and diabetes history within the study population were significantly associated ($p=0.050$). According to the lipid profile result, the triglyceride value was found to be significantly decreased in atherosclerosis patients when compared to healthy individuals ($p=0.012$). Additionally, factors including, gender, smoking status, and history of diabetes were significantly associated with serum cholesterol level ($p=0.015$).

Conclusion: Although no correlation was found between the serum IL-1β levels of the study groups, it was observed that the serum IL-1β expression alters in patients with atherosclerosis depending on age. In addition, low triglyceride levels may be a marker of disease in atherosclerosis patients. Studies involving different ethnic groups with a large sample and identifying the stages of the disease are necessary to confirm these results.

Keywords: Atherosclerosis, inflammation, IL-1β, triglyceride, lipid level, serum

INTRODUCTION

Atherosclerosis, which is considered to be one of the leading diseases among the causes of death worldwide, is a multi-stage disease in which many factors are involved (1). Due to the age-related development of atherosclerosis and the effect of inflammation on the formation of the disease, it has become clear through recent studies that age and inflammation can be counted among the risk factors of atherosclerosis (2, 3). There is increasing evidence that aging has not been associated with atherosclerosis as a one-way relationship (2). The changes that occur in the vessels, especially with increased age, are similar to several characteristic pathological processes

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seen in atherosclerosis presentations (4). It is reported that proinflammatory changes in monocytes/macrophages in the development of atherosclerosis change with age. One of the most important factors underlying the molecular infrastructure of atherosclerosis is the alteration in the expression of cytokines. An increase in the level of free radicals was parallelly observed with the activation and increased secretion of many cytokines, including interleukin-1 beta (IL-1β), within obtained data from patients with atherosclerosis (5).

The pathways that trigger or participate in inflammation determine the direction of atherogenesis, so risk factors linked with these pathways have long been associated with each stage of atherosclerosis and its complications. Although many molecular actors like interleukins (ILs) are detected in cardiovascular diseases, IL-1β has emerged as a therapeutic target among these ILs (6). A substantial number of studies have demonstrated the effect of IL-1β on atherosclerosis along with varying properties at different stages (7-9). The inflammatory response that develops in endothelial cells, which is known to occur especially in the primary stage of atherosclerosis, and the accumulation of inflammatory factors induced by this response in the vessels are promoted by IL-1β (6). IL-1β has a pivotal effect on atheroma growth. It is associated with a lower inflammatory response, increased IL-10 expression, and reduced plaque size when neuralized in studies on atherosclerosis disease (10). IL-1β, a member of the IL-1 family associated with coronary diseases, is significantly elevated in both mRNA and protein levels in patients with atherosclerosis as shown in various case-control studies (11, 12). The contribution of IL-1β in the development and progression of the disease is also related to the signals that cause its expression and the molecules involved in its synthesis. IL-1β is a product largely produced by macrophages and monocytes as part of a multi-step process. Activation of the precursor IL-1β is triggered by the accumulation of molecules such as cholesterol crystals and oxidized low-density lipoprotein (LDL). The maturation process is carried out by caspase-1 or various enzymes detected in human atherosclerotic plaques (6). Therefore, changes in the lipid profile are key factors in the course of the disease by contributing to the formation of a pro-inflammatory response in the development of atherosclerosis, and are important markers for understanding the molecular mechanism of this disease (6). In addition to cholesterol crystals and oxidized LDL, triglycerides are associated with the development of atherosclerosis. It has been reported that a high triglyceride level is a risk factor for coronary heart disease (13).

Since atherosclerosis is a disease that threatens public health worldwide, like other cardiovascular diseases, its exact molecular mechanism unknown, the expression level of IL-1β as a cytokine, aging, and lipid profiles that are reported to play important roles in the course of this disease have been investigated in the Turkish population.

MATERIALS AND METHODS

Study Population and Clinical Procedures

This study was performed per tenets of the Declaration of Helsinki. Ethical approval was obtained for this study from the Yeditepe University Medical Faculty Ethics Committee (Date: 01/30/2020 and No: 1793). All participants were selected and examined by the Department of Cardiology, Yeditepe University Hospital, and signed an informed consent form prior to the investigation. A total of 69 individuals of which thirty-nine were patients (31 males / 8 females) diagnosed with atherosclerosis were utilized. The control group involved thirty healthy subjects who do not have any disease including cardiovascular diseases (26 males / 4 females). Age ranges of subjects were between 30 and 80 years. Parameters such as age, gender, smoking status, diabetes history, and serum lipid levels (high-density lipoprotein (HDL), LDL, and triglyceride) of the study population were obtained from the hospital.

IL-1β Determination by ELISA

Following ethical procedures, the determination of serum IL-1β levels was performed using the commercial Human IL-1β Enzyme Linked Immunosorbent Assay (ELISA) assay kits (Biosource, Nivelles, Belgium), according to the manufacturer's instructions, and using the microplate reader with the ability to measure absorbance at 450 nm (WHYM201). The detection limit was 0.06 pg/ml for IL-1β.

Statistical Analysis

Statistical analysis was performed using the SPSS-22 software (SPSS, Inc, Chicago, IL, USA), and the values were expressed as mean ± standard deviation (SD). The value's significance level was $p < 0.05$. The demographic records were compared and the serum IL-1β levels of the study population were analyzed by the student's t-test, Chi-square test, one-way ANOVA, and the Mann-Whitney U test.

RESULTS

Table 1 shows the demographic characteristics of the study participants. Of the sixty-nine total individuals, thirty-nine were patients (79.0% male, 21.0% female) with ages of 62.95 ± 10.96 years, and 30 individuals were healthy controls (87.0% male, 13.0% female) aged 59.96 ± 9.36 years. The mean age ($p=0.143$) and gender ($p=0.533$) of the study population were not significantly different. In 33.0% of patients and 17.0% of healthy individuals, a history of diabetes was present. There was no significant difference between the study groups regarding this parameter ($p=0.698$). While the frequency of smoking was 38.0% among those with atherosclerosis, it was 53.0% in the group of healthy individuals. As shown in Table 1 smoking data of both groups was not statistically significant ($p=0.470$).

When the effect of serum lipid levels on disease progression in human serum samples with atherosclerosis and healthy

Table 1. Demographic data of the study subjects.

Characteristic	Patient (n=39)	Control (n=30)	p-value
Age (Years; mean± SD)	62.9 5± 10.96	59.96 ± 9.36	0.143
Gender (Male / Female; %)	79.0 / 21.0 (n=31) / (n=8)	87.0 / 13.0 (n=26) / (n=4)	0.533
History of Diabetes (Yes / No; %)	33.0 / 67.0 (n=13) / (n=26)	17.0 / 83.0 (n=5) / (n=25)	0.698
Smoking (Yes / No; %)	38.0 / 62.0 (n=15) / (n=24)	53.0 / 47.0 (n=16) / (n=14)	0.470

n=number of the sample; SD: Standard deviation

Table 2. Comparison of total cholesterol, triglycerides, LDL, HDL parameters of the study population.

Parameter	Patient (n=39)	Control (n=30)	p-value
Cholesterol (mg/dl)	185.15 ± 7.97	174.40 ± 6.25	0.34
Triglycerides (mg/dl)	132.31 ± 6.68	142.33 ± 14.63	0.012*
LDL (mg/dl)	120.61 ± 6.72	105.23 ± 6.19	0.329
HDL (mg/dl)	36.74 ± 1.14	40.23 ± 1.55	0.685

n=number of the sample; *p<0.05

individuals were examined, no relationship was found between cholesterol (p=0.34), LDL (p=0.329), and HDL (p=0.685) concentrations and atherosclerosis, except for triglycerides. In the group of patients with atherosclerosis, it was determined that the triglyceride level was significantly decreased (p=0.012) (Table 2). Although no difference was found in cholesterol levels between the patient and control groups in our study population, it was found that gender, smoking status, and diabetes history were associated with cholesterol levels (p=0.015).

Serum IL-1β levels were measured in both patient and control groups. Although lower IL-1β levels were detected in the patient group (12.90 ± 7.538 pg/ml) as compared to the control group (13.47 ± 19.565 pg/ml), no significance was found (p>0.05). Additionally, representative histograms of serum IL-1β and triglyceride concentrations are given in Figure 1 and 2.

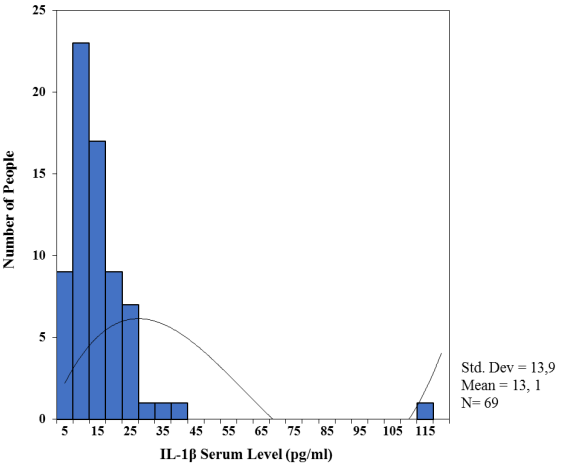

Figure 1. Representative histogram of IL-1β.

Further, to understand how the serum IL-1β level changes depending on age in both the patient and control groups, analyses were performed in three different age groups: less than 50 years old (<50), between 50-60 years old, and over 60 years old (60>). Out of thirty-nine patients with atherosclerosis, serum IL-1β expression levels at <50, 50-60, and 60> age groups were 17.23 ± 19.23 pg/mL, 10.93 ± 6.19 pg/mL, and 13.07 ± 6.47 pg/mL, respectively. Out of thirty healthy individuals, the IL-1β expression level results for <50, 50-60, and 60> age groups were as follows; 11.86 ± 6.28 pg/mL, 7.28 ± 5.27 pg/mL, and 19.11 ± 28.57 pg/mL. While the serum IL-1β level in the patient group was higher in the group aged 50 years and below, the opposite result was obtained in the control group. In the control group, the highest level of IL-1β was detected in the 60 years and older group (Figure 3).

Within the scope of our research, it was also investigated whether there was any association between the demographic and clinical data of the study groups and IL-1β expression level. Smoking and history of diabetes (p=0.143) and smoking and

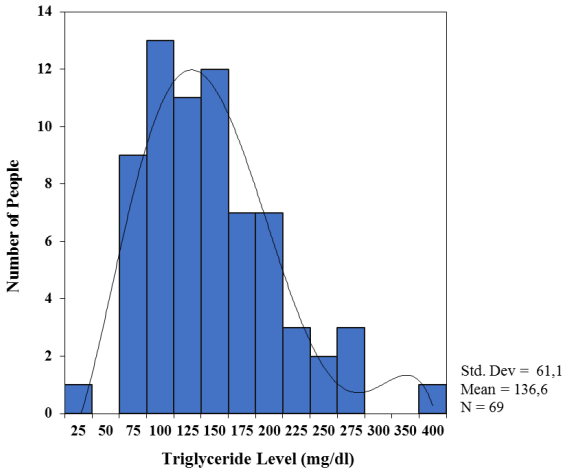

Figure 2. Representative histogram of serum triglyceride levels.

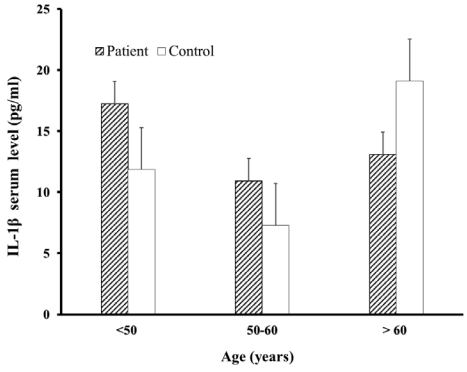

Figure 3. Distribution of serum IL-1β level in both patients with atherosclerosis and control groups by age.

gender (p=0.541) did not show any significant difference for IL-1β expression levels between the groups. Diabetes history alone was also not significantly related to serum IL-1β between the groups (p=0.833). On the other hand, serum IL-1β levels with gender, smoking, and diabetes history within the study population were significantly associated (p=0.050).

DISCUSSION

In literature, this is the first study to investigate the IL-1β expression level in human serum samples, its age-related change in atherosclerosis, and the serum concentration of triglyceride value in patients with atherosclerosis in the Turkish population.

In humans, the role of the proinflammatory cytokine IL-1β in atherosclerosis is not well-defined. By designing a case-control study in the Turkish population that included individuals with atherosclerosis and those without atherosclerosis, we examined the association of clinical data with the disease and the expression profile of the serum inflammatory cytokine IL-1β. Extensively investigated IL-1β levels by researchers all over the world was not a risk factor for atherosclerosis when compared to healthy individuals in the Turkish population. In this study population it showed that IL-1β alone does not play a role in the disease and cannot be a stand-alone biomarker for atherosclerosis.

The great majority of studies were conducted utilizing animal experiments, and very few population-based human studies have been performed. In addition, studies with the IL-1 family generally examined the consequences of their blockade of disease development and progression (14, 15). The common consensus that emerged from studies that have accumulated over the years was that inflammation has a visible link in coronary artery disease progression and each stage of the atherosclerosis process (16).

An animal study by Vromman et al. provided evidence that blocking IL-1β mainly brought blood monocytes to a less inflammatory state during atherogenesis, reduced atheroma size, and increased plasma levels of IL-10 (10).

One of the few population-based studies in the literature, Di Iorio et al. within the scope of the InCHIANTI project, measured IL-1β levels in 1,292 human samples collected from two different cities in Italy. As a result of the study, it showed that IL-1β did not change depending on age and gender but was associated with clinical conditions such as angina and serum calcium level (16). A cross-sectional study was performed by Joung et al. to determine whether IL-1β could be a marker for cardiovascular disease (15). As in our study, no significant relationship was found between the patients and study groups regarding serum IL-1β levels. However, when its relationship with other parameters in the study was examined, it could be an independent risk factor for newly diagnosed cardiovascular disease in smoking patients (16). Though the link of IL-1β expression with atherosclerosis development and progression has been shown in these studies, no significant relationship has been found between IL-1β expression and atherosclerosis in this study. Such contradictory results may be because the studies were conducted on different ethnic groups, as well as the sample size, human serum samples that were used, the nutritional preferences of the individuals included in the study, and the drugs used by the individuals.

In the context of this study, we examined whether the IL-1β expression levels change with regards to age. In patients with atherosclerosis, the IL-β serum concentration was highest in the age group of 50 and below (17.23 ± 19.23 pg/mL). IL-1β concentration reached the highest value in the 60-year and older group within the control group (19.11 ± 28.57 pg/mL). Despite these results, there was not a significant difference within or between the groups. Studies showing that aging is an independent risk factor for atherosclerosis are growing exponentially. In addition to the changes in the aging vessels, alterations in the expression profiles of the proinflammatory molecules, IL-1β, were observed (17). The most plausible explanation for the higher concentration of IL-1β expression in atherosclerosis patients under 50 years of age in our study

population may be that this cytokine is activated in the early stages of inflammation (18). In the control group, the high concentration measured in individuals aged 60 and older can be considered as sign of age-related inflammatory processes.

Parallel to the increased expression of proinflammatory cytokines, there was an increase in the level of atherogenic lipoprotein. Therefore, the lipid profiles of the study groups were measured, and their association with atherosclerosis was assessed. This study reported that triglyceride levels significantly decreased in atherosclerosis patients (p=0.012). Although many studies were conducted regarding high triglyceride levels and increased IL-1β activity in relation to the disease, few studies reported on the contribution of low triglyceride to the biology of atherosclerosis.

Although decreased serum triglyceride levels are predictors of cardiovascular death in patients with heart failure, the reasons for the low triglyceride levels remain unknown and require further investigation. This decline may be explained by various mechanisms. Studies that revealed these possibilities are available in the literature.

Research on chronic heart failure reported a link between low triglyceride levels and the disease. It showed that decreased triglyceride levels could be an independent marker of cardiac death (19). In addition, factors such as nutritional deficiency and increased inflammation could also be among the causes of changes in triglyceride levels. Since the triglyceride value of 150mg/dl is accepted as an indicator that does not pose a risk to human health, it is important to understand the atherosclerosis of patients in our selected population with an average of 132.31mg/dl level (20). Also, the decreased level of IL-1β (albeit non-significant) as an outcome of this study may have contributed to the low serum triglyceride levels in atherosclerosis by preventing lipolysis. This kind of change in cytokine activity can alter the molecular response to inflammation by affecting the serum triglyceride level, leading to a change in the lipid profile.

Study Limitations

This study contained some limitations that should be discussed. The most important of these is that the sample size was not large enough to achieve substantial results. In addition, the fact that only a single population was studied could have caused the results to differ from previous studies. The varied ages of the individuals in the study groups was another factor. Different age groups may cause different outcomes, so it may be necessary to narrow the age range in future studies. In addition, there is a close relationship between cardiovascular diseases and nutrition, therefore, the dominance of the Mediterranean diet in the Turkish population may have also affected the results.

CONCLUSION

Consequently, although a significant relationship could not be demonstrated, this study exhibited data showing that monitoring and analyzing serum IL-1β levels according to age groups could provide information regarding the course of atherosclerosis. In addition, this study is one of the few examining the relationship between serum IL-1β concentration and atherosclerosis amidst the Turkish population. It was found that the triglyceride level decreased in this patient group population. These results did not support the role of decreased IL-1β level in atherosclerosis in the Turkish population, but consequently showed that triglyceride levels decreased in patients with atherosclerosis. It will be important to carry out further studies utilizing larger sample groups and different ethnic populations regarding the possible elements of the molecular mechanism that caused this result.

Ethical Approval: All procedures performed in studies involving human participants were under the ethical standards of the 1975 Declaration of Helsinki guidelines and its later amendments. The research on humans study protocol was approved by the Yeditepe University Medical Faculty Ethics Committee (Date: 01/30/2020 and No: 1793).

Authors' Contributions: Conception/Design of Study – T.I., D.D.O.; Supervision – T.I., Data Acquisition – A.T.C.; Performing experiments – D.D.O., F.T.A.; Analysis and/or Interpretation – S.G.Y., F.T.A., D.D.O., Z.B., D.B.; Drafting Manuscript – T.I., D.D.O., D.B.; Critical Revision of Manuscript – T.I., S.G.Y., F.T.A., D.D.O., Z.B., A.T.C., D.B.; Final Approval and Accountability– T.I. All authors read, revised, and approved the final article.

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