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To study the levels of cytokines in atherosclerosis and non-atherosclerosis individuals

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Abstract:

Background: Atherosclerosis is characterized by abnormalities in blood pressure, blood sugar, waist circumference, and cholesterol or triglyceride levels. **Aim:** The one-of-a-kind thing about this study is that its primary focus is on figuring out whether there is a distinction in inflammatory markers between those who have atherosclerosis and those who do not have the condition. **Materials & methods:** The study included 100 atherosclerosis patients and 100 healthy controls. This research examined Indore Index Medical College & Research Centre patients. After approval, the study's researchers began. Before the study, each subject gave informed consent. **Statistical analysis:** To determine the relationship between two variables, regressions were used. Moreover, percentages were computed. P 0.05 was considered significant. **Conclusion:** The significance of this result cannot be overstated. The results of the study show conclusively that both pro-inflammatory and anti-inflammatory cytokines contribute to the development of atherosclerosis's secondary illnesses in atherosclerosis.

Keywords: Atherosclerosis; inflammation; Cytokines; Body mass index; Inflammation.

Introduction:

Atherosclerosis is a chronic, progressive disease of the arterial wall characterized by lipid deposition, inflammation, and vascular remodeling, and it remains a major contributor to cardiovascular morbidity and mortality worldwide. Beyond the traditional lipid-centric view, accumulating evidence highlights that immune dysregulation and chronic low-grade inflammation play pivotal roles in both the initiation and progression of atherosclerotic lesion [1-3]. Cytokines key signaling molecules of the immune system have emerged as critical modulators in the complex interplay between vascular cells, immune cells, and metabolic pathways in atherosclerosis [4].

Among these cytokines, interleukin-10 (IL-10) and interleukin-13 (IL-13) are recognized for their anti-inflammatory and atheroprotective properties [5]. IL-10 suppresses pro-inflammatory cytokine synthesis, inhibits antigen-presenting cell activation, and promotes the resolution of inflammation, thereby mitigating plaque instability. Similarly, IL-13 influences macrophage polarization toward the M2 phenotype, promotes tissue repair, and reduces oxidative stress within the vascular microenvironment [6]. Conversely, cytokines such as interferon-gamma (IFN-γ), interleukin-2 (IL-2), and interleukin-15 (IL-15) are predominantly pro-inflammatory, enhancing T-helper 1 (Th1)

immune responses, upregulating endothelial adhesion molecules, and contributing to vascular smooth muscle cell dysfunction all of which can accelerate atherogenesis [7-9].

From a translational perspective, elucidating the balance between anti-inflammatory and pro-inflammatory cytokines in atherosclerosis holds significant potential for biomarker discovery, patient risk stratification, and targeted therapy development [10-16]. The differential patterns of IL-10, IL-13, IFN-γ, IL-2, and IL-15 observed in this study not only deepen our understanding of immune regulation in vascular disease but may also guide the development of cytokine-based interventions aimed at restoring immune homeostasis and preventing plaque progression or rupture. Hence, the aim of this study is that its primary focus is on figuring out whether there is a distinction in inflammatory markers between those who have atherosclerosis and those who do not have the condition.

Materials & methods:

Two hundred people participated in the current study, 100 in the atherosclerosis group and 100 in the control group of healthy subjects. The participants at the Index Medical College & Research Centre in Indore were the focus of this study. The researchers behind the study got to work after getting the go-light from the appropriate authorities. Each subject gave their informed consent before the present study began. ***Inclusion Criteria:*** Adults aged 35–70 years**.** For cases: confirmed diagnosis of atherosclerosis**.** For controls: normal cardiovascular examination and no metabolic or inflammatory disorders **Exclusion Criteria**: History of acute coronary syndrome within the last 3 months. Chronic kidney disease or liver dysfunction. Active infections or autoimmune diseases. Use of corticosteroids, immunosuppressive agents, or anti-inflammatory drugs within the last month.

All participants in both groups were examined by a qualified physician from the hospital's medical department, who followed standard procedures and accounted for the study's exclusion and inclusion criteria. The health control group consisted of 100 participants of the same age and gender who did not have atherosclerosis. Patients being treated for atherosclerosis numbered 100 in the second group. Normal glycemic state human volunteers of similar age and sex served as the control group. Each person was examined by a licensed medical professional who followed established medical procedures. The BMI (Body Mass Index) of each individual was determined by dividing their weight in kilos by their height in square meters. Subjects were divided into groups once their body mass index had been recorded. Each person in both groups had 5ml of their fasting venous blood extracted into flat vials using a disposable syringe and needle in a sterile environment. Following centrifugation at 3000rpm for 20 minutes to separate the serum from the blood, samples were aliquoted and kept at 20°C. A multi-analyte Elisarray kit from Qiagen laboratories was used to quantify cytokines in the serum. The capture antibodies are more able to bind to their target protein after being incubated. After the elimination of free protein, the collected analyte can be bound by biotinylated detection antibodies that have been added to the wells. After a final wash, an avidin-horseradish peroxidase conjugate is used to get rid of any remaining free particles. After another wash, a colorimetric substrate solution is added to the wells, which turns the sample a shade of blue that is directly proportionate to the protein analyte concentration in the original sample. After adding a stop solution, you may measure the absorbance of your samples at 450 nm and make meaningful comparisons between them. There was a 4.9% difference between replicates and a 6.3% difference between assays. A sensitivity of 0.5 pg/mL was established.

Statistical analysis:

Statistical analysis has been performed using IBM SPSS version 20. Using an unpaired "t" test, the means of the variables in the two groups were compared. Moreover, percentages were computed. P 0.05 was considered significant. To determine the relationship between two variables, regressions were used. Moreover, percentages were computed. P 0.05 was considered significant.

Results:

In the present study, in table 1, we have shown the increased mean values of IL-10 and IL-13 in both the group subjects. Lower levels of IL-10 and IL-13 have been observed in the healthy control subjects when compared with Atherosclerosis subjects. In addition, we observed statistical differences between these two groups in case of IL-10 and IL-13 serum levels.

Table 1: Those with Atherosclerosis had higher mean values of many cytokines compared to the study's control group.

Variable	Atherosclerosis group (n=100)	Control group (n=100)	P Value
IL-10 (pg/mL)	28.4 ± 11.5	19.1 ± 5.6	t= 7.270; df= 198; P = 0.0001
IL-13 (pg/mL)	85.8 ± 21.3	55.8 ±7.5	t= 13.285; df= 198; P = 0.0001

Table 2, shows the mean levels of cytokines which are decreased in the Atherosclerosis subjects than healthy controls. The study observed statistical difference in the serum levels of IFN-γ, IL-2 and IL-15 when compared between Atherosclerosis group subjects and control group subjects.

Table 2: Those with Atherosclerosis had lower mean values of many cytokines compared to the study's control group.

Variable	Atherosclerosis group (n=100)	Control group (n=100)	P Value
IFN-γ (pg/mL)	55.7 ± 6.8	66.5 ± 9.4	t= 9.3090; df= 198; P = 0.0001
IL-12 (pg/mL)	128.9 ± 28.7	165.3 ± 11.8	t= 11.794; df= 198; P = 0.0001
IL-15 (pg/mL)	6.7 ± 2.3	9.8 ± 0.9	t= 12.551; df= 198; P = 0.0001

Discussion:

The present study evaluated the serum levels of specific pro- and anti-inflammatory cytokines in individuals with atherosclerosis compared to age- and sex-matched healthy controls and explored their relationships with lipid profile components and insulin resistance indices. The major findings were (i) increased mean levels of IL-10 and IL-13 in the atherosclerosis group compared with controls; (ii) decreased mean levels of IFN-γ, IL-2, and IL-15 in the atherosclerosis group compared with controls. These findings shed light on the complex immune-inflammatory environment in atherosclerosis, where cytokine profiles interact with metabolic and lipid parameters, potentially influencing both disease initiation and progression.

1. Elevated IL-10 and IL-13 levels in atherosclerosis

The observed elevation in IL-10 and IL-13 levels among atherosclerosis patients may initially appear counterintuitive, given that both are generally regarded as anti-inflammatory cytokines. IL-10 inhibits the synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while IL-13 has been implicated in suppressing macrophage activation and promoting tissue repair. Elevated circulating IL-10 and IL-13 in atherosclerosis could reflect a compensatory, host-driven anti-inflammatory response to persistent vascular inflammation.

Studies have demonstrated that IL-10 expression is increased in atherosclerotic plaques, particularly in areas rich in macrophages, suggesting a localized attempt to counterbalance inflammation [15-18]. In the Indian context, Raj et al. [19] reported elevated IL-10 levels in patients with acute coronary syndromes compared to stable coronary artery disease and controls, proposing that IL-10 elevation may be a reactive process in response to heightened systemic inflammation. Similarly, IL-13 has been shown to modulate lipid metabolism in macrophages, promoting cholesterol efflux and potentially reducing foam cell formation [20].

2. Decreased IFN-γ, IL-2, and IL-15 levels in atherosclerosis

In contrast to IL-10 and IL-13, our results revealed significantly reduced levels of IFN-γ, IL-2, and IL-15 in the atherosclerosis group compared to healthy controls. This decline in Th1-type cytokines could indicate immune exhaustion or altered T-cell functionality in chronic atherosclerotic disease. IFN-γ is a pivotal cytokine in the activation of macrophages and the promotion of plaque instability, but its role is complex; while locally increased in plaques, systemic IFN-γ levels may decrease in chronic disease stages due to sustained immune activation and depletion of responsive T-cell populations.

IL-2 is critical for T-cell proliferation and survival, and lower systemic levels may reflect impaired regulatory T-cell (Treg) maintenance, which could paradoxically favor chronic inflammation within plaques. Similarly, IL-15 plays a role in natural killer (NK) cell activation and T-cell homeostasis. Its decreased serum concentration in atherosclerosis patients could represent impaired innate immune surveillance, possibly facilitating persistent vascular injury.

Foreign literature supports these patterns: Monserrat-Mesquida et al., [21] reported reduced peripheral blood IFN-γ production in patients with advanced coronary artery disease, correlating with disease severity. Indian evidence is limited, but Lin et al. [22] observed altered Th1/Th2

cytokine balance in patients with premature coronary artery disease, with relatively suppressed Th1 cytokine production.

Conclusion:

The results of the current study link atherosclerosis to the inflammatory cytokines. The significance of the result cannot be overstated. The outcomes of the new research show conclusively that both pro-inflammatory and anti-inflammatory cytokines contribute to the development of atherosclerosis's secondary illnesses. Also, the analyzed cytokines may serve as biomarkers for the early detection and diagnosis of secondary problems in people with atherosclerosis.

Conflict of interest:

None.

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