The Effect of Vitamin D Level on Some Biomarkers of Inflammation and Oxidative Stress in Atherosclerotic Patients

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Abstract
Atherosclerosis is a chronic disease around the world and is the main cause of death risk for patients with atherosclerosis and type 2 diabetes. The study aimed to evaluate the effect of vitamin D on a number of non-traditional biomarkers overlapping in the processes of inflammation and oxidation in patients with atherosclerosis. The study was conducted on patients referred to specialized clinics in Kirkuk and Cardiology Hospital in Sulaymaniyah for the period from August 2021 to January 2022 and included sixty samples of men whose ages ranged between 40 and 70 years, distributed into three groups, the first included twenty men with atherosclerosis, the second group included twenty men with atherosclerosis and type II diabetes, and the third group included twenty healthy men within the same age range. The results showed a significant decrease in the groups of atherosclerosis and atherosclerosis with diabetes compared to the control group for the level of vitamin D, and there was a significant increase in the levels of PTX3, Interlukin 10, OxLDL and hsCRP in the groups of atherosclerosis and atherosclerosis with diabetes compared to the control group. It is concluded that vitamin D deficiency may be associated with increased production of reactive oxygen species, poor antioxidant capacity, and low anti-inflammatory properties that are extremely important in the development of atherosclerosis event.

Keyword: Atherosclerosis, Vitamin D, PTX3, Interlukin 10, OxLDL, hsCRP .

1. Introduction

Atherosclerosis is a disease that is characterized by the accumulation of lipids, fibrous elements, and calcification within the large arteries. This process is initiated by endothelium activation, followed by a cascade of events, which implies the vessel narrowing and activation of inflammatory pathways leading to atheroma plaque formation. Altogether, these processes result in cardiovascular complications that remain as the leading cause of death worldwide [1]. Cardiovascular diseases (CVD) are a major health concern worldwide. According to the World Health Organization (WHO), coronary artery disease (CAD), and this disease mainly occurs due to the development of arteriosclerosis, which is the biggest risk factor [2]. Cardiovascular disease continues to grow as an enormous global health burden, with coronary artery disease being one of its most deadly. Atherosclerosis causes changes in the blood vessels and the extracellular matrix (ECM) in each vascular layer , altering the ECM homeostasis has significant modulatory effects on the inflammatory response, proliferation and migration of vascular smooth muscle cells, neointimal formation, and vascular fibrosis seen in atherosclerosis , understanding the different effects of ECM modifications opens up a large number of therapeutic options that would mitigate the significant health toll of atherosclerosis on the global population [3]. Atherosclerosis is initiated by endothelial injury due to oxidative stress associated with cardiovascular risk factors including diabetes mellitus, hypertension, cigarette smoking, hyperlipidemia, obesity and metabolic syndrome [4]. Oxidative stress converts low-density lipoproteins (LDL-C) into oxidative lipoproteins (ox-LDL-C), which are devoured by macrophages and stimulate endothelial cells to produce cytokines and chemokines [5]. In the search to develop reliable biomarkers for atherosclerotic disease, researchers have focused on increasing insights into the pathology that underlies propagation of atherosclerotic disease. Atherosclerosis has become increasingly recognized as a pathological state, characterized by the accumulation of oxidative stress and inflammation in association with lipids in the artery wall [6–7]. These processes have been implicated at all stages of the disease, from the very early appearance of endothelial dysfunction, through the propagation and rupture of atherosclerotic plaque and, ultimately, tissue injury, in the settings of both ischemia and reperfusion. Accordingly, there has been considerable interest in the monitoring of these pathways as potential biomarkers for CVD, which will herein be studied.

2. Materials and Methods

Venous blood was collected for men with arteriosclerosis aged between (40-70) years from patients who attended the Specialized Cardiology Center in the city of Sulaymaniyah for the period from (August 2021 to January 2022), who were diagnosed with atherosclerosis clinically and

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laboratory. Vascular calcification by specialists in the same center, based on the health staff's tests, based on the CT-Scan device, which also gives a picture or assesses the severity of the patient's arterial stiffness level and the study included three groups, the first of which was a control group with 20 healthy men from the disease, the second group included Patients with atherosclerosis only, and the third group included people with atherosclerosis and type 2 diabetes. The study focused on knowing the extent of the effects on the level of vitamin D and Patients' lipid profile.

Blood Samples

Blood was taken from the humeral vein by withdrawing (5 ml / sample) and leaving the sample at room temperature (25 °C) for a period of (20 min) for the purpose of coagulation until the blood clotted, then placed in a centrifuge at a speed (3000 rpm) for (15-20) minutes, and the blood serum was withdrawn by means of a micropipette to the eppendrof tubes after dividing them into small samples and serum was frozen at (-20 °C) until biochemical tests were performed, and the information on each sample was recorded on it.

Biochemical measurements:

Vitamin D, Pintraxin (PTX3), Interleukin 10 (IL-10), Oxidized low density lipoprotein (OxLDL) and high sensitivity C-Reactive Protein (hs-CRP) was estimated using ELISA Kit (Sunlong) according to manufacturer instructions [8].

3. Statistical Analysis

The results were statistically analyzed using the Minitab program. In order to extract the differences between the experimental groups with emphasis on these differences by extracting the standard error (Stander Error) SE Statistical analyzes were conducted according to Duncan were identified the probability level (P≤0.05).

4. Results and Discussion

The results of the current study showed a significant decrease at the level (P≤0.05) in the concentration of vitamin D in the group affected by atherosclerosis and diabetes mellitus compared with the healthy group, as well as a significant decrease in the concentration of vitamin D in the group affected by atherosclerosis compared with the healthy group , and there are no significant differences in the concentration of vitamin D when comparing the group affected by atherosclerosis and diabetes mellitus and the group affected by atherosclerosis only, the results of the current study showed a significant increase at the level (P≤0.05) in Pintraxin (PTX3) , Interleukin 10 (IL-10) , Oxidized low density lipoprotein (OxLDL) and high sensitivity C-Reactive Protein (hs-CRP) concentrations in the group affected by atherosclerosis and diabetes mellitus compared with the group affected by atherosclerosis and the healthy group, as well as a significant increase in Pintraxin (PTX3) , Interleukin 10 (IL-10) , Oxidized low density lipoprotein (OxLDL) and high sensitivity C-Reactive Protein (hs-CRP) concentrations in the group affected by atherosclerosis compared with the healthy group, as shown in the table (1).

Table (1) : Sample specification and studied parameters in the group affected by atherosclerosis and diabetes mellitus and the group with atherosclerosis only and the healthy group of diseases.

Sample specification		Healthy	Atherosclerosis	Atherosclerosis and DM
Number		20	20	20
Systolic pressure mm/Hg		118	168	191
Diastolic pressure mm/Hg		78	93	102
Smoking	smokes	3	14	15
	Nonsmoking	17	6	5
BMI Kg/m^2		22	31	28
Age (years)		53	54	57
Sex		male	male	male
Parameters				
Vitamin D ng/ml		14.679 a	5.173 b	4.521 b
PTX3 pg/ml		77.875 c	203.875 b	988.708 a
IL-10 pg/ml		4.731 c	7.475 b	11.111 a
OxLDL pg/ml		278.95 c	402.592 b	481.654 a
hs-CRP ng/ml		1.391 c	3.5 b	7.254 a

Vitamin D is also involved in atherosclerosis by inhibiting the proliferation of vascular smooth muscle cells. Furthermore, vitamin D deficiency is associated with a persistent incidence of cardiovascular disease, such as myocardial infarction, unstable angina, stroke, cardiovascular death, and increased mortality after acute stroke [9], and a few different studies indicated that low levels of vitamin D were associated with poor endothelial function [10], and It is attributed to the fact that vitamin D deficiency has a significant and direct effect on the state of blood vessels, including promoting nitric oxide formation and inhibiting anti-inflammatory and oxidative stress [11]. In addition, vitamin D deficiency increases miR-21 expression in the artery, which leads to the development of atherosclerosis [12], and Vitamin D deficiency has a direct negative effect on the endothelial function of the blood vessels, which increases the incidence of atherosclerosis [13], and the reason for the decrease in vitamin D in patients with atherosclerosis is due to the existence of an

actual correlation between the levels of the vitamin and the levels of the lipid profile in the blood, as well as its immune role, which allows for the occurrence of inflammation in the inner lining and the occurrence of atherosclerosis. It is attributed that diabetes increases the liver's production of lipoproteins rich in triglycerides, which leads to an increase in the formation of VLDL that causes atherosclerosis.

PTX3 is predominantly produced in vascular cells in response to inflammatory signals, dendritic cells, endothelial cells, smooth muscle cells, macrophages, and fibroblasts [14]. PTX3 has been suggested as a marker of inflammatory activity and instability of weak plaques in the artery wall. Immunofluorescent microstudies showed increased expression of PTX3 in atherosclerotic plaques compared with that in non-atherogenic arteries [15], and it has been attributed that an elevated level of PTX3 is a possible indicator of weakness in the carotid plaque in the arterial walls [16], and by the apparent effect of pentaxene PTX3 on lipid metabolism by increasing uptake of oxidized low-density lipoprotein cholesterol and inhibiting cholesterol efflux pathways such as peroxisome proliferator-activated receptor (PPARγ), liver X receptor alpha (LXRα) and ATP-binding cassette 1 (A-A-A transporter ABCA1). In contrast, HDL-c molecules and antioxidants have been shown to modulate innate immunity, including activation of the complement system and expression of PTX3 and this is a clear indication of the onset of atherosclerosis and the onset of endothelial wall dysfunction [17]. In addition, another study confirms that the high levels of the PTX3 pentraxin protein are due to weakness and rupture of the arterial plaque, and this indicates the identification of the stages of development of vascular diseases and atherosclerosis [18]. Inflammatory factors reduce the risk of coronary heart disease and atherosclerosis. The multidirectional interleukin-10 (IL-10) reduces the risk of atherosclerosis. It is known that a genetic polymorphism of the IL10 gene region is associated with varying levels of IL-10 production, which is indicative of a decrease in arterial elasticity in men [19], and it is attributed that elevated levels of interleukin IL-10 may be predictive markers for the progression of coronary atherosclerosis [20], and diabetes mellitus is a disorder of carbohydrate metabolism characterized by hyperglycemia, so diabetes depends on the immune system, where altered expression patterns of cytokines and anti-inflammatory factors such as IL-10 play an essential role in inflammation, as well as being an important inhibitor against the action of cytokines. Inflammatory and potentially high levels of IL-10 may also be risk factors for diabetes [21]. In addition, interleukin IL-10 has an effective role in the production of many pro-inflammatory molecules and is closely related to the increase in harmful fats in the blood, as well as diabetes mellitus and is related to risk factors for cardiovascular disease and atherosclerosis [22], and this may explain that the elevated IL-10 level in the group with atherosclerosis and diabetes is associated with these diseases, which increases the inflammatory process within arterial walls when deposits of fat, cholesterol, etc., may accumulate. It is a predictive marker for the stages of atherosclerosis and diabetes.

Under oxidative stress, low-density lipoprotein (LDL) oxidation occurs by a process of lipid peroxidation that primarily involves phospholipid molecules. They are oxidized by ROS in which case the LDL particles are transformed into OX-LDL [23], and atherosclerosis is the number one cause of death in the world, and over the past two decades, several evidence have accumulated, indicating the central role of inflammation in the development of atherosclerosis. High sensitivity C-reactive protein (hsCRP) is a well-established marker of cardiovascular disease (CV), as well as an association of high levels of hsCRP with negative CV outcomes after acute coronary syndrome (ACS), hsCRP in the initiation and development of atherosclerotic plaque , hsCRP has been progressively considered a real risk factor for atherosclerosis and similarly for low-density lipoprotein cholesterol (LDL-C), expanding the concept of inflammatory risk for heart disease and atherosclerosis [24], and in our current study, it may be attributed that high levels of hsCRP jointly increase the risk of atherosclerosis and this may be from creating an inflammatory environment in the plaque of the arterial walls [25]. Our finding of an association between hsCRP concentration and atherosclerosis regardless of other factors, hsCRP is a major inflammatory factor produced by hepatocytes, SMCs, macrophages, ECs, lymphocytes, and fat cells in the body. In addition, hsCRP levels were significantly higher in individuals with atherosclerotic disease [26]. High sensitivity C-reactive protein (HS-CRP) is a marker of inflammation and an independent risk factor for atherosclerosis. It may be an important predictor of atherosclerosis, as well as an effective indicator when treating atherosclerosis, as the risk of cardiovascular disease is observed by lowering the level of inflammatory markers, including hs-CRP, when taking medications.

5. Conclusions

The results of the study concluded that there is a correlation between vitamin D deficiency and atherosclerosis and a decrease in the level of vitamin D corresponds to an increase in inflammatory and oxidative stress indicators in the blood serum. In addition, the decrease in the level of vitamin D coincided with the increase in the level of Pentraxin and hs-CRP and more in the atherosclerosis group with diabetes, which gives a clear indication of the progression of the disease and damage to the endothelium of the blood vessel.

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