

## ORIGINAL ARTICLE

# HYPERLIPIDEMIA CONNOTED VITIATION OF SERUM ADIPOKINES AND REDOX IMBALANCES

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### Summary

**Background and objectives:** Atherosclerosis is the underlying pathology of ischemic heart disease. There are many aggravating metabolic and oxidant parameters which are participating together overwhelming the pathology of vascular stenosis. Adipokines play a positive metabolic effect in healthy individuals and oxidation reaction greatly impacts the lipid metabolism and might negatively impact the condition. In the present study, we aimed to characterize the level of adiponectin, obestatin, and redox parameters in atherosclerotic patients.

**Methods:** Serum was collected from atherosclerotic patients and froze to be ready for analysis.

**Results:** The results indicated that hyperlipidemia significantly reduced adiponectin, obestatin, and antioxidant enzymes (catalase, glutathione, glutathione-S-transferase, and glutathione peroxidase) together with a significant increase in oxidant byproduct (malondialdehyde) and modulated lipid parameters (cholesterol, triglyceride, and high-density lipoprotein).

**Conclusion:** The study concluded that atherosclerosis is associated with reduced antioxidant enzymes, obestatin, and adiponectin levels and increased lipid levels. These parameters play a great role in the pathological status of coronary stenosis.

*Key words: Adipokines; Adiponectin; Antioxidant enzymes; Hyperlipidemia; Obestatin*

### Introduction

The coronary artery is the major vascular system responsible for oxygen supply to the myocardium. Long-term hyperlipidemia might lead to continuous lipid precipitation in intima-media of blood vessels, such as the coronary artery leading to atherosclerosis (1). Atherosclerosis is one of the major health problems and the first most common cardiovascular disease due to ischemia, it's part of angina pectoris and myocardial infarction (2). The underlying pathology is based on vascular narrowing due to lipid deposition, metabolic disturbances, oxidative stress, and inflammatory reactions (3). The outcome of these cardiovascular diseases is mainly based on the systemic metabolic condition and oxidative status of the patient at the site of injury or stenosis (4). The fate of the patients is based on the stabilization of the atherosclerotic plaque which is eventually negatively affected by the metabolism of biomolecules and systemic redox reaction (5).

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Oxidative stress plays a role in the overall health status of an individual. Hyperlipidemia is associated with the metabolic and oxidative reaction resulting in lipid precipitation in vascular wall layers (6). The increase in lipid profile is indicated by changes in the level of lipid biomolecules, such as triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) (7). The elevated level of these lipid parameters together with increased oxidative status is associated with increased formation of oxidized lipid biomolecules resulting in increased lipid precipitation in vascular layers ending with atherosclerosis (8). The metabolism of carbohydrates and lipid is key processing in the formation and accumulation of a high amount of lipid biomolecule and their subsequent vascular precipitation (9). Many regulatory factors contribute to the metabolism of carbohydrates and lipids and might be linked to the pathology of atherosclerosis, such as insulin (10). The role of insulin has been studied extensively, however, some other biomolecules might be equivocal in their role with insulin including adiponectin. Adiponectin plays a role in the catabolism of lipids and carbohydrates and has a positive role in our body reducing the risk of cardiovascular diseases (11-14).

The three following cellular events define atherosclerotic cellular responses: monocyte attachment to endothelial via binding proteins, macrophage oxidized LDL uptake via scavenger receptors, and multiplication of transitioned smooth muscles via platelet-derived growth factors or heparin-binding endothelial growth factor-like growth factor (4). The activation of binding proteins such as intracellular adhesion molecule-1, vasculature cell adhesion molecule-1, and E-selectin was shown to be substantially inhibited at physiological levels of adiponectin (15). TNF-induced nuclear factor-B stimulation was inhibited by adiponectin via inhibiting phosphorylation, which could be a primary molecular mechanism for the disease (16).

Numerous harmful variables exist in people, namely oxidized LDL, inflammatory stimuli, and organic molecules that might cause vascular damage (4). Adiponectin, a protein released by adipose tissues, may then enter the wounded arteries and rescue them from atherogenic vascular alterations (11, 4). Additionally, obestatin has been linked to diabetes, metabolic syndrome, and early hyperlipidemia (17-19). In the present study, we demonstrate the role of adiponectin, obestatin, and redox parameters in atherosclerotic patients.

## Materials and Methods

**Sample collection:** The present study was based on the recruitment of 88 subjects (45 healthy controls and 43 newly diagnosed hyperlipidemic patients) from private clinics. Demographic information has been recorded for the individual subject in the questionnaire form as outlined in Table 1. The age and sex of subjects were approximately matched between the control and patients group. Blood has been withdrawn from overnight fasting subjects (patients and healthy individual). The blood was allowed to clot and the serum separated and stored at -20°C for further analysis.

**Table 1.** Demographic parameters of participants included in the current study.

Demographic parameter	Control (n=45)	Patient (n=43)	P- value
Age (years)	34-66	34-65	----
Sex (M, F)	(21, 24)	(24, 19)	----
BMI (kg/m <sup>2</sup> )	26 ± 1.6	27 ± 1.8	0.001

**Estimation of a serum lipid profile:** Serum total cholesterol (TC), triglycerides (TG), and serum high-density lipoprotein cholesterol (HDL) were measured by the colourimetric enzymatic method (Biomerieux, France).

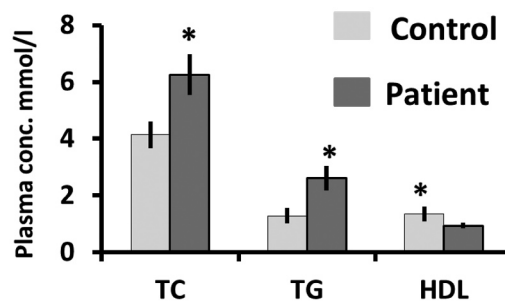
**Estimation of serum adiponectin and obestatin hormones:** ELISA technique has been used to quantify human adiponectin (USBIOLOGICAL kit, USA) and Human OB (Obestatin) ELISA Kit (Elabscience), the principle of an assay based on immunoassay technique; in which case the target protein was held between a sandwich of two antibodies. The whole test was conducted in the dark and at room temperature. To conduct the ELISA test, standard 96-well plates were pre-coated with capture antibody (polyclonal anti-human adiponectin or obestatin antibody);

after 12 hours elapsed, the plate was washed with a washing buffer and then plasma samples or serial concentrations of the standard were loaded into the microplate and incubated for 2 hours, followed by washing cycle. In the subsequent step, the detection antibodies were loaded into individual wells and incubated for 1 hour followed by washing and next the wells were loaded with horse-radish peroxidase enzyme for 1 hour. An enzymatic reaction continues followed by the addition of a substrate for half-hour producing a light green colour and a stop solution to stop the enzymatic reactions changing the colour to light yellow which will be measured spectrophotometrically at 450 nm. The washing cycle involve four washing times and the fourth step was tapped against a paper towel. The washing buffer was prepared by dilution of the washing solution provided by the supplier with a dilution buffer. The concentration of the samples was extrapolated from the standards.

**Estimation of serum malondialdehyde:** A laboratory examination of plasma Malondialdehyde (MDA, E-BC-K025-S) levels was performed. The MDA detection test was caused by the interaction of MDA with thiobarbituric acid, which resulted in the production of a red-coloured molecule whose absorbance was determined at 532 nm.

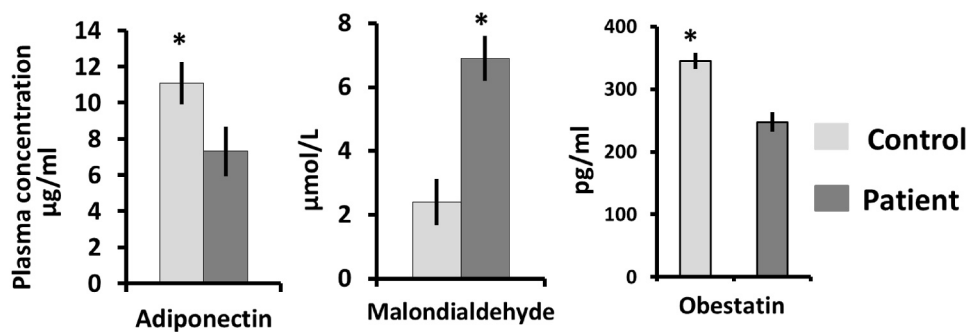
## Results

Analysis of the results obtained from serum samples of hyperlipidemia patients revealed a significant increase ( $p<0.001$ ) of TC and TG ( $6.26\pm0.72$ ;  $2.6\pm0.43$  mmol/l) in patients compared to control ( $4.14\pm0.46$ ;  $1.3\pm0.28$  mmol/l), respectively. On the other hand, HDL levels were significantly reduced ( $p<0.001$ ) in patients compared to control ( $0.94\pm0.1$ ;  $1.36\pm0.26$  mmol/l), respectively (Figure 1).



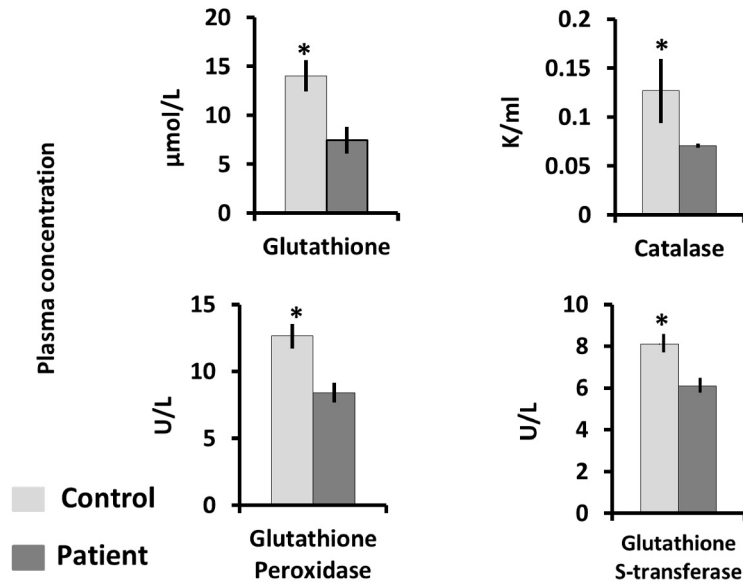
**Figure 1.** Measured lipid parameters of studied groups. TC=total cholesterol, TG=triglyceride, HDL=high-density lipoprotein. Data expressed as mean±SD, \* $p<0.001$ .

The results showed that hyperlipidemia was associated with a significant ( $p<0.001$ ) reduction in plasma concentration of adiponectin and obestatin ( $11.08\pm1.2$ ;  $7.3\pm1.4$  ug/ml), ( $345\pm13$ ;  $247\pm16$  pg/ml), respectively (figure2). On the other hand, there was a significant ( $p<0.001$ ) elevation of plasma MDA in patients compared to the control group ( $2.4\pm0.72$ ;  $6.9\pm0.71$  umol/l) (Figure 2).



**Figure 2.** Hyperlipidemia is associated with increased plasma MDA and reduced adiponectin and obestatin levels. Data expressed as mean±SD and \* $p<0.001$ .

Measurement of antioxidant enzymes revealed that hyperlipidemia was associated with a significant reduction of antioxidant enzymes in the patient group compared to the control group, including catalase, glutathione (GSH), glutathione peroxidase (GPX), glutathione-s-transferase (GST) (figure 3).



**Figure 3.** Hyperlipidaemia is associated with the modulation of the antioxidant enzyme. Comparison between results analysed from the serum of the both groups indicated that Hyperlipidaemia reduced catalase, glutathione, glutathione peroxidase, and glutathione-s-transferase. Data expressed as mean $\pm$ SD, \* $p$ <0.001.

## Discussion

The present study confirmed that the initial stages of hyperlipidemia have a great impact on the modulation of important parameters which could influence the prognosis of the disease and the fate of the therapy. The initial steps of hyperlipidemia were scrutinized by recruiting patients who were visiting private clinics for a check-up. We found out that the initial steps of hyperlipidemia were associated with a significant reduction in adiponectin concentration, obestatin concentration, and redox enzymatic chains, alongside a significant increase in malondialdehyde.

Atherosclerosis is a bi-armed disease involving inflammation and oxidative reaction; these two steps are cardinal for the overwhelming picture of cardiovascular morbidity. Early stages of atherosclerosis involve inflammatory reactions at atherosclerotic lesions mediated by inflammatory cytokines-tumour necrosis-alpha (TNF- $\alpha$ ) (20, 21).

Adipose tissue secretes a mediator named Adiponectin, which is released by fatty tissue (adipose tissue). Adiponectin activates 5'AMP-initiated protein kinase alongside a bunch of other protein kinases. Furthermore, the AMPK cascade can boost glucose metabolism and usage, as well as fatty acids (FFA) metabolism, in myocytes, hepatocytes (liver), and fatty tissue (adipocytes), hence influencing the metabolic rate and insulin resistance (22-24). Adiponectin could also protect blood circulation by preventing the deformation of vessel walls, particularly endothelial cells, by provocative elements (20, 25). As the study reveals, adiponectin is associated with overweight, glucose intolerance, T2DM, and other diseases (26). Adiponectin, a plasma protein exclusive to adipose tissues, has previously been reported to exert anti-inflammatory effects on capillary wall cellular processes.

Adiponectin can stimulate different cells, including skeletal muscles and hepatocytes, to respond to insulin by boosting muscle-free fat oxidation and inhibiting glucose output from the liver, according to Maeda *et al.* (27). This was demonstrated in an animal model by genetic modification, specifically knocking out the Adiponectin gene in mice. Furthermore, adiponectin has the ability to regulate glucose uptake and increase insulin sensitivity in peripheral tissues (28).

It is suggesting that the cytotoxic effects of ROS are due to high level of MDA in hyperlipidemic patients. Also, the high MDA levels are correlated with the increased production of free radicals, lipid peroxidation, and cell oxidative damage (29). Therefore, in the present study, the increased oxidant and decreased antioxidant enzyme activities represent the atherosclerosis development. The imbalance between oxidants and antioxidants is leading to increased peroxidation of lipids. GSH and GPX are the first lines of defense against oxidative cellular injury by scavenging hydrogen peroxide. Low levels of antioxidant enzymes may lead to insufficient scavenging of ROS, which leads to redox imbalance. Also, ROS cause the inactivation of GSH and GPX enzymes through damaging their proteins (30).

Nilsson PM *et al.* came to the same conclusion, claiming that Adiponectin blood levels were inversely related to coronary artery intima-media thickness, implying that Adiponectin was a biochemical variable and accurate marker for asymptomatic cases of atherogenesis and could predict the risk of coronary heart disease and cerebral stroke (29). Jiang *et al.* demonstrated that perhaps the serum concentration of adiponectin was inversely related to illness in patients with peripheral vascular disease (30).

To see if congenital hypoadiponectinemia causes biochemical problems including insulin sensitivity and atherosclerotic alterations, researchers looked at the diagnostic people's profiles who had recently discovered adiponectin gene mutations. In a knockout (KO) mouse model for the adiponectin gene to confirm this theory. When neither high-fat nor high-sucrose meal was given to the KO mice, they showed no particular phenotype. Insulin sensitivity was also evident in the mouse model, as measured by an insulin tolerance test following a high-fat, high-sucrose meal. The addition of adiponectin via adenovirus transfection significantly reduced insulin sensitivity (27, 31).

Enhanced intimal vascular smooth muscle growth was also detected in the wounded aorta of the mouse model, which was reversed by adiponectin replacement in adenovirus-transfected KO animals, implying that adiponectin may play a role in the pathogenesis of systemic inflammation during tissue dysfunction (32). Recombinant adenovirus-expressed human adiponectin was given to apolipoprotein E-deficient mice. Blood adiponectin values vary 48 times in adenovirus-adiponectin-treated mice compared to control mice. When compared to untreated apolipoprotein E KO mice, plaques development in the aortic inlet was reduced by 30% on the 14 days following adenovirus-adiponectin inoculation (33). In comparison to nontreated mice, adiponectin-treated mice's lesions and lipid droplets shrank (31).

We found out that obestatin has been reduced in hyperlipidemia patients, these findings could preclude obestatin as a biomarker for detection of atherosclerosis, which further confirms the study conducted by Gu *et al.*, 2013 (17), who has reported a positive correlation between obestatin levels and intima-media thickness or atherosclerosis alongside strong positive correlation with glycemic control and obestatin levels. In agreement with our results, obestatin has been reduced in overweight individuals compared to normal healthy control (18) in a study conducted by Szentpeteri *et al.*, 2017. (18). Nevertheless, Celik *et al.*, 2016 has found a non-significant difference between obestatin levels in obese versus normal healthy individual, this disagreement with our results could be due to the small age group sample used in this children aged 10-18 years (19).

The total sample is mostly drawn from a certain geographical region, and additional methodological limitations such as MDA and adiponectin levels in the blood may affect the result in a section of the population (29). Additionally, the morphological and cellular mechanisms by which Adiponectin and its ligand signaling pathway regulate carbohydrate metabolism, lipid metabolism, immunological effect, anti-proliferative effect, anti-thrombotic action, and Type 2 diabetes still need to be studied in depth.

## Conclusion

The crux points in the pathogenesis of hyperlipidemia are represented by their vitiation of important physiological biomolecules. Adipokines are influenced by high serum lipid profile resulting in a reduction of adiponectin level and parallel effects demonstrated on serum redox biomolecules represented by measured parameters (catalase, glutathione, glutathione-s-transferase, and glutathione peroxidase). It's important to balance these parameters and potentiate patient intake of antioxidants. Restoration of normal plasma lipid level and subsequent mitigation of oxidative damage is jointly advised with the maintenance of balanced antioxidant status to prevent the initiation or progression of plaque formation and its consequences.

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## Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

## Adherence to Ethical Standards

The study has received ethical approval from Nineveh Health- Iraqi Ministry of Health, the study approval number and date (32775 on 18/9/ 2021).

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