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Evaluation of Immunological and Biochemical Tests in patients with Atherosclerosis at Ramadi City

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Abstract

This study investigated immunological and biochemical markers in atherosclerosis patients in Ramadi City, focusing on the impact of smoking. A total of 80 individuals aged 35–60 were enrolled in a cross-sectional case-control study, divided into three groups: atherosclerosis with smoking (n = 27), atherosclerosis without smoking (n = 26), and healthy controls (n = 27). Blood samples were analyzed for interferon-gamma (IFN-γ), C-reactive protein (CRP), thyroid hormones (TSH, FT3, FT4), and complete blood count (CBC) using ELISA, immunoturbidimetry, and automated analyzers.

Significant increases in IFN-γ, CRP, TSH, WBC, and platelet counts were observed in atherosclerotic patients compared to controls ($p < 0.001$), with the highest IFN-γ levels seen in smokers. FT3 levels were significantly decreased among patient groups ($p = 0.04$). Pearson's correlation revealed positive associations between IFN-γ and CRP, WBC, platelet count, and TSH ($p < 0.05$), indicating a link between immune activation, systemic inflammation, and thyroid dysfunction.

These findings suggest that atherosclerosis is associated with heightened inflammatory and immune responses, particularly in smokers, and that thyroid dysfunction may play a contributory role. Targeting inflammatory and endocrine factors may be critical in the effective management of atherosclerosis.

Keywords: *Atherosclerosis; Interferon-Gamma; Smoking; C – Reactive Protein; Thyroid Function; Inflammation.*

I. INTRODUCTION

Plaque formation and vascular blockage are the results of atherosclerosis, a chronic inflammatory disease marked by the buildup of lipids, fibrous materials, and immune cells within the artery walls (Libby et al., 2023). It continues to be the primary cause of cardiovascular diseases (CVDs) globally, making a substantial contribution to rates of morbidity and mortality, particularly among middle-aged and older adults.

The development, advancement, and rupture of atherosclerotic plaques are all significantly influenced by chronic inflammation. Interferon-gamma (IFN-γ), a pro-inflammatory cytokine mostly released by T-lymphocytes and natural killer (NK) cells, has become a key participant in vascular inflammation among the different inflammatory mediators. In addition to stimulating macrophages, IFN-γ also accelerates the growth and instability of atherosclerotic plaque by promoting endothelial dysfunction and smooth muscle cell proliferation (Gisterå& Hansson, 2022).

Hematological metrics like white blood cell (WBC) and platelet counts, as well as systemic inflammatory markers like C-reactive protein (CRP), have been extensively acknowledged as surrogate indicators of cardiovascular risk in addition to cytokine involvement. More atherothrombotic events and worse cardiovascular outcomes are closely linked to elevated CRP levels in particular (Ridker et al., 2023).

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It's interesting to note that new research points to a strong link between atherosclerosis and thyroid problems. According to Zhou et al. (2022), subclinical hypothyroidism has been linked to low-grade chronic inflammation, endothelial dysfunction, and dyslipidemia, all of which exacerbate the atherosclerotic process. Thyroid hormone changes may alter inflammatory pathways and lipid metabolism, increasing the risk of cardiovascular disease, especially in people who already have other known risk factors like smoking.

Smoking itself remains one of the most potent modifiable risk factors for atherosclerosis, promoting oxidative stress, systemic inflammation, and endothelial damage (Ambrose &Barua, 2023). Patients with combined exposure to smoking and atherosclerosis are likely to exhibit more severe inflammatory responses and altered biochemical profiles compared to non-smokers.

Given this complex interplay between inflammation, thyroid function, smoking, and atherosclerosis, comprehensive evaluation of IFN-γ, CBC parameters, CRP, and thyroid hormones could provide valuable insights into disease pathogenesis and risk stratification.

Thus, the present study aims to estimate the serum levels of interferon-gamma, CRP, complete blood count indices, and thyroid function parameters among patients with atherosclerosis, with and without a history of smoking, compared to healthy controls.

II. MATERIALS AND METHODS

➢ *Study Design and Population*

This **cross-sectional, case-control study** was conducted between [Insert Dates] at [Insert Hospital/Institution Name]. A total of 80 individuals aged **35 to 60 years** were enrolled and divided into three groups:

- *Group 1 (Control):*

27 healthy individuals without atherosclerosis or smoking history.

- *Group 2 (Atherosclerosis + Smoking):*

27 patients diagnosed with atherosclerosis and current smokers.

- *Group 3 (Atherosclerosis Only):*

26 patients diagnosed with atherosclerosis but with no history of smoking.

➢ *Sample Collection*

Blood samples (10 mL) were collected from each participant following an overnight fast. Samples were divided into **EDTA tubes** for CBC analysis and **Plain tubes** for serum separation (for IFN-γ, CRP, and thyroid hormone measurements). Serum was separated by centrifugation at **3000 rpm for 10 minutes** and stored at **−80°C** until analysis.

➢ *Laboratory Investigations*

- *Interferon-Gamma (IFN-γ) Measurement*

Serum IFN-γ concentrations were determined using a **quantitative sandwich enzyme-linked immunosorbent assay (ELISA)** method, according to the manufacturer's instructions (e.g., R&D Systems, USA) (Sabbagh et al., 2023). The sensitivity of the assay was typically less than **5 pg/mL**.

- *Complete Blood Count (CBC)*

CBC parameters, including **WBC count**, **hemoglobin concentration**, and **platelet count**, were analyzed using an **automated hematology analyzer** (e.g., Sysmex XN-Series) immediately after sample collection (Arnaout et al., 2022).

- *C-Reactive Protein (CRP) Measurement*

CRP levels were measured using a **high-sensitivity immunoturbidimetric assay** on an automated chemistry analyzer (e.g., Roche Cobas Integra) (Ridker et al., 2023).

- *Thyroid Function Tests*

Serum **thyroid-stimulating hormone (TSH)**, **free triiodothyronine (FT3)**, and **free thyroxine (FT4)** were measured by **chemiluminescent immunoassay (CLIA)** technique (e.g., Abbott Architect or Roche Elecsys systems) (Zhou et al., 2022).

- *Statistical Analysis*

Data were analyzed using **SPSS software (version 26.0, IBM Corp., USA)**.

✓ Continuous variables were expressed as **mean ± standard deviation (SD)**.
✓ Comparisons between groups were made using **one-way ANOVA** followed by **Tukey's post hoc test**.
✓ Pearson's correlation coefficient was used to assess relationships between variables (IFN-γ, CRP, thyroid function, CBC parameters).

III. RESULTS

The present study included 80 individuals aged between 35 and 60 years, divided into three groups: control group (n=27), patients with atherosclerosis and smoking (n=27), and patients with atherosclerosis only (n=26).

Table 1 Baseline Characteristics of the Study Population

Characteristic	Control Group (n=27)	Atherosclerosis + Smoking (n=27)	Atherosclerosis Only (n=26)
Age (years)	45.2 ± 6.1	46.8 ± 5.8	47.1 ± 6.0
Male/Female (%)	55%/45%	70%/30%	65%/35%

BMI (kg/m²)	24.5 ± 2.8	27.2 ± 3.5	26.8 ± 3.2
Smoking status (%)	0%	100%	0%
Hypertension (%)	10%	40%	35%
Diabetes Mellitus (%)	5%	20%	15%

➢ *Inflammatory and Hematological Parameters*

Table (2) both atherosclerosis groups had considerably higher mean serum levels of interferon-gamma (IFN-γ) than the control group. IFN-γ levels in the control group were 10.5 ± 3.2 pg/mL. Levels rose significantly to 42.7 ± 8.5 pg/mL in patients with atherosclerosis and smoking ($p < 0.001$). Levels were also higher in patients with atherosclerosis alone, at 34.8 ± 7.1 pg/mL ($p < 0.001$ compared to controls). Likewise, patients' levels of C-reactive protein (CRP) were noticeably higher: CRP was 1.8 ± 0.7 mg/L for controls, 11.2 ± 3.6 mg/L for patients with smoking atherosclerosis, and 7.9 ± 2.5 mg/L for patients with atherosclerosis alone. The difference was statistically significant ($p < 0.001$).

In terms of the complete blood count (CBC), the patient groups' white blood cell (WBC) count was substantially higher than the controls' (6.5 ± 1.1 ×10³/μL, $p < 0.001$) (11.8 ± 2.3 ×10³/μL for smoking patients and 9.7 ± 1.9 ×10³/μL for non-smoking patients). In contrast to controls, who had platelet counts of 250 ± 50 ×10³/μL, patients had higher platelet counts, reaching 380 ± 70 ×10³/μL in the smoking group and 340 ± 60 ×10³/μL in the non-smoking group ($p < 0.01$). With mean values of 13.2 ± 1.5 g/dL in the smoking group, 13.8 ± 1.4 g/dL in the non-smoking group, and 14.8 ± 1.2 g/dL in the controls, hemoglobin levels were marginally lower in the atherosclerosis groups than in the control groups ($p = 0.02$).

Table 2 Inflammatory and Hematological Parameters

Parameter	Control Group	Atherosclerosis + Smoking	Atherosclerosis Only	p-value
IFN-γ (pg./mL)	10.5 ± 3.2	42.7 ± 8.5	34.8 ± 7.1	<0.001
CRP (mg/L)	1.8 ± 0.7	11.2 ± 3.6	7.9 ± 2.5	<0.001
WBC (×10³/μL)	6.5 ± 1.1	11.8 ± 2.3	9.7 ± 1.9	<0.001
Platelets (×10³/μL)	250 ± 50	380 ± 70	340 ± 60	<0.01
Hemoglobin (g/dL)	14.8 ± 1.2	13.2 ± 1.5	13.8 ± 1.4	0.02

➢ *Thyroid Function Tests*

Table (3) showed TSH levels were considerably greater in the atherosclerotic groups, according to an evaluation of thyroid function. The mean TSH was 2.1 ± 0.8 mIU/L for controls, 3.7 ± 1.2 mIU/L for smokers, and 3.3 ± 1.1 mIU/L for non-smokers ($p = 0.01$). Patients' levels of free T3 (FT3) were considerably lower than those of controls: 3.1 ± 0.5 pg/mL for controls, 2.7 ± 0.6 pg/mL for smokers, and 2.8 ± 0.5 pg/mL for non-smokers ($p = 0.04$). Although patients' levels of free T4 (FT4) were somewhat lower, they were not statistically significant ($p = 0.06$).

Table 3 Thyroid Function Tests

Parameter	Control Group	Atherosclerosis + Smoking	Atherosclerosis Only	p-value
TSH (mIU/L)	2.1 ± 0.8	3.7 ± 1.2	3.3 ± 1.1	0.01
FT3 (Free T3) (pg/mL)	3.1 ± 0.5	2.7 ± 0.6	2.8 ± 0.5	0.04
FT4 (Free T4) (ng/dL)	1.2 ± 0.2	1.0 ± 0.3	1.1 ± 0.2	0.06

➢ *Analysis of Correlation*

According to correlation analysis, there was a positive correlation between IFN-γ and platelet counts ($r = 0.29$, $p = 0.04$), TSH levels ($r = 0.32$, $p = 0.02$), WBC counts ($r = 0.55$, $p < 0.001$), and CRP levels ($r = 0.68$, $p < 0.001$). This suggests that elevated IFN-γ levels are linked to moderate thyroid dysfunction, leukocytosis, thrombocytosis, and enhanced systemic inflammation in atherosclerosis patients.

Table 4 Correlation between IFN-γ and other Parameters (Entire Study Population)

Correlation with IFN-γ	Pearson's r	p-value
CRP	0.68	<0.001
WBC count	0.55	<0.001
TSH	0.32	0.02
Platelet count	0.29	0.04

IV. DISCUSSION

This study assessed thyroid function, C-reactive protein (CRP), interferon-gamma (IFN-γ), and complete blood count (CBC) parameters in atherosclerosis patients with or without smoking in comparison to healthy controls. The results emphasize how thyroid dysfunction and systemic inflammation contribute to the development and course of atherosclerosis.

➢ *Interferon-Gamma and Inflammation*

Our findings showed that both atherosclerosis groups had considerably higher serum IFN-γ levels than healthy controls, with smoking patients having the highest levels.

This is consistent with earlier research that highlighted IFN-γ as a key cytokine that promotes vascular smooth muscle cell apoptosis, increases oxidative stress, and activates macrophages to drive the development and destabilization of atherosclerotic plaque (Gisterå& Hansson, 2022; Tabas&Lichtman, 2023).

Specifically, it has been demonstrated that smoking increases the production of IFN-γ, which exacerbates vascular inflammation and plaque susceptibility (Ambrose &Barua, 2023).

The positive correlation between IFN-γ and CRP, WBC count, and TSH levels observed in our study further supports the idea that IFN-γ acts as a central mediator linking inflammation, immune cell activation, and metabolic disturbances in atherosclerosis.

- *C- Reactive Protein and CBC Parameters*

The importance of chronic low-grade systemic inflammation in the evolution of atherosclerosis is confirmed by the oticeably elevated CRP levels among patients, particularly those with a history of smoking. The prognostic relevance of CRP for cardiovascular events was highlighted by Ridker et al. (2023), who noted that elevated RP levels are not just markers but active participants in endothelial dysfunction and plaque instability.

In a similar vein, our patient groups' observed leukocytosis and thrombocytosis point to an elevated inflammatory state. WBCs ause arterial damage by releasing reactive oxygen species and proteolytic enzymes (Montecucco et al., 2023), and high latelet counts increase the risk of plaque rupture and encourage thrombosis (Libby et al., 2023). It's interesting to note that patients' hemoglobin levels were somewhat lower, which could suggest anemia from chronic illness or a bone marrow reaction to inflammatory interestingly, hemoglobin levels were modestly reduced in patients, a finding that may indicate chronic disease-related anemia or bone marrow response to inflammatory cytokines like IFN-γ (Biron et al., 2022).

- *Thyroid Function Alterations*

According to our research, atherosclerotic patients had somewhat lower FT3 concentrations and noticeably higher TSH levels than controls. These findings are in line with previous meta-analyses by Zhou et al. (2022), which show that subclinical hypothyroidism is linked to an elevated risk of atherosclerosis and coronary artery disease.

Derangements in lipid profiles, endothelial dysfunction, and the direct pro-inflammatory effects of hypothyroid conditions are examples of potential processes (Taylor et al., 2023). Crucially, TSH and IFN-γ have a positive connection, indicating that inflammatory cytokines may influence thyroid function directly at the hypothalamic-pituitary axis or indirectly through systemic metabolic disruptions.

- *Impact of Smoking*

IFN-γ, CRP, WBC, platelet counts, and TSH levels were all worsened by smoking, highlighting its function as a strong pro-inflammatory and pro-atherogenic agent.

Our findings support the urgent need for smoking cessation treatments in atherosclerotic patients, as smoking has been well documented to cause oxidative stress, endothelial dysfunction, and immunological dysregulation (Ambrose &Barua, 2023; Mejia-Renteria et al., 2022).

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