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Assessing Vitamin D3 in Patients with a Therosclerosis and Their Correlation with Interferon Gamma and Biochemical Markers in the Salah al-Din Governorate

May Khalil Ghaffar , Iktifa Abdul Hamid Saeed

Department of Biology, College of Education for Girls, Tikrit University, Tikrit, Iraq

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ABSTRACT

The study aimed to assess vitamin D3 levels particularly interferon-gamma (IFN-γ), and their correlation with various biochemical and physiological markers. This study involved 180 males aged 45–75 years. Group1 had long-term atherosclerosis, Group 2 had recently developed atherosclerosis, Group 3 had both atherosclerosis and diabetes, and for each group (n=40), Group 4 (n=35) had atherosclerosis and were receiving vitamin D3 supplements, and Group 5 (n=25) was the healthy group. Blood samples were collected at Salah al-Din General Hospital after diagnosis by specialist doctors.

Results significant (P≤0.01) in IFN-γ levels in Groups 1, 2, and 3. Aspartate aminotransferase (AST) levels showed a significant increase (P≤0.01) in groups 2, 3, and 4. aminotransferase (ALT) levels showed a significant increase (P≤0.01) in group 2.

These findings suggest a strong association between atherosclerosis and altered IFN-γ and liver enzyme levels, indicating potential biomarkers for disease progression and treatment monitoring. Vitamin D3 plays a crucial role in preventing atherosclerosis, particularly by reducing the incidence of high-risk atherosclerotic plaques and decreasing the burden of non-calcified plaques.

Keywords: Atherosclerosis, Diabetes, Interferon-γ, Liver enzymes, Vitamin D3.

Name: **May Khalil Ghaffar** **E-mail:** maykhalel04@gmail.com

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تقييم فيتامين و D3 إنترفيرون جاما لدى مرضى تصلب الشرايين وعلاقتها بعدد من المؤشرات البيوكيميائية والفسيولوجية في محافظة صلاح الدين

مي خليل غفار، اكتفاء عبد الحميد سعيد

قسم علوم الحياة، كلية التربية للبنات، جامعة تكريت، تكريت، العراق

الملخص

شملت هذه الدراسة 180 ذكرًا تتراوح أعمارهم بين 45 و75 عامًا. المجموعة الأولى مصابة بتصلب الشرايين طويل الأمد، والمجموعة الثانية مصابة به مؤخرًا، والمجموعة الثالثة مصابة بتصلب الشرايين والسكري معًا. وفي كل مجموعة (عددهم 40)، كانت المجموعة الرابعة (عددهم 35) مصابة بتصلب الشرايين وتتلقى مكملات فيتامين د3، بينما كانت المجموعة الخامسة (عددهم 25) مجموعة صحية. جُمعت عينات الدم من مستشفى صلاح الدين العام، بعد تشخيصها من قبل أطباء متخصصين. هدفت الدراسة إلى تقييم مستويات فيتامين د3 وبعض الإنترلوكينات، وخاصةً الإنترفيرون جاما(IFN-γ) ، وارتباطها بمختلف المؤشرات البيوكيميائية والفسيولوجية. أظهرت النتائج دلالة إحصائية ($P\leq0.01$) في مستويات إنترفيرون–جاما (IFN-γ) في المجموعات 1 و2 و3. وأظهرت مستويات ناقلة أمين الأسبارتات (AST) زيادة ملحوظة ($P\leq0.01$) في المجموعات 2 و3 و4. وأظهرت مستويات ناقلة أمين الأسبارتات (ALT) زيادة ملحوظة ($P\leq0.01$) في المجموعة 2.

تشير هذه النتائج إلى دور محتمل لفيتامين D3 الإنترفيرون غاما في فسيولوجيا مرض تصلب الشرايين، وتدعم استخدامها كمؤشرات بيولوجية لمراقبة تقدم المرض.

INTRODUCTION

Cardiovascular diseases, particularly atherosclerosis, pose significant challenges to global health [1]. The underlying pathological mechanism of cardiovascular diseases is atherosclerosis [2]. The increasing prevalence of atherosclerotic lesions resulting from heart diseases, which are classified as age-related conditions, is one of the leading causes of death worldwide. This represents a major health crisis affecting the cardiovascular system. Atherosclerosis is described as a chronic condition that can lead to myocardial infarction, ischemic cardiomyopathy, stroke, and peripheral artery diseases [3, 4]. Atherosclerosis is defined as a progressive inflammatory disease characterized by the accumulation of fats in the walls of arterial blood vessels. It involves the deposition of atherosclerotic plaques in the inner walls of arteries, which leads to the gradual accumulation of lipids, followed by the recruitment of T-cells and macrophages to the intima of the arteries, resulting in narrowing of the vascular lumen [5]. Vitamin D plays a key role in the structure and function of the vascular endothelium, with some studies indicating a potential relationship between low serum vitamin D levels and atherosclerosis, as well as an increase in the thickness of the arterial intima[6]. Interferon (IFN-γ) can influence several critical steps in the development of atherosclerosis, including pro-inflammatory gene expression, the recruitment of monocytes from the blood to the activated endothelium, and plaque stability. The central role of IFN-Y in atherosclerosis makes it a promising medical target [7]. The abnormal levels of alkaline phosphate (ALP) in the blood indicate liver and gallbladder damage, bone disorders, heart disease, type 2 diabetes, hypertension and dislipidemia[8, 9]. The exact mechanism associated with vitamin D3 deficiency and the exact mechanisms involving atherosclerosis

include anti -inflammatory effects. This pro-inflammatory cytokinet inhibits the production of IFN IS, reduces the activation of immune cells involved in the disease, and reduces inflammatory loads in the arterial walls by modifying the inflammatory response and disrupting the growth and progress of atherosclerosis [10].

The purpose of this study is to estimate the concentration of interferon (IFN-)), measure Alleninaminotransferase (alt) enzyme, assess the level of alkaline phosphate (ALP) and aspartate measure aminotransferase (AST) enzyme.

MATERIALS AND METHODS

Sample Collection and Storage:

A total of 180 male blood tests were collected from patients admitted to the intensive care unit after intensive diagnosis of special doctors. The diagnosis was based on clinical symptoms, detailed patient questionnaire, and specialized clinics in Tikrit. A total of 180 male blood tests were collected from patients admitted to the intensive care unit after intensive diagnosis of special doctors. Diagnosis was based on clinical symptoms, detailed patient questionnaire

1. Immunological Tests

The concentration of interferon IFN-Y in the blood serum was measured using the sandwich element method, which is with a set of a Chinese manufacturer, a set produced by Sanlong. The optical density (OD) was measured using a spectrophotometer on a wavelength of 450 nm [11].

2. Chemical Tests

1. Measurement of Alanine Aminotransferase (ALT) Enzyme Concentration. According to the protocol from Giesse Diagnostics, Italy [12], the all concentration was measured using the Ultraviolet (UV) method without pyridoxal phosphate activation.

2. Measurement of Alkaline Phosphatase (ALP) Enzyme Concentration

According to the protocol from Giesse Diagnostics, Italy [12], all concentration was measured using the Ultraviolet (UV) method without pyridoxal phosphate activation.

3. Aspartataminotransferase (AST) Enzyme concentration measurement AST concentration according to the GIESSE diagnostic protocol was measured using the Ultraviolet (UV) method without pyridoxal phosphate activation [12], Italy.

The data were statistically analyzed using one-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to determine significant differences among means at a significance level of $P \leq 0.01$ [13].

$$\text{L- aspartate} + \alpha \text{ oxoglutarate} \xrightarrow{\text{AST}} \text{L- glutamate} + \text{oxoglutarate}.$$

RESULTS AND DISCUSSION

The results (Figure 1 and Table 1) showed a significant increase ($P \leq 0.01$) in the IFN-Y concentration in the group of patients with long-term atherosclerosis, the group with short-term atherosclerosis, the group with atherosclerosis and diabetes. and the group with atherosclerosis who were taking vitamin D3, compared to healthy group.

The results (Figure 1 and Table 1) showed a significant increase ($P \leq 0.01$) in the IFN-Y concentration in the group of patients with long-term atherosclerosis, the group with short-term atherosclerosis, the group with atherosclerosis and diabetes. and the group with atherosclerosis who were taking vitamin D3, compared to healthy group.Compared with other groups, D3 for the group with atherosclerosis who took ($P \leq 0.01$) and a significant decrease. Helper T cells secrete IFN-γ, which plays a supporting role in atherosclerosis Helper T cells secrete IFN-γ, which plays a supporting role in atherosclerosis [14]. Vitamin D3 levels have anti-inflammatory effects on risk factors associated with atherosclerosis by modulating immune responses, improving endothelial cell function, and influencing lipid metabolism. All of these factors play a critical role in the development of atherosclerosis and plaque stability [15].

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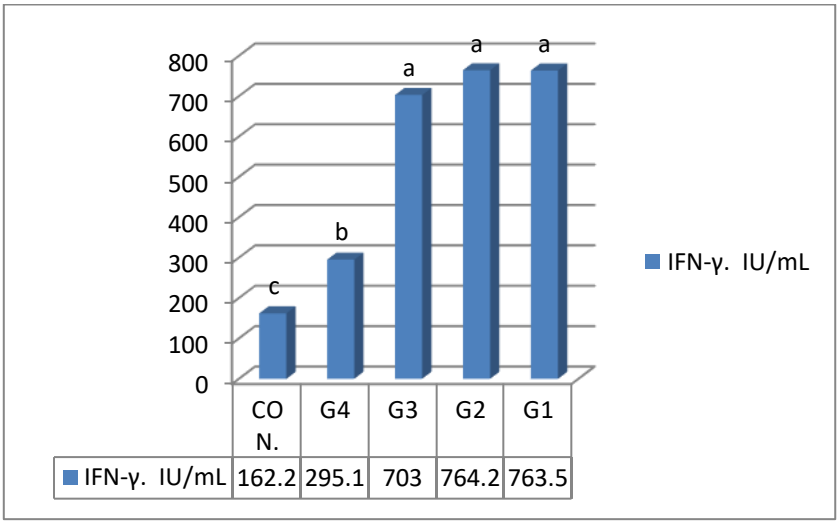

Fig. 1: The IFN-γ concentration levels in patient groups compared to healthy individuals. It illustrates the concentration levels for a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), and a group of patients with atherosclerosis who are taking vitamin D3 (G4), compared to healthy group. (G5).

Table 1: The concentration levels of ((IFN-γ)) in patient groups compared to healthy individuals. It presents the mean ± standard deviation of ((IFN-γ)) concentrations for a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), and a group of patients with atherosclerosis who are taking vitamin D3 (G4), compared to healthy group. (G5).

Sequence	Study Group	N	M ± S.D	Significance Letters	P-value
1	G1	40	81.4 ± 763.5	a	0.00007
2	G2	40	84.1 ± 764.2	A	
3	G3	40	94.6 ± 703.0	a	
4	G4	35	49.9 ± 295.1	b	
5	G5	25	32.8 ± 162.2	c	
Total		180			

*Different letters horizontally indicate significant differences at (P≤0.05) and (P≤0.01)

The results in (Figure 2 and Table 2) showed a significant increase (P≤0.01) in AST concentration in the group of patients with short-term atherosclerosis, the group with atherosclerosis and diabetes, and the group with atherosclerosis who were taking vitamin D3, compared to healthy group.There was no significant difference between the group with long-term atherosclerosis, and healthy controls.Studies have confirmed a relationship between elevated AST levels and cardiovascular diseases and metabolism. Epidemiological studies strongly support a connection between elevated AST levels and increased risk of death from all causes, including cardiovascular diseases [16].

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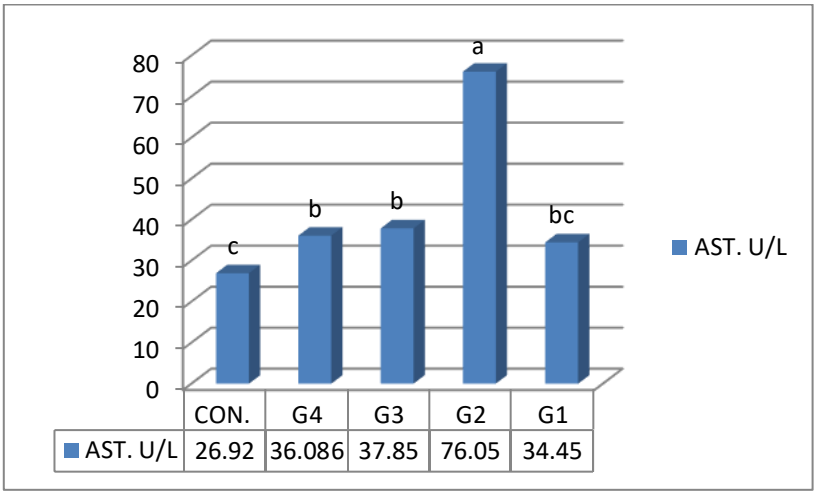

Fig. 2: The AST concentration levels in patient groups compared to healthy individuals. It illustrates the AST concentration levels for a group of patients with long-term atherosclerosis (G1),a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), and a group of patients with atherosclerosis who are taking vitamin D3 (G4), compared to healthy group. (G5).

Table 2: Comparing the concentration of AST in patient groups versus healthy controls. It shows the mean ± standard deviation of AST levels in a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), a group of patients with atherosclerosis who are taking Vitamin D3 (G4), compared to healthy group. (G5).

No.	Study Group	N	M ± S.D (U/L)	Significance Letters	P-value
1	G1	40	34.450 ± 4.771	bc	0.000003
2	G2	40	76.050 ± 7.800	A	
3	G3	40	37.850 ± 3.800	B	
4	G4	35	36.086 ± 4.252	B	
5	G5	25	26.920 ± 4.030	C	
	Total	180			

*Different letters horizontally indicate significant differences at (P≤0.05) and (P≤0.01)."

The results in (Figure 3 and Table 3) for ALT (Alanine Aminotransferase) showed a significant increase (P≤0.01) in ALT concentration in the group of patients with short-term atherosclerosis, and a significant decrease (P≤0.01) in the group with long-term atherosclerosis and the group with atherosclerosis who were taking vitamin D3. No significant differences were observed in the group with atherosclerosis and diabetes, compared to healthy group. Elevated ALT levels are associated with increased mortality from cardiovascular diseases. Alkaline phosphatase (ALP) is a commonly used marker for liver function and cardiovascular disease risk [17]. It is well-known that liver enzyme markers and the risk of coronary artery disease are elevated in cases of myocardial ischemia. AST peaks approximately 24 hours after a myocardial infarction, while ALT peaks later, between 1-6 days, with both enzymes declining by the sixth day after infarction. Both AST and ALT are also correlated with the creatine kinase (MB) isoenzyme levels in circulation, and elevated transaminase levels are an independent indicator for 30-day outcomes, contributing to the emergence and progression of atherosclerosis[18].

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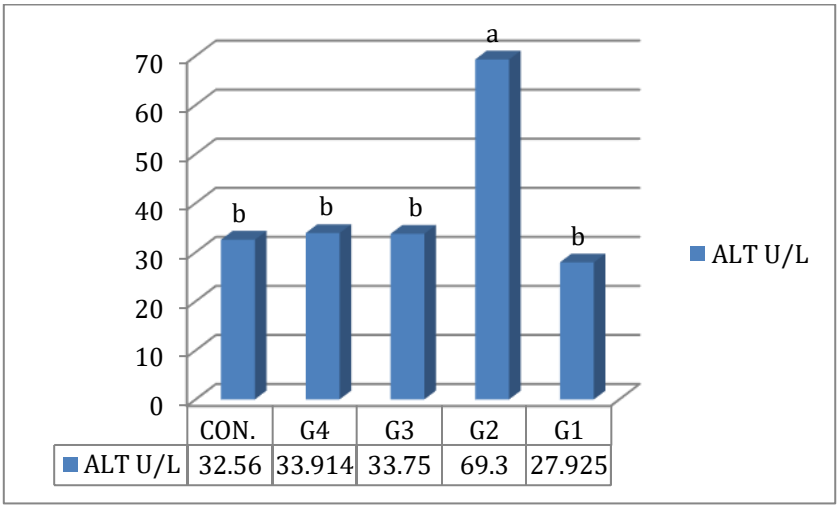

Fig. 3: ALT concentration levels in patient groups compared to healthy controls. It shows the ALT concentration levels in a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), a group of patients with atherosclerosis who are taking Vitamin D3 (G4), compared to healthy group. (G5).

Table 3: ALT concentration in patient groups compared to healthy controls. It shows the mean ± standard deviation of ALT levels in a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), a group of patients with atherosclerosis who are taking Vitamin D3 (G4), compared to healthy group. (G5).

No.	Study Group	N	M ± S.D (U/L)	Significance Letters	P-value
1	G1	40	27.925 ± 4.166	B	0.000004
2	G2	40	69.300 ± 13.21	a	
3	G3	40	33.750 ± 3.184	b	
4	G4	35	33.914 ± 3.721	B	
5	G5	25	32.560 ± 4.321	b	
	Total	180			

*Different letters horizontally indicate significant differences at (P≤0.05) and (P≤0.01)."

The results in (Figure 4 and Table 4) showed a significant increase (P≤0.01) in ALP concentration in the group with short-term atherosclerosis, the group with atherosclerosis and diabetes, and the group with atherosclerosis who were taking vitamin D3. No significant difference was observed in the group with long-term atherosclerosis compared to healthy group. Strong evidence suggests a potential link between elevated ALP levels and an increased risk of atherosclerosis. Elevated ALP levels are associated with negative outcomes in atherosclerosis [19]. The cardiovascular damage is more pronounced with a correlation between ALP and C-reactive protein (CRP), indicating a shared biological pathway [20]. It has been established that serum alkaline phosphatase (ALP) levels are linked to the risk of cardiovascular diseases (CVD), with inflammation initiating the process of atherosclerosis. Over the course of atherosclerosis, ALP levels increase, and the risk of developing atherosclerosis increases with elevated ALP levels. This relationship becomes more evident in inflammatory cases and varies by sex and age [21, 22].

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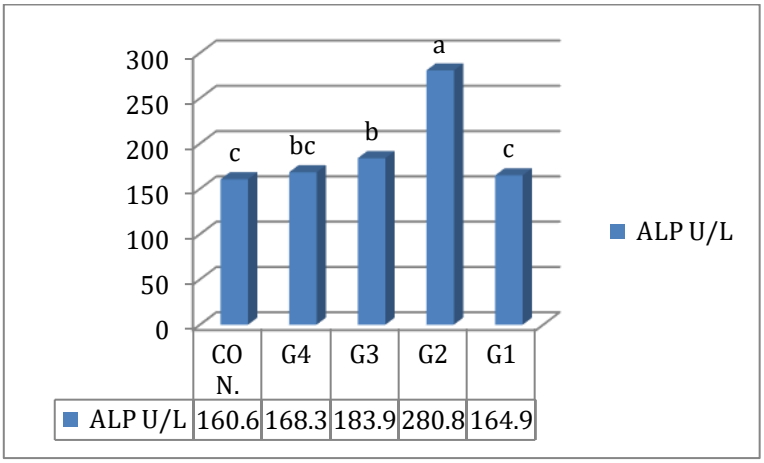

Fig. 4: ALP concentration levels in patient groups compared to healthy controls. It shows the ALP concentration levels in a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), a group of patients with atherosclerosis who are taking Vitamin D3 (G4), compared to healthy group. (G5).

Table 4: ALP concentration in patient groups compared to healthy controls. It shows the mean ± standard deviation of ALP concentration levels in a group of patients with long-term atherosclerosis (G1), a group of patients with short-term atherosclerosis (G2), a group of patients with atherosclerosis and diabetes (G3), a group of patients with atherosclerosis who are taking Vitamin D3 (G4), compared to healthy group. (G5).

No.	Study Group	N	M ± S.D (U/L)	Significance Letters	P-value
1	G1	40	164.93 ± 17.53	c	0.00006
2	G2	40	280.75 ± 29.75	a	
3	G3	40	183.88 ± 10.25	B	
4	G4	35	168.34 ± 12.34	Bc	
5	G5	25	160.56 ± 15.77	C	
	Total	180			

*Different letters horizontally indicate significant differences at (P≤0.05) and (P≤0.01).

The significant elevation of IFN-γ across all atherosclerotic groups, suggests a critical role of immune-mediated inflammation in disease pathology. the biochemical variations indicate systemic inflammatory responses and hepatic stress. Vitamin D3 supplementation showed distinct effects on ALT and IFN-γ levels, suggesting a modulatory role in inflammation and liver function.

Conclusions

In light of the research and the current findings, the following conclusions were reached:

1- Vitamin D3 levels were low in the long-term atherosclerosis group, the short-term atherosclerosis group, and the atherosclerosis group with diabetes, except for the atherosclerosis group taking Vitamin D3, as a reduction in symptoms was observed.

2- Regarding liver enzymes, ALP, AST, and ALT concentrations, no significant difference was found in the long-term atherosclerosis group compared to healthy individuals. However, a significant increase was observed in all concentrations in the short-term atherosclerosis group compared to healthy individuals. A significant increase in AST concentration was found in the short-term atherosclerosis group, the atherosclerosis group

with diabetes, and the atherosclerosis group taking Vitamin D3.

3- A significant increase in INF-Y concentrations was found in all study groups—the long-term atherosclerosis group, the short-term atherosclerosis group, the atherosclerosis group with diabetes, and the atherosclerosis group taking Vitamin D3—compared to healthy individuals.

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Author contribution: Authors contributed equally in the study.

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