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STUDY THE EFFECT OF PLASMINOGEN ACTIVATOR INHIBITOR-1 AND CERTAIN BIOCHEMICAL PARAMETERS ON PATIENTS WITH AORTIC ATHEROSCLEROSIS

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Abstract

This study involved 140 cases that belong to both males and females aged between 30 and 73 years old, divided as follows: 70 patients who suffer from aortic atherosclerosis, and 70 healthy individuals as control samples. Concentrations of plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-Alpha), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were estimated for each participant.

The results indicate a significant increase ($p \leq 0.01$) in PAI-1, IL-6, TNF-Alpha, TC, TG, and LDL-C levels for patients compared to the control group. Gender analysis showed significant differences in PAI-1, IL-6, TNF-α, and LDL-C levels. Age categorization (30-45, 46-60, and over 60 years) revealed significant differences in IL-6, PAI-1, TC, TG, and LDL-C concentrations for the age group over 60 years compared to other age groups. This study explored correlations between PAI-1 and other parameters, showing low positive correlations with IL-6, TNF-Alpha, and highly significant positive correlations with TC, TG, and LDL-C. Additionally, a little negative correlation was found between PAI-1 and HDL-C.

Keywords: aortic atherosclerosis, Plasminogen Activator Inhibitor-1 (PAI-1), age, gender.

Introduction

Vascular diseases (VD), including aortic atherosclerosis, are a leading cause of death in many countries. Global rates of VD have increased over the past three decades, with approximately 523 million adults worldwide affected by VD (1). Any condition that affects the lymphatic system, blood circulation, blood disorders detrimental to the vascular system, and arterial diseases such as acute aortic dissection (AAD) and

aortic atherosclerosis (AA), as well as vein disorders, are all considered forms of vascular diseases (2,3). This study will specifically focus on aortic atherosclerosis.

Aortic atherosclerosis

Arteriosclerosis, originating from Greek roots, signifies the stiffening or hardening of artery walls (4). Atherosclerosis, viewed as a chronic inflammatory ailment, implicates both innate and adaptive immunity, involving macrophages and lymphocytes in the atherosclerotic mechanism. The progression is typically gradual, characterized by the formation of lipid-laden plaques within large and medium-sized arteries like aorta (5). The disease may be asymptomatic for extended periods until the development of a blood clot, leading to artery blockage and subsequent tissue damage which causes ischemia, plaque erosion, or rupture (6). risk factors for aortic atherosclerosis include obesity, a significant contributor to vascular diseases such as arteriosclerosis (7). Additionally, diabetes mellitus, hypertension, smoking, alcohol consumption, elevated homocysteine levels with advancing age, gender, family history, and heredity are other factors linked to the disease occurrence (8).

Plasminogen Activator Inhibitor-1 (PAI-1)

(PAI-1) is a single-chain glycoprotein (9) PAI-1 inhibits plasminogen activation and belongs to the serine protease inhibitor family (10). This protein promotes blood clot formation by inhibiting the activity of Plasminogen Activators (PAs), enzymes responsible for breaking down blood clots. Inhibiting PAs leads to reduced fibrin breakdown and increased clot formation, potentially contributing to arteriosclerosis development. Additionally, PAI-1 enhances inflammation and oxidative stress, both major driving factors in arteriosclerosis (11). IL-6 stimulates antibody production, activates coagulation, and elevated levels indicate vascular diseases (12). TNF-α, produced by immune cells, plays a crucial role in inflammation and atherosclerosis development, disrupting blood vessels and leading to vascular diseases (13).

Cholesterol, essential for human life, is crucial for cellular functions while elevated levels lead to aortic atherosclerosis, (14). LDL, a harmful cholesterol transporter, forms atherosclerotic plaques, reducing artery elasticity (15). HDL, or "good cholesterol," removes excess cholesterol from tissues, transporting it to the liver for elimination (16, 17, 18) Triglycerides, transported in the bloodstream, can lead to diseases like atherosclerosis when elevated (19).

Methods

In this study, 140 blood samples were collected from 70 patients with aortic atherosclerosis and 70 individuals in the control group. The patients were diagnosed at specialized medical centers in Mosul between 11/4/2023 and 15/6/2023, with ages ranging from 30 to 73 years, including both males and females. to prepare serum, 5 ml of venous blood was drawn and incubated at 37°C for 10 minutes, followed by centrifugation at 1006 x g for 15 minutes (20). The non-dissolving serum was withdrawn using a micropipette.

Blood serum levels of PAI-1, TNF-Alpha, and IL-6 were measured using ready-made kits from Bioassay Technology Laboratory, China, employing the ELISA method (21, 22). total cholesterol (TC), triglycerides (TG), were measured using ready-made kits provided by Biolabo France. (20), HDL-C were measured using the Enzymatic Method and Biolabo Ready-to-Use Kit, following the method described by Kostner in 1976 (23). LDL-C LDL-Cholesterol levels were calculated using the formula: LDL-Cholesterol (Conc.) = Conc. of Total Cholesterol - Conc. of HDL-C, based on the concentration of low-density lipoprotein cholesterol, following the previously described method (24).

Statistical Analysis:

All clinical test results were analyzed using SPSS19 statistical software to determine the following:

1. **Standard Statistical Methods:** Mean and standard errors (SE) were calculated using one-way analysis of variance. Duncan's test was used to identify specific differences between groups, considering a significance value of p (p-value) where ($p \leq 0.05$) indicated a significant difference (25).

Results and discussion:

The data presented in Tables (4) reveal a significant increase in PAI-1 concentration ($p \leq 0.01$) in the serum of atherosclerosis patients (11.27 ± 0.35) compared to the control group (1.20 ± 0.032). These findings support the previous research to indicate that elevated PAI-1 levels are associated with a higher risk of vascular diseases, particularly atherosclerosis. Increased PAI-1 expression in arteries hampers local fibrinolysis by tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA), leading to persistent arterial wall clots and an escalation in atherosclerosis occurrences (26,27).

This study shows significantly elevated IL-6 levels in patients, attributed to IL-6 transportation via blood vessels and subsequent secretion of PAI-1 by endothelial cells. This aligns with research linking dysregulated IL-6 to chronic vascular inflammation and atherosclerosis (28,29). Additionally, elevated TNF-α levels in patients were found, emphasizing its active role in atherosclerosis development. A previous study showed of TNF-α involvement in fibrosis induction, arterial wall weakening, and regulation of PAI-1 expression (30).

The current study reveal high significantly in (TC) levels in patients (9.73±0.70) at the probability ($P \leq 0.01$) compared to control group (3.89±0.05). due to disturbances in lipoprotein metabolism. This disruption led to increased cholesterol storage within cells and reduced synthesis of LDL receptors. Elevated TC levels were linked to the development of aortic stenosis, indicating their potential contribution to the disease (31,32).

(TG) levels significantly higher in patient (4.19±0.43) at a probability ($P \leq 0.01$) compared to the healthy cases (1.14±0.006), possibly due to decreased effectiveness of the lipoprotein lipase (LPL) enzyme responsible for triglyceride removal. High TG were associated with elevated (PAI-1) levels, suggesting a role in blood clot formation and impaired fibrinolysis (33).

The results in Table (1) indicate a significant decline in (HDL-C) levels in blood serum of patients (0.50±0.002) compared to control samples (1.35±0.007), potentially due to reduced production of nascent HDL particles. Low HDL-C levels compromised reverse cholesterol transport, leading to cholesterol accumulation in arteries, increased inflammation, and oxidative stress, contributing to aortic atherosclerosis (34,35).

This study refers to a significant increase in (LDL-C) levels in patients (6.39 ± 0.08) at a probability ($P \leq 0.01$) (compared to the control group (0.71 ± 0.16). This elevation might be attributed to defects in apoB100 structure on LDL particles or its receptors on liver cells and macrophages. Dysfunctional LDL receptors and altered LDL uptake by macrophages contributed to cholesterol accumulation and foam cell formation, promoting atherosclerosis. The current study highlighted the connection between blood cholesterol levels and the development of aortic stenosis, indicating a potential relationship between elevated cholesterol and the disease (36, 37)

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Table (1): Comparing PAI-1 Concentration and Various Biochemical Variables Between Patients with Aortic Atherosclerosis and the Control Group.

Biochemical Variables	Patient Group (n=70)		Control Group (n=70)		P value
	Mean	Standard error	Mean	Standard error	
Plasminogen activator inhibitor-1 (pg/ml)	11.27	0.35	1.20	0.032	**
Interleukin-6 (pg/ml)	32.83	0.94	4.71	0.13	**
Tumor necrosis factor-alpha (pg/ml)	316.12	5.57	7.37	0.14	**
Total cholesterol (mmol/L)	9.73	0.07	3.89	0.05	**
Triglycerides (mmol/L)	4.19	0.43	1.14	0.006	**
High-density lipoprotein cholesterol (mmol/L)	0.50	0.002	1.35	0.007	**
Low-density lipoprotein cholesterol (mmol/L)	6.39	0.08	0.71	0.016	**

(**) indicates a significant difference at the probability level (P ≤ 0.01).

The effect of certain factors on biochemical variables in patients with aortic atherosclerosis:

Gender Influence: The results of current study, presented in Table (2), revealed a significant difference in PAI-1 levels between males and females affected by atherosclerosis, consistent with a previous study (38). This disparity may be attributed to hormonal differences, other risk factors, and genetic factors. Female patients exhibited higher IL-6 levels compared to males, Additionally, TNF-α concentration differences between genders were noted, influenced by sex hormones like estrogen and testosterone, contributing to distinct disease patterns (39,40). A previous study has shown differences in cholesterol levels between men and women during aging, with lower levels of low-density lipoprotein (LDL) cholesterol in women compared to men (41). This aligns with the findings of the current study. Variations in cholesterol concentrations between men and women may contribute to differences in the risk of developing aortic atherosclerosis. Women with familial hypercholesterolemia (FH) are more susceptible to elevated cholesterol levels compared to men with familial hypercholesterolemia (42).

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Table (2): illustrates the comparison of PAI-1 concentration levels and some biochemical variables in the serum of male and female patients with aortic atherosclerosis.

Biochemical Variables	Male group (N=33)		Female group (n=37)	
	Mean	**Standard error**	**Mean**	**Standard error**
Plasminogen activator inhibitor-1 (pg/ml)	a 12.50	0.48	b 10.17	0.43
Interleukin-6 (pg/ml)	b 28.22	1.04	c 39.94	0.70
Tumor necrosis factor-alpha (pg/ml)	b 267.56	0.53	a 359.43	1.42
Total cholesterol (mmol/L)	b 9.50	0.10	a 9.93	0.08
Triglycerides (mmol/L)	b 3.84	0.06	a 4.51	0.83
High-density lipoprotein cholesterol (mmol/L)	a 0.504	0.003	a 0.502	0.003
Low-density lipoprotein cholesterol (mmol/L	a 6.54	0.11	a 6.26	0.12

Horizontally similar letters indicate no significant difference at the probability level ($P \leq 0.05$).

The Effect of age

The results in Table (3) demonstrate the impact of Aging significantly influenced certain biochemical parameters in patients, particularly in the (over 60 years), where PAI-1 concentration was significantly higher (14.44±0.302) compared to (46-60 years) (10.03±0.30) and (30-45 years) (8.27±0.24). These findings align with prior research linking aging to PAI-1 (43). Elevated PAI-1 levels hinder plasmin production, promoting clot formation (44).

The data indicate a significant increase in IL-6 levels, particularly in the age group over 60 years, indicating an association between IL-6 and aging, consistent with previous research (45). elevated IL-6 levels in patients are linked to factors like chronic inflammation and immune responses, contributing to various age-related inflammatory diseases. Additionally, significant differences in TNF-α levels were observed among age groups, with age-related increases in TNF-α potentially contributing to atherosclerosis development. Studies suggest TNF-α's role in vascular dysfunction and atherosclerosis, with anti-TNF-α treatment showing promise in improving atherosclerosis in patients with inflammatory joint disorders.

The study's findings revealed reduced TNF-α concentration in certain categories, possibly due to treatments lowering TNF-α levels. Interestingly, research on elderly mice lacking TNF-α receptors demonstrated a reduction in atherosclerosis (46, 47, 48).

This study found a significant difference in TC, TG, and (LDL-C) concentrations, with an increase in aging among atherosclerosis patients. These findings corroborate previous research linking elevated total cholesterol, triglycerides, and LDL-C with atherosclerosis in older adults, attributed to their atherogenic properties and roles in inflammation and arterial clotting (49, 50). Additionally, low levels of HDL-C) are associated with atherosclerosis in the elderly due to its anti-atherosclerotic properties (51).

Table (3): shows the comparison of PAI-1 concentration levels and some biochemical variables between patients with atherosclerosis and the control group across different age categories: (30-45 years), (46-60 years), and (over 60 years).

Biochemical variables	(30-45) years old N=16		46-60) years old(N=28		<60) years(N=26	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Plasminogen activator inhibitor-1 (pg/ml)	c 8.27	0.24	b 10.03	0.307	a 14.44	0.302
Interleukin-6 (pg/ml)	b 32.49	1.89	b 31.64	1.41	a 38.59	1.30
Tumor necrosis factor-alpha (pg/ml)	a 333.63	9.75	b 310.66	9.23	b 311.22	9.33
Total cholesterol (mmol/L)	b 9.39	0.13	b 9.56	0.07	a 10.12	0.12
Triglycerides (mmol/L)	a 3.48	0.065	b 3.62	0.054	b 4.11	0.043
High-density lipoprotein cholesterol (mmol/L)	b 0.518	0.003	b 0.514	0.002	c 0.48	0.002
Low-density lipoprotein cholesterol (mmol/L)	c 5.77	0.12	b 6.13	0.09	a 7.05	0.07

Horizontally different letters indicate a significant difference at the probability level ($P \leq 0.05$).

The relationship (PAI-1) and the biochemical variables (Pearson correlation coefficients) is as follows:

The correlation analysis Table (4) shows weak positive correlations between (PAI-1), (IL-6) and (TNF-α), indicating a direct relationship. Previous research supports the role of TNF-α in regulating PAI-1 expression, while IL-6 and other cytokines stimulate PAI-1 production. (52, 53). Previous studies indicate a relationship between TC, TG, LDL-C, and PAI-1, with elevated PAI-1 levels associated with

clotting, and vascular problems. Managing cholesterol and triglycerides can reduce disease risks (54, 55). The results align of current study, showing a significant positive correlation ($P \leq 0.01$) with TC, TG, and LDL-C and a low negative correlation ($P \leq 0.01$) with HDL. Elevated PAI-1 levels are linked to increased vascular disease risk, including aortic atherosclerosis (56, 57)

Table (4): display the correlation analysis between the levels of certain biochemical variables and the concentration of PAI-1 in the serum of individuals with aortic atherosclerosis for Patient group.

Parameters	plasminogen activator inhibitor-1
Interleukin-6 (pg/ml)	0.125
Tumor necrosis factor-alpha (pg/ml)	0.384**
Malondialdehyde (µmol/L)	0.765**
Peroxynitrite (µmol/L)	0.779**
Total cholesterol (mmol/L)	0.350**
Triglycerides (mmol/L)	0.679**
High-density lipoprotein cholesterol (mmol/L)	-0.653**
Low-density lipoprotein cholesterol (mmol/L)	0.696**

****Significant differences at the probability level ($p \leq 0.01$).**
*** Significant differences at the probability level ($p \leq 0.05$).**

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