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RESEARCH ARTICLE - CHEMISTRY

Evaluating the Association between Oxidized Low-Density Lipoprotein (OxLDL) and Nitric Oxide Levels in Iraqi Atherosclerosis Patients

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Abstract

Atherosclerosis is a chronic condition where plaques composed of lipids build up in arterial walls, causing them to harden and narrow. Oxidized low-density lipoprotein (OxLDL) plays a crucial role in this process by triggering inflammatory responses and contributing to plaque formation. Nitric oxide (NO) is essential for maintaining vascular health, but its bioavailability is often reduced in atherosclerosis, exacerbating the condition. ***Objective:*** Evaluation of levels of oxLDL as an indicator of oxidative stress and assessment its effect on nitric oxide levels. ***Methods:*** This study included determination of the levels of oxLDL and nitric oxide in sera samples from 100 atherosclerosis patients in comparison with age and sex matched healthy donors. ***Results:*** The results showed that there is a statistically significant increase ($p<0.01$) in oxLDL levels in atherosclerotic individuals as compared to control group, while a statistically significant reduction ($p<0.01$) was observed in nitric oxide levels in patients' group. ***Conclusion:*** The data indicates that elevated levels of oxidized low-density lipoprotein (OxLDL) and reduced levels of nitric oxide (NO) are significant markers in the pathogenesis of atherosclerosis. Oxidative stress acts as a critical trigger for the formation of OxLDL, which in turn amplifies pro-inflammatory responses within the vascular system. High OxLDL levels contribute to endothelial dysfunction and promote inflammation, exacerbating plaque formation. Concurrently, decreased NO levels impair vascular relaxation and increase oxidative stress, further aggravating the condition. This interplay between oxidative stress, high OxLDL, and decreased NO highlights the complex mechanisms driving atherosclerosis and underscores the potential for therapeutic interventions targeting these pathways.

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Keywords: Atherosclerosis, Oxidized Low-Density Lipoprotein, Nitric Oxide, Oxidative stress, Inflammation.

1. Introduction

Atherosclerosis, a major cause of mortality in developed nations, arises from lipid dysregulation and oxidative processes. This chronic vascular disease is marked by the accumulation of atherosclerotic plaques, [1, 2] consisting of a lipid-rich core encased in a fibrous cap, which leads to arterial thickening and impaired blood flow. Hyperlipidemia and hyperglycemia contribute to its pathogenesis by increasing oxidative stress,[3] impairing antioxidant defenses, and disrupting lipoprotein metabolism. [4]Oxidative stress influences atherosclerosis through the overproduction of reactive oxygen species (ROS), [5, 6]which cause vascular damage and trigger inflammatory responses.[7] This inflammatory state is characterized by elevated levels of mediators such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-6, IL-18), and C-reactive protein (CRP), [8, 9] all associated with cardiovascular risk and plaque instability. Consequently, there is a considerable likelihood of oxidized low-density lipoprotein (oxLDL) formation, which may contribute to endothelial dysfunction and foam cell formation, processes integral to disease progression. Measuring oxLDL levels may help elucidate the role of oxidative mechanisms in atherosclerosis**.** [10]Similarly, nitric oxide (NO), a regulator of endothelial function and vascular tone, is linked to inflammation and plaque development, highlighting its relevance in understanding

disease progression. [11]This study further examines the interactions among oxidative stress, inflammation, and lipid status in atherosclerosis, focusing on their roles in the progression of the disease and their impact on cardiovascular risk.

2. Methods

A cohort of 100 atherosclerosis patients, comprising 48 males and 52 females, was gathered between December 2023 and March 2024 from Gene Lab Laboratory and Al-Naaman Hospital in Baghdad. This group was compared with 40 healthy individuals (20 males and 20 females). The age range for both patients and controls was 50 to 70 years, and all were free from any additional diseases.

Biochemical tests were carried out on both patients and control subjects, specifically targeting those diagnosed with atherosclerosis. The tests included measuring Human Oxidized Low-Density Lipoprotein (OxLDL) via ELISA technique, and quantifying Nitric Oxide (NO) using a colorimetric assay. The purpose of these analyses was to explore potential biomarkers linked to atherosclerosis and oxidative stress.

The Elabscience Nitric Oxide (NO) Colorimetric Assay protocol[12] and the Elabscience Human OxLDL (Oxidized Low-Density Lipoprotein) ELISA Kit protocol were meticulously adhered to, [13] following the manufacturer's guidelines for each respective kit.[14]

2.1. Statistical Analysis and calibration

OxLDL standard curve were used to ascertain the concentration in unknown samples. as shown in Figure 1. This curve was generated by plotting the mean optical density (O.D.) readings at 450 nm against a series of eight standard concentrations (4000, 2000, 1000, 500, 250, 125, 62.5, and 0 pg/mL). Prior to interpretation, the O.D. readings for each sample are adjusted by deducting the average value of the zero standard.

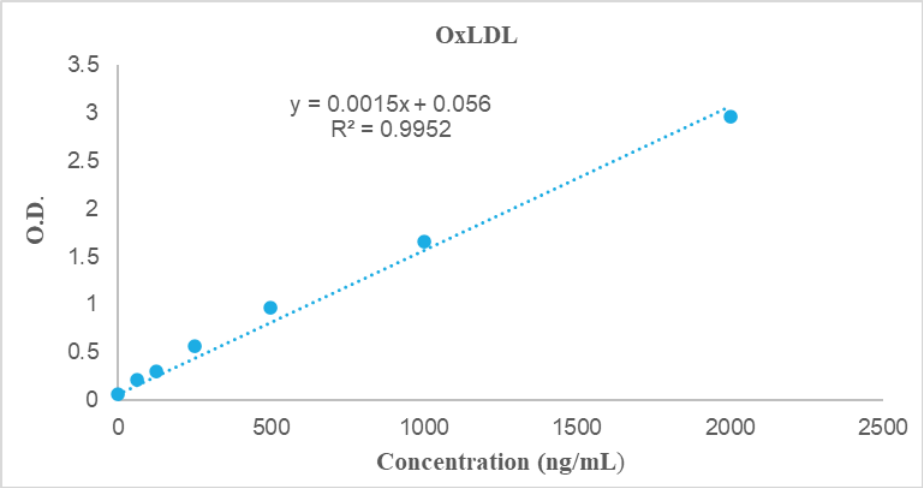

Figure 1. Standard carve of OxLDL determination.

To determine the nitric oxide content, the calculation was conducted using the following parameters after measuring the absorbance of the samples and the standard solution:

$$\text{NO content } (\mu\text{mol/L}) = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

ΔA1: Absorbance of Sample - Absorbance of Blank

ΔA2: Absorbance of Standard - Absorbance of Blank

c: Concentration of sodium nitrite, which is 40 μmol/L.

f: Dilution factor of the sample prior to testing.

The Statistical Analysis System (SAS, 2018) was utilized to assess the impact of different groups (patients and controls) on study parameters. The T-test was applied to assess significant

differences between means, while the Chi-square test was employed for the significant comparison of percentages at 0.05 and 0.01 probability levels. Additionally, the correlation coefficient between parameters in this study was estimated.[15]

3. Result and discussion

The patient and control groups were matched for their anthropometric parameters, including age, weight, height, and BMI to ensure comparability. The average age was 60.5 years for the patient group and 60.07 years for the control group, with a non-significant difference ($p>0.01$). Similarly, the mean values for weight, height, and BMI were closely aligned between the two groups, also yielding an insignificant difference ($p>0.01$). These data confirm the reliability of the selection process in minimizing confounding variables, thereby providing a robust basis for the comparative analysis of atherosclerosis cases and controls, as shown in Table 1.

Table 1. Comparison between the anthropometric parameters for patient and control groups.

Group	**Anthropometric parameter (mean ±SD)**	
	Age (yrs)	**BMI (kg/m^2)**
Patients	60.50 ±7.88	31.30 ±6.98
Control	60.07 ±8.41	31.02 ±6.94
T-test	2.973 NS	2.581 NS
P-value	0.778	0.827
NS: Non-Significant		

The results of this study clearly show a significant difference in oxLDL levels between the control group and patient groups. The mean values ± SD for the control and atherosclerosis patients were (107.92 ± 3.91 ng/ml) and (4.869 ± 3.37 ng/ml), respectively. All changes in the serum level of oxLDL between the two study groups are summarized in Table 2 and depicted in Figure 2.
These findings indicate that the mean serum level of oxLDL in the patient group was significantly higher ($p<0.01$) compared to the control group.

Table 2. Means of oxLDL levels in patient and control groups**.**

Group	**oxLDL mean±SD (ng/mL)**	**T-test**	**P-value**
Patients	89.65 ±38.33	12.361	0.0001*****
Control	28.90 ±14.86		
***(P≤0.01) statistically significant**			

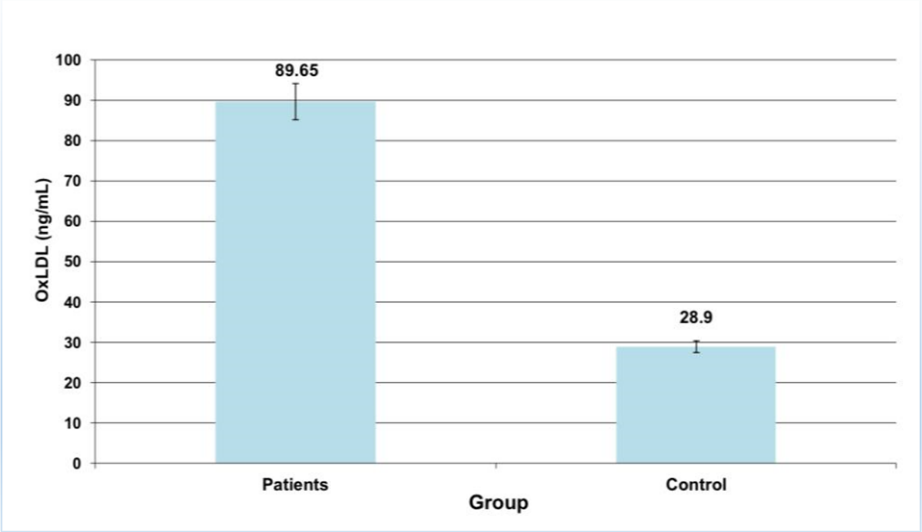

Figure 2. Represantative comparison between patients and control groups in OxLDL levels.

Oxidized low-density lipoprotein (oxLDL) plays a crucial role in the onset of atherosclerosis, contributing to the buildup of plaques and heightening the risk of cardiovascular incidents such as heart attacks and strokes. LDL becomes oxLDL when it is oxidatively modified, mainly by reactive oxygen species (ROS), turning it into a more pro-inflammatory and atherogenic substance compared to its native form. Individuals with atherosclerosis often exhibit high levels of oxLDL, which are associated with oxidative stress and lipid peroxidation, both of which are fundamental to the disease's advancement. Furthermore, oxLDL levels might act as indicators of early atherosclerosis and endothelial dysfunction. Approaches that focus on oxLDL and oxidative stress, including the use of antioxidants and modifications in lifestyle, may offer promising avenues for treating atherosclerosis. [10]

The study's results reveal a marked difference in nitric oxide (NO) levels between the control group and the patient groups. The mean values plus standard deviation for the control group were (107.92 ± 3.91 ng/ml), while those for patients with atherosclerosis stood at (4.869 ± 3.37 µmol/L). A detailed summary of the serum NO level variations between the two groups is provided in Table 3 and depicted in Figure 3.

Table 3. Means of NO levels in patient and control groups.

Group	Nitric oxide means ±SD (µmol/L)	T-test	P-value
Patients	4.869 ±3.37	3.972	0.0001*
Control	107.92 ±3.91		
*** (P≤0.01) statistically significant**			

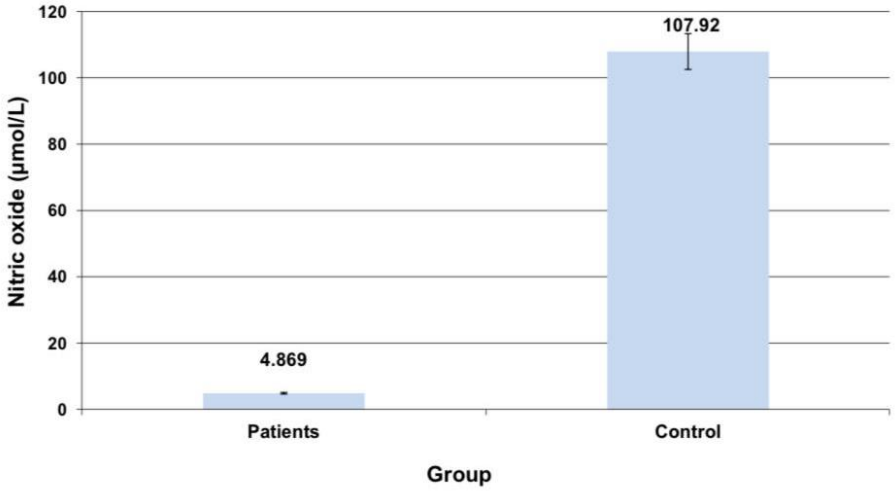

Figure 3. Represantative comparison of NO levels in patients and control groups.

The results of this study clearly show a significant difference in oxLDL levels between the control group and patient groups. The mean values ± SD for the control and atherosclerosis patients were (107.92 ± 3.91 ng/ml) and (4.869 ± 3.37) ng/ml, respectively. All changes in the serum level of oxLDL between the two study groups are summarized in Table 3 and depicted in Figure3. These findings indicate that the mean serum level of oxLDL in the patient group was significantly higher ($p<0.01$) compared to the control group.

In addition, the results reveal a statistically significant decrease in nitric oxide (NO) levels between the control group and the patient groups. The mean values plus standard deviation for the control group were (107.92 ± 3.91 ng/ml), while those for patients with atherosclerosis stood at (4.869 ± 3.37 µmol/L). A summary of the serum NO level variations between the two groups is provided in Table 3. and depicted in Figure 3.

Oxidative stress as a result of imbalance between oxidants and antioxidants have been previously recognized as critical factor in the development of atherosclerosis,[16] a condition characterized by chronic inflammation of the arterial walls. In healthy conditions, antioxidants have been shown to counterbalance free radicals and prevent cellular damage. However, under pathological conditions, where there is an increase in production of reactive oxygen species (ROS), this balance is greatly disrupted.One of the key consequences of oxidative stress is the modification of low-density lipoproteins (LDL) to form oxidized LDL (oxLDL) turning it into a more pro-inflammatory and atherogenic substance compared to its native form. As oxLDL accumulates, it triggers inflammatory responses and contributes to endothelial dysfunction, lipid deposition, and the recruitment of macrophages.[17, 18] Notably, oxLDL has been shown to bind to the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which is subsequently upregulated under conditions of oxidative stress, hypoxia, and mechanical strain.[19, 20] This interaction has been demonstrated to significantly trigger pro-inflammatory cascades, including the production of cytokines such as TNF-α, IL-1β, and IL-6, thereby amplifying vascular inflammation and endothelial dysfunction .[8, 9]

Moreover, the activation of LOX-1 by oxLDL has frequently been associated with endothelial cell apoptosis through oxidative stress-induced mitochondrial dysfunction, further weakening the vascular lining. This apoptotic effect has also been recognized as a critical contributor to plaque instability.

Simultaneously, the activation of the nuclear factor-kappa B (NF-κB) signaling pathway by elevated oxLDL levels has been demonstrated to regulate the transcription of adhesion molecules, pro-inflammatory cytokines, and matrix metalloproteinases (MMPs), all of which are central to leukocyte infiltration and extracellular matrix remodeling.[21] These effects could significantly reinforce the chronic inflammation and structural instability associated with atherosclerotic plaques.

Additionally, oxLDL has been found to promote vascular calcification through its effect on vascular smooth muscle cells (VSMCs), inducing their osteogenic transformation. This process, mediated by pathways such as BMP-2 and RUNX2, has been increasingly recognized as a hallmark of advanced atherosclerotic lesions.[22]

On the other hand, the function of endothelial nitric oxide synthase (eNOS), the enzyme responsible for producing NO, has been shown to be impaired by high oxLDL levels resulting in disrupting NO homeostasis thereby contributing to vascular damage and creating a feedback loop that accelerates the subsequent pathological effects. [23, 24]

Collectively, these findings underscore the multifaceted role of oxLDL in promoting inflammation, vascular damage, and calcification, while simultaneously impairing protective mechanisms such as NO-mediated vasodilation. These insights help understanding some aspects of the molecular basis of atherosclerotic events and highlight the importance of restoring oxidative balance as a potential therapeutic approach for preventing and managing atherosclerosis and related cardiovascular diseases. Despite both oxidized low-density lipoprotein (OxLDL) and nitric oxide (NO) being associated with the pathogenesis of atherosclerosis; the data indicates a weak negative correlation between the two variables (Table) which implies there is almost no linear relationship between OxLDL and NO levels. This weak correlation suggests that variations in one variable are unlikely to have a significant or predictable impact on the other.

Several factors could explain this result. The relationship between these biomarkers is likely to exhibit greater complexity, potentially following a non-linear pattern. For instance, their interaction may involve threshold dynamics, or adhere to exponential or logarithmic relationships, rather than a straightforward inverse correlation. Additionally, while both biomarkers might be involved in triggering the atherosclerotic events, they could be influenced by distinct mechanisms. OxLDL could be primarily associated with oxidative stress, whereas NO could be more closely linked to inflammatory processes. These distinct mechanisms may not consistently align, indicating that their interplay does not necessarily follow a straightforward inverse relationship. Furthermore, atherosclerosis is inherently multifactorial, with biomarkers potentially acting in parallel but not necessarily in a direct inverse relationship. The presence of biomarker saturation effect could also distort the observed relationship. If either biomarker approaches a saturation threshold or exhibits limited variability within the sample population, this constraint may obscure the true nature of their interaction. [11, 25]

Despite the weak linear correlation observed, the data nonetheless suggest meaningful insights. This underscores the necessity of considering the underlying complexities and diverse pathways through which these biomarkers contribute to the onset of atherosclerosis.[26]

4. Acknowledgement

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