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Non Invasive Early Predication of Endothelial Dysfunction in Obese Subjects

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Abstract: **Obesity is a public health problem that has reached epidemic proportions with an increasing worldwide prevalence. Endothelial dysfunction represents an early phase of vascular changes that eventually lead to atherosclerosis with all its unfavorable complications. The purpose of this study was to evaluate the endothelial dysfunction and oxidative stress as potential biomarkers to assess whether these markers are considered non invasive early predication of CVD. Two groups of subjects were included in this study (obese and non obese). Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), E-selectin (E-selectin), oxidized LDL (oxo-LDL) and 8-iso-prostaglandinF2*α* (8-iso-PGF2*α*) were assessed by enzyme linked immunosorbent assay. In comparison with control group, a significantly elevation of serum sICAM-1, sVCAM-1, E-selectin, oxo-LDL and 8-iso-PGF2*α* levels ($p < 0.001$) in obese population as comared with none obese. In obese group, Pearson analysis showed a positive correlation between E-selectin and with albumin ($r= 0.313, p= 0.036$) but negative correlation between 8-iso-PGF2*α* and albumin ($r= -0.430, p= 0.003$), ICAM-1 concentration were negative correlated with T.BIL ($r= -0.307, p= 0.040$) and D.BIL ($r= -0.324, p= 0.030$) but positive correlated with AST ($r= 0.323, p= 0.030$) and LDH ($r= 0.348, p= 0.019$). Endothelial dysfunction and oxidative stress marker are significantly elevated in obese population with poor performance status.**

Keywords: **Obesity, oxidative stress biomarker, Saudi Arabia**

INTRODUCTION

Obesity is a public health problem that has reached epidemic proportions with an increasing worldwide prevalence. It has been demonstrated that obesity is associated with chronic systemic inflammation; this status is conditioned by the innate immune system activation in adipose tissue that promotes an increase in the production and release of pro-inflammatory cytokines that contribute to the triggering of the systemic acute-phase response which is characterized by elevation of acute-phase protein levels (1). **S**audi Arabia is one of the fastest growing economies of the world. The growth and prosperity, however, have brought pronounced changes in the lifestyle of our people. Most notably, eating habits are less healthful and the level of physical activity has declined. Consequently, obesity is increasing in the Kingdom at an alarming rate (2).

Endothelial dysfunction represents an early phase of vascular changes that eventually lead to atherosclerosis with all its unfavorable complications (3).
Obesity is associated with greater arterial stiffness (4) and, as expected, visceral adiposity is particularly detrimental (5). In accordance with a causal role for obesity in the pathogenesis of early atherosclerosis, weight loss improves both endothelial function (6) and arterial stiffness (7).
Oxidative stress (OS) due to the imbalance between pro-oxidant/antioxidant status results in generation of reactive oxygen species (ROS) and subsequent modification of biomolecules such as protein, lipids and nucleic acids.

Oxidative stress (OS) is implicated in the development of diabetic complications (8) by its association with peroxidation of membrane lipids and LDL-cholesterol. These peroxidation products can impair beta cell function and induce apoptosis (9).

Primary end products of lipid peroxidation include conjugated dienes and lipid hydroperoxides, while secondary end products include thiobarbituricthiobarbituric reactive substances (TBARS), gaseous alkanes and a group of prostaglandin (PG) F2-like products termed F2- isoprostanes (F2-IsoPs) (10). F2-IsoPs are prostaglandin-like compounds formed in-vivo from free radical catalyzed peroxidation of arachidonic acid and have emerged as novel and direct measures of oxidative stress.

The goal of the present study is to evaluate the endothelial dysfunction and oxidative stress as biomarkers in obese population. These biomarkers may help for non invasive predication of endothelial dysfunctions and a major risk in developing obesity related medical illnesses, including diabetes and cardiac events.

2. SUBJECTS AND METHODS

Subjects:

A cross-sectional study in which ninety volunteers obese (Female and male) (BMI>30) were included in the present study with age ranging between (19-50) years and were all inpatients attending the Diabetic and Hypertension Care Center and King Fahad Hospital, Jeddah, Saudi Arabia. In addation 45 non obese (control group) with BMI (18.5–24.9) All subjects gave informed consent, and the ethical committee of the king Abdulaziz university approved the study protocol.

Blood Sampling:

Blood samples were collected after a 12-h fast in plain tubes .The samples were allowed to clot at room temperature and then centrifuged at 2,000×g for 15 min to obtain serum. Routine clinical analysis was performed on the same day blood was taken. Subsequently, 5 ml of serum was stored at −80 °C until measurement of Biomarker was performed.

Methods:

The serum concentrations of routine biochemistry tests were determined using the commercially available assay kits (Boehringer Mannheim GmbH, Mannheim, Germany) on a Dimension® clinical chemistry system. LDL cholesterol (LDL-C) level was obtained using the following formula: LDL-C=TC−HDL-C−TG/5.

Serum ICAM-1, VCAM-1, E-Selectin, oxo-LDL and 8-iso PGF2α concentration weremeasured byquantitative sandwich enzyme immunoassay technique this kit is available in a PharmPak (R&D Systems,USA). Assays were carried out according to the manufacturer's instruction.

Statistical analysis:

All statistical analysis was performed using SPSS statistical software package (SPSS for windows, version 17, SPSS Inc, and USA). Numerical data were expressed as mean value for each parameter ± standard deviations (SD). One way analysis of variance (ANOVA) was carried out to test the significance of difference between groups mean value for each parameter. In case of significant *p*-value, multiple comparisons using Latin Square Design (LSD) was carried out to test which group's mean value differ from which. For all comparisons, *p*-values of < 0.05 were considered statistically significant. Correlation coefficients (r) for pairs of variables were determined by Pearson's method to test the strength of association between any two variables in the same group. Differences were statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

3. RESULTS

Table 1 showed a significant elevation in the level of ICAM-1 in obese 22.37±7.26 as compared to control 17.26±3.22 ($p < 0.001$) , The mean VCAM-1 was increased in obese 65.05±25.38 as compared to control 28.22±9.37 ($p < 0.001$) .The mean E-selectin was also significantly higher in obese 2.69±1.01 as compared to control 1.88±0.98 ($p < 0.001$) . Furthermore, there was increased mean levels of oxo-LDL in obese 491.25±207.23 as compared to control 87.66 ±28.93 ($p < 0.001$) .Moreover, mean 8-iso-PGF2α levels were significantly elevated in obese (22.53±14.04) as compared to

control (6.00±4.33) ($p < 0.001$) . In Table (2) highly positive correlation were noted between ICAM-1 and VCAM-1 ($p<0.0001$). In obese group, Pearson analysis showed a positive correlation between E-selectin and with albumin ($r= 0.313, p= 0.036$) but negative correlation between 8-iso-PGF2α and albumin ($r= -0.430, p= 0.003$), ICAM-1 concentration were negative correlated with T.BIL ($r= -0.307, p= 0.040$) and D.BIL ($r= -0.324, p= 0.030$) but positive correlated with AST ($r= 0.323, p= 0.030$) and LDH ($r= 0.348, p= 0.019$) (Table 3) (figures 16).

4. DISCUSSION

In the resent study serum ICM-1, VCAM-1 and E-selectin levels were significantly raised in obese population as compared to control ($p < 0.001$). Furthermore, sVCAM-1 reached the highest levels in obese.

It was demonstrated that the early phases of abdominal obesity are characterized by coronary endothelial dysfunction in association with vascular oxidative stress, hypertension, and mild lipid profile abnormalities in the absence of a state of insulin resistance. These changes are accompanied by a systemic increase in leptin levels and decreased NO end products. In contrast, no systemic inflammation or oxidative stress was observed, suggesting that the early abnormalities induced by obesity are mainly localized at the vascular wall level (11).

Endothelial dysfunction of the peripheral vasculature also has prognostic value. Noninvasive endothelial function testing predicted cardiovascular events in patients with coronary artery disease (12), peripheral artery disease (13), and hypertension (14) and in patients who underwent vascular surgery (15). Moreover, there is increasing evidence that links markers of endothelial dysfunction and vascular inflammation with cardiovascular events. It was suggested that, obesity is a well-established independent risk factor for coronary artery disease, the mechanisms that relate fat mass to vascular health are poorly understood. Excess fat and particularly visceral fat, predispose to the major components of the metabolic syndrome that influence cardiac risk. Obesity might also promote preclinical atherosclerotic changes via a direct effect on vascular physiology.

In the early stages of atherosclerosis biomarkers of endothelial dysfunction, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, are increased in response to subclinical inflammation and play an important role in the formation of atherosclerotic plaque (16). Numerous studies documented increased circulating levels of soluble adhesion molecules in obesity (17), but the mechanisms are still not completely clear (18).

The increased in level ICAM-1 and VCAM-1 in visceral adipose tissue (VAT) in obesity women. Circulating levels of soluble adhesion molecules are markers of endothelial activation (19), being elevated in obese individuals in majority (20), but not all (21)

This study investigated the relationship between oxo-LDL, a marker of systemic oxidative stress and obesity. We found a significant increase in mean values of oxo-LDL concentration in obese as compared to healthy group. Our results are in agreement with those reported by (22) who found that oxo-LDL level were significantly higher in overweight/obese vs. normal weight children also oxo-LDL is significantly associated with adiposity and with insulin resistance, independent of body fatness, in children.also reported by (23) suggest that systemic oxidative stress may be a novel risk factor for T2D and obesity. Increased lipid peroxidation has been reported in obese people (24). It has also been reported that, in obese subjects with the metabolic syndrome and T2D, oxidative stress is increased and the redox state is a potentially useful therapy (25) The possible explanation for the positive relationship between obesity and oxLDL is the impaired antioxidant defense of HDL in abdominally obese subjects as the antioxidant action of HDL components prevents LDL oxidation and renders LDL resistant to oxidation (26).

Our results are in agreement with those reported by (27) who found the release of free radicals is enhanced from conditions such as hyperglycaemia, ischaemia, infection and would be available to oxidize the particle .It was found that,the amount of oxidized phospholipid on LDL apo B100 appears to be a good marker of atherosclerotic progression. Increased oxidative stress, reflected by elevated levels of oxidized LDL, may precede the development of insulin resistance (28) and therefore be important in the early pathophysiology of type 2 diabetes mellitus.

IsoPs, derived from the non-enzymatic peroxidation of arachidonic acid are associated with hyperglycemia, vasoconstriction and diabetic nephropathy (29). F2-Isops, in urine or plasma, provide a highly precise and reliable approach to assess lipid peroxidation in vivo (30). F2-IsoPs have been found to be increased in both type I and type II diabetes (31). Increased isoprostane levels were observed in plasma and urine of type 2 diabetes (NIDDM) (32).

Ou results showed that mean values of 8-iso PGF was elevated significant in obese as compared to healthy group. It was reported that 8-iso-PGF2α (a major F2-IsoPs) was found to be threefold higher in type 2 diabetics than in healthy individuals. Plasma F2-IsoP concentrations were found to be increased by 34% in acute hyperglycemia and this is similar to other models of oxidative damage. Increased plasma esterified 8-epi-F2α-IsoPs were reported in heavy smokers by (33). 8-epi-F2α-IsoPs possess biologically important proatherogenic actions, and serves as well as a marker for free radical damage.

In accordance with the LDL oxidation hypothesis of atherosclerosis, levels of F2-IsoPs should be higher in atherosclerotic plaques than in normal vascular tissue.

Levels of esterified F2-IsoPs in vascular tissue normalized to both wet weight and dry weight were significantly higher in atherosclerotic plaques compared to normal vascular tissue (34).

A better measure of the actual extent of oxidation, however, may be obtained by normalizing the data to the amount of arachidonic acid present in the tissue since it is the substrate for IsoP formation. When the data was normalized to arachidonic acid content, the F2-IsoP/arachidonic acid ratio was ~4-fold higher in diseased tissue than the ratio in normal vascular tissue ($p = 0.009$). This finding indicates that unsaturated fatty acids in atherosclerotic plaques are more extensively oxidized than lipids in normal vascular tissue. Increase amounts of F2-IsoPs in human atherosclerotic lesions and the localization of F2-IsoPs in atherosclerotic plaque tissue to foam cells and vascular smooth muscle cells (35). While 8-iso-PGF2α, plays a pivotal role in patients with insulin resistance and hyperglycemia its role is still unclear in subjects without evident hyperglycemia.

5. CONCLUSIONS

In this study, in comparison to a control group, the endothelial dysfunction and oxidative stress marker are significantly elevated in obese population which could have worrisome health consequences. Those can be utilized as biomarkers for possible non invasive early prediction of endothelial dysfunction that is share in poor health performance.

Table 1: comparison of Endothelial dysfunction and oxidative stress marker between obese and control subjects (Mean±SD)

Biomarkers	Control (n=45)	Obese (n=45)	p-value
ICAM-1 ng/ml	17.26±3.22	22.37±7.26	<0.001
Range	(10.61-20.92)	(12.13-44.39)	
VCAM-1 ng/ml	28.22±9.37	65.05±25.38	<0.001
Range	(16.17-47.04)	(35.27-130.20)	
E-selectin ng/ml	1.88±0.98	2.69±1.01	<0.001
Range	(0.79-3.40)	(0.29-3.83)	
Oxo-LDL ng/ml	87.66±28.93	491.25±207.23	<0.001
Range	(32.52-138.64)	(262.31-900)	
8-iso-$PGF_{2\alpha}$ pg/ml	6.00±4.33	22.53±14.04	<0.001
Range	(0.30-25.60)	(1.77-50.50)	

(ICAM-1: intercellular adhesion molecule 1, VCAM-1: vascular cell adhesion molecule-1,Oxo-LDL: oxidized low density lipoprotein, 8-iso-PGF2α: 8-iso-prostaglandin F2α).

Values were represented as the mean ± SD; the data were statistically analyzed using ANOVA followed by LSD test.

P-value ≤ 0.05 was used as a criterion of significance.

P-value ≤ 0.01 was used as a criterion of highly significance.

NS: no significant.

Table 2: Pearson correlation coefficients biochemical marker in control group and obese group

Markers	Control group n=45 **r-coefficient**	**p-value**	Obese group n=45 **r-coefficient**	**p-value**
ICAM-1 ng/ml	**NS**	**NS**	**0.610****	0.000
VCAM-1 ng/ml	**NS**	**NS**	**0.610****	0.000
E-SELECTIN ng/ml	**NS**	**NS**	**NS**	NS
OXO-LDL ng/ml	**NS**	**NS**	**NS**	NS
8-iso-PGF$_{2\alpha}$ pg/ml	**NS**	**NS**	**NS**	NS

(CRP: C-reactive protein, IL-6: interleukin 6, TNF-α: tumor necrosis factor alpha, ICAM-1: intercellular adhesion molecule 1, VCAM-1: vascular cell adhesion molecule-1, oxo-LDL: oxidized low density lipoprotein, 8-iso-PGF2α: 8-iso-prostaglandin F2α)

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

NS: Non significant

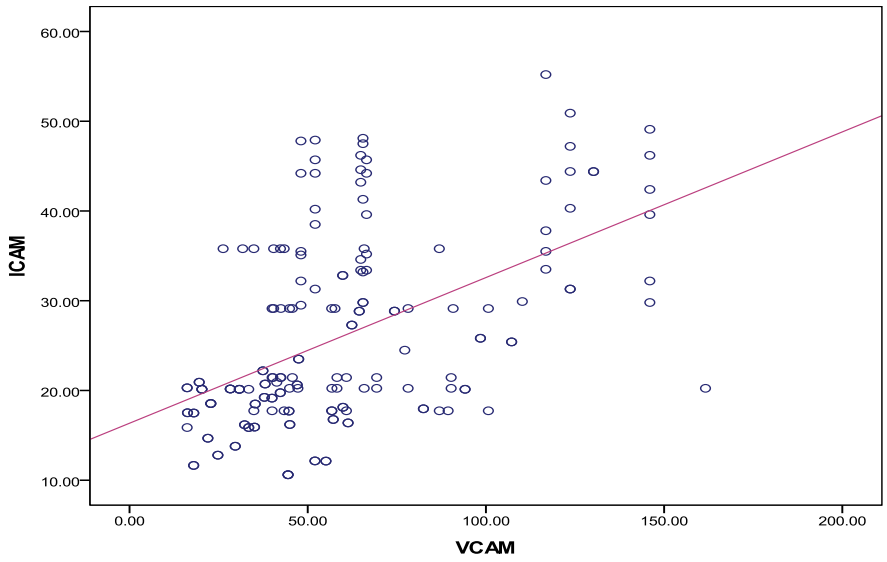

Figure 1: Correlation between ICAM-1 and VCAM-1 in obese group (r = 0.610, p = 0.000**)

Table 3: Pearson correlation coefficients biochemical marker in control group

Pearson's Correlation	ICAM	VCAM	E-SELECTIN	OXO-LDL	8-iso-PGF$_{2\alpha}$
	r- p-value	r- p-value	r- p-value	r- p-value	r- p-value
K (mmol/l)	-----	----	-----	0.351 0.018*	----
Cl (mmol/l)	0.397 0.007**	----	-----	-----	----
U.A mg/dl	----	----	-----	----	----
CA mg/dl	----	----	----	0.302 0.044*	----
ALB g/dL	----	-----	-----	0.368 0.013*	----
Total-cholesterol (mg/dl)	-0.366 0.014*	----	-----	-----	-----
TG (mg/dl)	-0.439 0.003**	----	-----	----	-----
HDL-cholesterol (mg/dl)	----	----	0.320 0.032*	-----	----
LDL-cholesterol (mg/dl)	----	----	------	-----	----
CK (u/l)	-0.341 0.022*	---	------	-----	----
LDH (u/l)	-0.445 0.002**	----	-----	----	-----

(CREAT, createnine, BUN: blood urea nitrogen, Na: sodium, K: potassium,Cl: chloride, U.A: uric acid, CA: calcium,T.BIL: total bilirubin, D.BIL: direct bilirubin, T.P: total protein, ALB: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein CK: creatine phosphate, LDH:lactatedehydrogenaseCRP: C-reactive protein, IL-6: interleukin 6, TNF-α: tumor necrosis factor alpha, ICAM-1: intercellular adhesion molecule 1, VCAM-1: vascular cell adhesion molecule-1, oxo-LDL: oxidized low density lipoprotein, 8-iso-PGF2α: 8-iso-prostaglandin F2α)

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.
NS: Non significant

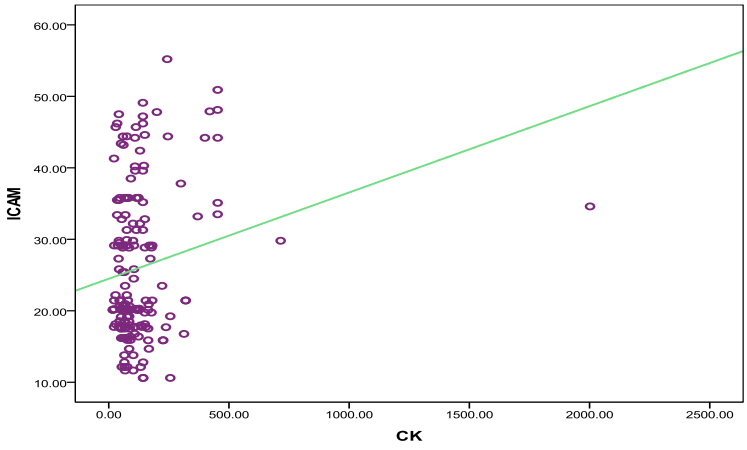

Figure 2: Correlation between ICAM-1 and CK in control group (r = -0.341, p = 0.022*)

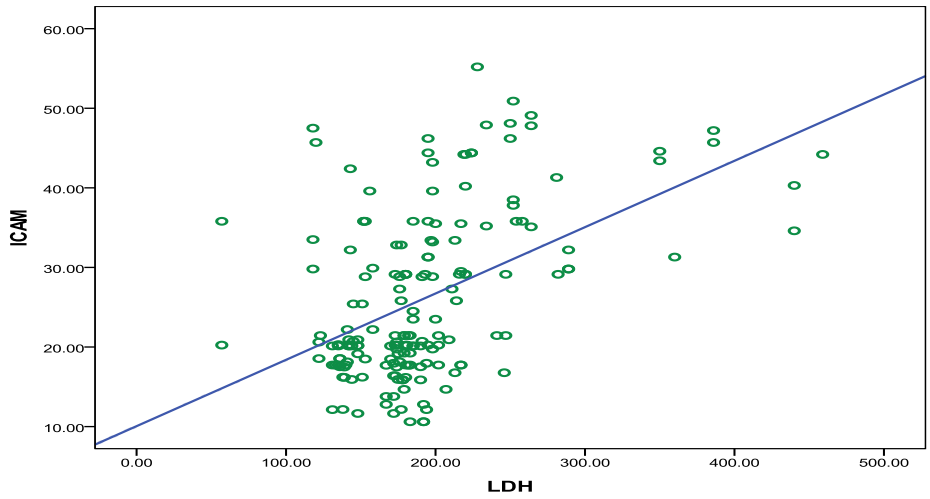

Figure 3: Correlation between ICAM-1 and LDH in control group (r = -0.445, p = 0.002**)

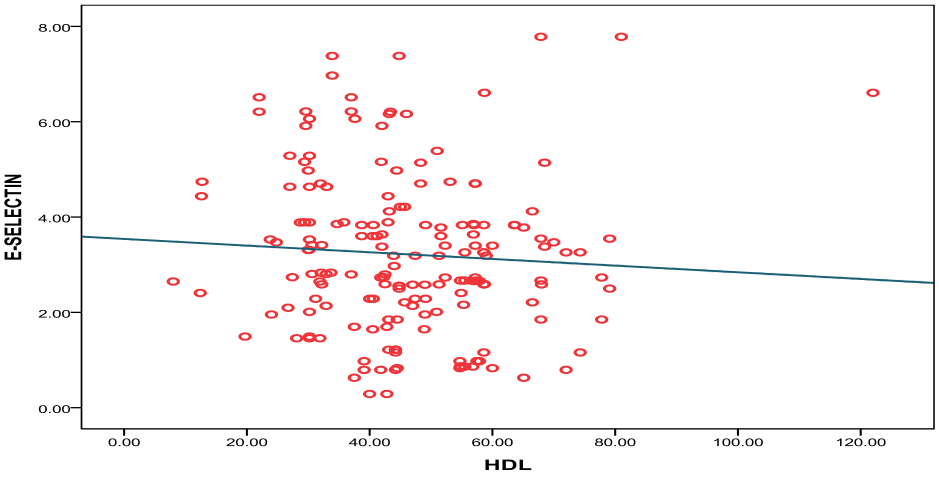

Figure 4: Correlation between E-selectin and HDL in control group (r = 0.320, p = 0.032*)

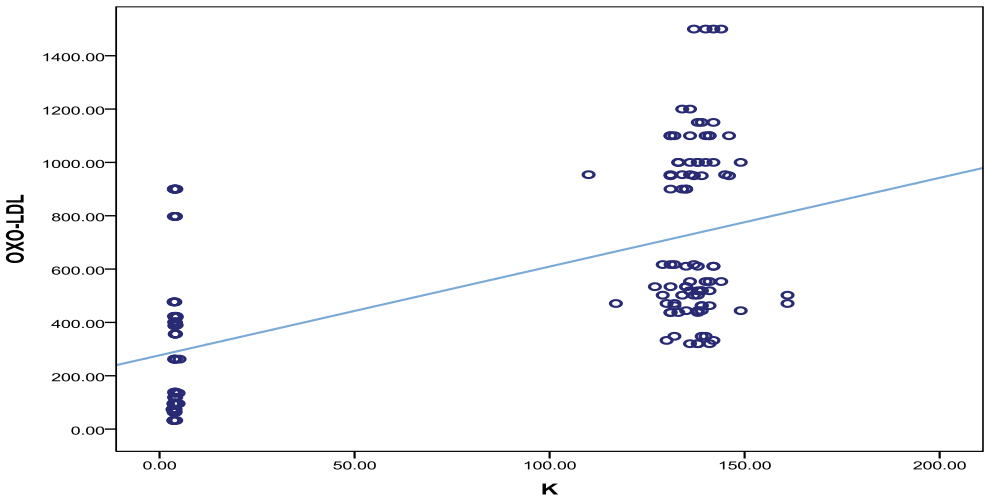

Figure 5: Correlation between Oxo-LDL and K in control group (r = 0.351, p = 0.018*)

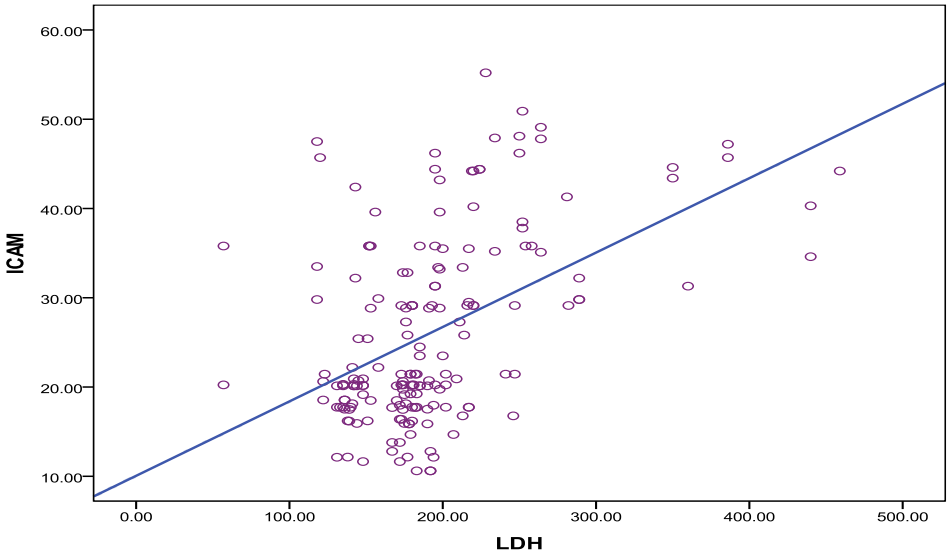

Figure 6: Correlation between ICAM and LDH in obese group (r = 0.348, p = 0.019*)

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