Serum Eotaxin as a marker in Prediction of Atherosclerosis in Obese Patients with NAFLD

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Abstract: **Background*:*** Atherosclerosis and its clinical manifestations in coronary heart disease, stroke, and peripheral artery disease are the leading cause of morbidity and mortality worldwide. Atherosclerosis is considered to be a chronic inflammatory disease and scientific interest has focused on the role of cytokines as possible predictor agents for early atherosclerosis and as a therapeutic target for atherosclerosis. As obese patients with nonalcoholic fatty liver disease (NAFLD) are vulnerable to many risk factors for atherosclerosis that related to metabolic syndrome and NASH prevalence in those patients. **Objective*:*** The aim of this study is to study the role of serum Eotaxin, in early prediction of atherosclerosis in obese patients with nonalcoholic fatty liver disease (NAFLD). **Materials and Methods:** The study included 90 participants 60 of them were obese with NAFLD **(cases)** recruited from Tanta University Hospitals The patients were subdivided into 3 groups 30 obese patients with NAFLD without vascular ischaemic complications, 30 obese patients with NAFLD with vascular ischaemic complications. Serum Eotaxin level, hsCRP, Interleukin 1β(IL-1β) and Vascular endothelial growth factor (VEGF) were measured. Abdominal ultrasound, Muscle ultrasound and Duplex on common carotid, the carotid bulb, and the near and far wall segments of the internal carotid to measure CIMT and evaluate early atherosclerosis also were done. **Results*:*** There was significant increase of serum Eotaxin levels in ischaemic obese patients than non ischaemic obese patients and it was positively correlated with HOMA for insulin resistance, IL1β (proatherogenic cytokine) and VEGF (angiogenic factor) Also, there was positive correlation between CIMT and serum Eotaxin. CIMT was predicted by serum Eotaxin level. Also, it was significantly higher in obese patients with MetS than obese patients without MetS. ROC curve showed that it has higher sensitivity and specificity in detection and prediction of atherosclerosis in obese patients. **Conclusions*:*** From this study, it is concluded that despite few studies investigated the role of serum Eotaxin in the pathogenesis of atherosclerosis, it was significantly higher in ischaemic obese patients than non ischaemic obese and its levels in obese patients are positively correlated with subclinical atherosclerosis.

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1. Introduction

The prevalence of obesity has increased dramatically worldwide over the last decades and has now reached epidemic proportions (1). It has important consequences for morbidity, disability and quality of life and entails a higher risk of developing type 2 diabetes, cardiovascular diseases, several common forms of cancer, osteoarthritis and other health problems (2).

Patients with non alcoholic fatty liver disease (NAFLD) usually have features of the metabolic syndrome (MetS) and also have a myriad of other emerging CVD risk factors (3), (4). This finding has important clinical implications for the development of future CVD events among these patients.

There is search for additional mechanism of initiation and progression of atherosclerosis-related disorders. Recent advances in the pathobiology of atherosclerosis suggest that this malady may be an immuno-inflammatory disorder. This concept has evolved based on the identification of accumulation of inflammatory cells from the beginning of plaque formation to the development of acute flow restricting disorders resulting in acute myocardial infarction and stroke. There is also much interest in development of therapies targeting inflammatory signals (5).

Cytokines are one of the major subset of mediator contributing in the interaction between inflammatory, endothelial and smooth muscle cells and the subsequent perpetuation of the inflammatory reaction (6) and inflammatory biomarkers appear to have an important prognostic value in patients with cardiovascular disease and may be useful in the diagnosis of apparently healthy subjects without known CAD who cannot be assessed with conventional risk factors.

Despite some criticisms (7), findings suggest that increased circulating levels of Eotaxin, an eosinophil chemoattractant cytokine implicated in allergic responses, are detected in the serum of patients with coronary artery disease (CAD) (8). Unstable atheromatous lesions have an abundant inflammatory infiltrate, including macrophages, T cells, and mast cells (9). Mediators produced by leukocytes may be critical determinants of plaque stability (10).

2. Subjects and Methods

Subjects of the study:

Our study included 90 participants 60 of them were obese with NAFLD **(cases)** recruited from Tanta University Hospitals, Internal medicine department (endocrinology, diabetes & metabolism outpatient clinics and inpatient wards) and cardiology department (outpatient clinic and inpatient wards) from **December 2014 to June 2017**, and 30 participants were age and sex matched healthy subjects **(control group)**. Obesity was confirmed as BMI was more than 30 with established obesity more than 3 years.

Ethical approval was obtained and each subject gave a written informed consent.

The patients were subdivided into 3 groups:

- **Group (I) (non ischaemic group)**:30 obese patients with NAFLD without vascular ischaemic complications.
- **Group (II) (ischaemic group):** 30 obese patients with NAFLD with vascular ischaemic complications if form of ischaemic heart disease presented with recent or previous MI, unstable angina or stable angina.
- **Group (III) (control group):** 30 healthy subjects as a control group.

➢ **Exclusion criteria:**

-Patients on steroid therapy

-Hepatic comorbidities as HCV/HBV infection, alcohol abuse, primary biliary cirrhosis and metabolic diseases.

-Autoimmune diseases as SLE, RA, autoimmune hepatitis.

-Allergic diseases as allergic dermatitis and asthmatic patients

-Diabetes

All participants in the study were subjected to:

1- History taking:

2- Clinical examination:

Measurement of body weight and height with calculation of body mass index (BMI), waist circumference, hip circumference W/H ratio, systolic and diastolic blood pressure.

The correct position for measuring waist circumference is midway between the upper most border of the iliac crest and the lower border of the costal margin (rib cage). The tape was placed around the abdomen at the level of this midway point and a reading taken when the tape was snug but does not compress the skin (11).

Hip circumference was measured around the widest part of the buttocks, with the tape parallel to the floor, and waist to Hip ratio (WHR) was calculated. Normal reference values of the waist circumference were set at 94 cm for men and 80 cm for women;

W/H ratio for general risk and obesity 0.94 for men and 0.80 for women (12).

Routine laboratory investigations

- CBC
- ALT, AST, GGT, alkaline phosphatase, albumin, bilirubin, prothrombin time
- Fasting glucose, fasting basal insulin
- Post prandial blood glucose.
- HbA1C
- Serum total cholesterol, triglycerides (TG), HDL and LDL
- ESR
- HBVsAg, HCV Ab
- ANA, Anti dsDNA, ALKMA, ASMA and AMA

Specific investigations

- Serum Eotaxin level
- High sensitive C-reactive protein (hsCRP)
- Interleukin 1β(IL-1β)
- Vascular endothelial growth factor (VEGF)

Radiological investigations

- Electrocardiogram (ECG)
- Echocardiography
- Abdominal ultrasound
- Muscle ultrasound (Ms U/S)
- Duplex on common carotid, the carotid bulb, and the near and far wall segments of the internal carotid.

✓ **Sample collection:**

Ten ml of venous blood sample was withdrawn from each subject after over night fasting (12h) via proper venipuncture technique under complete aseptic condition.

- One ml was delivered into EDTA tube for CBC analysis using automated counter, Sysmex KX-21 (USA), and HbA1c testing using fast ion exchange resin supplied by Human (Germany).
- Five ml were delivered into EDTA tube then centrifuged and the clear plasma was kept frozen at –20 °c till analysis of Serum Eotaxin, IL1B, VEGF and h s CRP.
- The rest of the sample was left to clot then centrifuged and the clear none hemolyzed sera were used for traditional laboratory investigation (FBG, fasting insulin, total cholesterol, triglycerides, LDL,

HDL, ALT, AST) using commercially available kits supplied by Spin react (Spain).

- Plasma Serum Eotaxin, IL1B, VEGF and h s CRP level was assayed by ELISA kits according to **1- Human Eotaxin 1 (CCL11)**

Human Eotaxin 1 (CCL11) ELISA kit catalogue No 201-12-0113 supplied by Sun Red company.

Test principle

The kit uses a double- antibody sandwich enzyme-linked immunosorbentassay (ELISA) to assay human eotaxin 1. sample was added to monoclonal antibody enzyme well which was pre-coated with Human Eotaxin 1 monoclonal antibody, incubated then Eotaxin 1 antibody labelled with biotin was added and combined with streptavidin-HRP to form immune complex then incubation and washing out again to remove uncombined enzyme. Then chromogen solution A, B was added and the color of the liquid changed into blue and at the effect of acid the finally became yellow. the chroma of colour and concentration of the human Eotaxin of sample were positively correlated.

Insulin Resistance status was determined by the HOmeostatic Metabolic Assessment (HOMA), which was assessed by the formula: fasting insulin (mU/mL) x fasting glucose (mg/dL) /405.

- **NAFLD fibrosis score**

The NAFLD fibrosis score is based on six readily available variables (i.e. age, body mass index (BMI), hyperglycemia, platelet count, albumin, and AST: ALT ratio) and is recognized as a clinically useful tool to identify NAFLD patients with a higher likelihood of having bridging fibrosis and/or cirrhosis (13).

A score <–1.455 had 90% sensitivity and 60% specificity to exclude advanced fibrosis, whereas a score >0.676 had 67%sensitivity and 97% specificity to identify the presence of advanced fibrosis (14).

Radiological investigations

- **Abdominal ultrasound**

Abdominal ultrasound was performed by high resolution ultrasound probe (Siemens machine with a convex probe3 MHz) and the classification of Hepatic steatosis, commonly known as ‘‘bright liver’’, was based on the following scale of hyperechogenity: grade 0= absent, grade 1= light, grade 2= moderate, grade 3 = severe, indicating the difference between the densities of the liver and the right kidney (15).

- **Muscle ultrasound**

Muscle ultrasound was performed by high resolution ultrasound probe (Siemens machine with a linear probe11 MHz) at the level of the biceps muscle of the left superior arm, is a feasible and reliable technique to visualize altered muscle tissue, as in case of ImTG, since it is non-invasive and provides results in real-time. Infiltration of fat and fibrous tissue increases muscle echo intensity, i.e., the muscles appears whiter at the ultrasound image. To describe muscle echo intensity (16). There are a four grade visual scale, where grade I represented

- **Duplex on common carotid, the carotid bulb, and the near and far wall segments of the internal carotid**

Before initiation of measurements, all participants were fasting (8-12h) and lying in a supine position on an examination table for 10 minutes and remained lying down for the rest of the examination (17)**.**

Examination of the right common carotid artery was performed with high resolution ultrasound probe (Siemens machine with a linear probe7.5 MHz). Subjects were having their head tilted a bit to the opposite side to optimize the image access to the arteries. The ultrasound probe was placed just below the carotid bifurcation bulb and the arteries were scanned in the longitudinal plan(18)**.**

3. Results

Table (1): Demographic data of the studied groups.

Data		Non ischaemic obese GI	Ischaemic obese G II	Control G III	Test	P value
Age (years)	**Range**	45 – 57	45 – 58	45 – 55	**F:** 2.832	0.123
	Mean	**48.77**	**50.73**	**49.83**		
	S. D	3.65	3.19	2.69		
Sex	**Male (%)**	13 **(43.3%)**	17 **(56.7%)**	16 **(53.3%)**	**X^2:** 1.156	0.561
	Female (%)	17 **(56.7%)**	13 **(43.3%)**	14 **(46.7%)**		

The mean age of patients in the non ischaemic obese group (48.77±3.65 years) was not significantly different from either the ischaemic obese group or control groups (50.73 ± 3.19, 49.83 ± 2.695 years, respectively) ***(P= 0.123).***

Table (2): Laboratory data of the studied groups (Part I).

			Non ischaemic obese G I	Ischaemic obese G II	Control G III	F. test	P. value		
FBG mg/dl		**Range**	78 – 125	70 – 120	70 – 98	17.149	**0.001***	**P1**	0.688
		Mean	**97.37**	**96.07**	**80.37**			**P2**	**0.001***
		S. D	15.12	13.48	7.73			**P3**	**0.001***
Fasting insulin mIu/ml		**Range**	2.8 – 27.6	5 – 28	2.6 – 14	24.267	**0.001***	**P1**	**0.001***
		Mean	**12.93**	**18.02**	**7.91**			**P2**	**0.001***
		S. D	7.26	5.78	2.94			**P3**	**0.001***
HbA1c (%)		**Range**	4.2 – 6.4	4.7 – 6.3	4.9 – 5.6	0.535	0.588	**P1**	0.310
		Mean	**5.26**	**5.38**	**5.30**			**P2**	0.713
		S. D	0.63	0.43	0.21			**P3**	0.516
HOMA IR		**Range**	0.57 – 8.5	1.05 – 8	0.46 – 2.9	21.253	**0.001***	**P1**	**0.025***
		Mean	**3.35**	**4.33**	**1.58**			**P2**	**0.001***
		S. D	2.24	1.69	0.62			**P3**	**0.001***
TC mg/dl		**Range**	140 – 270	165 – 300	145 – 195	28.848	**0.001***	**P1**	**0.001***
		Mean	**201.70**	**230.47**	**168.37**			**P2**	**0.001***
		S. D	30.72	43.64	12.86			**P3**	**0.001***
TG mg/dl		**Range**	100 – 300	120 – 450	100 – 140	15.397	**0.001***	**P1**	**0.005***
		Mean	**171.90**	**225.33**	**121.77**			**P2**	**0.009***
		S. D	69.88	103.24	11.77			**P3**	**0.001***
LDL mg/dl		**Range**	80 – 150	80 – 195	56 – 88	95.838	**0.001***	**P1**	**0.001***
		Mean	**109.15**	**145.69**	**72.93**			**P2**	**0.001***
		S. D	19.05	28.39	8.60			**P3**	**0.001***
HDL mg/dl	**Male**	**Range**	41 – 55	20 – 45	45 – 70	72.130	**0.001***	**P1**	**0.001***
		Mean	**47.0**	**29.47**	**54.06**			**P2**	**0.003***
		S. D	4.64	6.15	6.88			**P3**	**0.001***
	Female	**Range**	35 – 55	25 – 36	50 – 78	79.912	**0.001***	**P1**	**0.001***
		Mean	**43.71**	**30.77**	**64.21**			**P2**	**0.001***
		S. D	7.10	4.21	8.62			**P3**	**0.001***

P: probability. **SD**, standard deviation. **FBG**, fasting blood glucose. **HbA1c**, glycosylated hemoglobin. **TC** total cholesterol **TG** triglyceride **LDL** low density lipoprotein **HDL** high density lipoprotein **HOMA IR** HOmeostatic Metabolic Assessment (HOMA) for insulin resistance**. P1:** between on ischaemic obese and ischaemic obese**, P2:** between on ischaemic obese and control **P3:** between ischaemic obese and control**.**

Table (3): Laboratory data of the studied groups (Part II).

		Non ischaemic obese G I	Ischaemic obese G II	Control G III	F. test	P. value		
ALT IU/L	**Range**	12 – 50	12 – 65	12 – 34	11.793	**0.001***	**P1**	**0.030***
	Mean	**27.90**	**33.53**	**21.17**			**P2**	**0.010***
	S. D	11.64	11.06	5.89			**P3**	**0.001***
AST IU/L	**Range**	12 – 55	12 – 75	13 – 37	9.370	**0.001***	**P1**	0.057
	Mean	**29.70**	**35.07**	**23.03**			**P2**	**0.019***
	S. D	9.64	14.72	6.27			**P3**	**0.001***
Platelet count (×1000/cmm)	**Range**	155 – 410	150 – 350	150 – 400	7.716	**0.006***	**P1**	**0.001***
	Mean	**293.83**	**223.17**	**253.33**			**P2**	**0.027***
	S. D	72.27	57.17	78.58			**P3**	0.098
Albumin mg/dl	**Range**	3.5 – 5.5	3.5 – 5	3.5 – 5.5	5.481	**0.006***	**P1**	0.052
	Mean	**4.18**	**3.92**	**4.35**			**P2**	0.192
	S. D	0.52	0.43	0.57			**P3**	**0.001***

ALT alanine transaminase **AST** aspartate transaminase **P1:** between obese non ischaemic and obese ischaemic**, P2:** between obese non ischaemic and control **P3:** obese ischaemic and control

Table (4): Result of NAFLD fibrosis score of obese patients.

Data			Obese Non Ischaemic G I	Obese ischaemic G II	Test	P. value
NAFLD score	Range		-4.22 – 1.18	-2.25 – 2.95	F 19.339	0.001*
	Mean		-1.64	0.00		
	S. D		1.45	1.44		
NAFLD score result interpretation	SFU	N (%)	14(46.7%)	6 (20.0%)	X^2 5.925	0.052
	I	N (%)	12 (40.0%)	14 (46.7%)		
	SFL	N (%)	4 (13.3%)	10 (33.3%)		

NAFLD non alcoholic fatty liver disease **SFU:** significant fibrosis unlikely **I:** intermediate **SFL:** significant fibrosis likely. As regard to NAFLD fibrosis score calculation there was

Table (5): Inflammatory markers levels in studied groups.

		Non ischaemic Obese G I	Ischaemic Obese G II	Control G III	F. test	P. value		
Hs CRP ng/ml	Range	1.9 – 111	32 – 110	7.9 – 98	25.626	0.001*	P1	0.280
	Mean	86.36	93.30	50.74			P2	0.001*
	S. D	26.26	16.47	29.50			P3	0.001*
EOTAXIN pg/ml	Range	300 – 790	520 – 1276	8.5 – 520	307.753	0.001*	P1	0.001*
	Mean	462.23	965.60	89.15			P2	0.001*
	S. D	132.76	174.02	93.09			P3	0.001*
IL1β pg/ml	Range	356 – 3693	505 – 10345	0 – 985	20.833	0.001*	P1	0.001*
	Mean	1333.90	3791.6	431.50			P2	0.098
	S. D	854.90	3496.64	327.45			P3	0.001*
VEGF ng/l	Range	388 – 1681	474 – 5032	0 – 935	39.069	0.001*	P1	0.001*
	Mean	836.63	2487.3	438.63			P2	0.109
	S. D	370.45	1581.27	283.84			P3	0.001*

Ppropability, **SD** standard deviation **hsCRP** high sensitive C reactive protein **IL1β** interleukin 1β, **VEGF** vascular endothelial growth factor transaminase **P1:** between obese non ischaemic and obese ischaemic**, P2:** between obese non ischaemic and control **P3:** obese ischaemic and control

As regard serum hsCRP level, there was no significant difference between non ischaemic obese and ischaemic obese group (P1=0.280) but it was significantly higher in both obese groups in comparison to control group (P2, P3=0.001) with mean values (86.36 ± 26.26, 93.30 ± 16.47 and 50.74 ± 29.50 ng/ml respectively).

As regard serum Eotaxin, there was significantly higher levels in ischaemic obese group than non ischaemic obese group (P1 =0.001) and it was also significantly higher in both obese groups in comparison to control group (P2, P3=0.001). Mean Eotaxin levels in all groups were (462.23±132.76, 965.60 ± 174.02 and 89.15± 93.09pg/ml respectively).

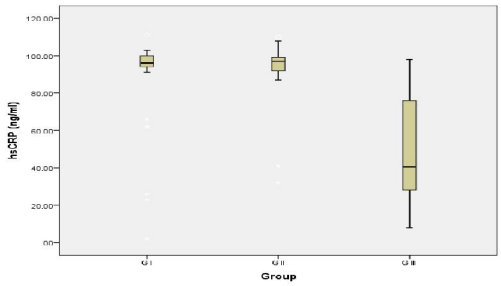

Figure (1): Serum hsCRP in different groups.

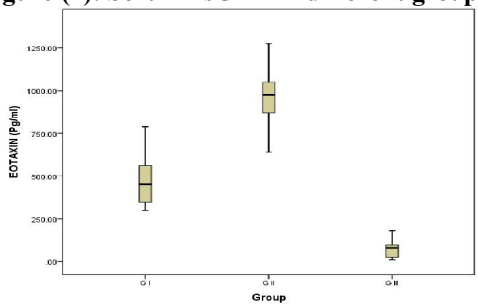

Figure (2): Serum Eotaxin levels in studied groups.

As regard serum **IL 1β** level, it was significantly higher in ischaemic obese group than non ischaemic obese group ***(P1=0.001)*** and it was significantly higher in obese ischaemic group than control group (***P3 =0.001***). But, there was no significant difference between non ischaemic obese group in comparison with control group ***(P2= 0.098)***. Mean **IL 1β** levels in all groups were (1333.90 ± 854.90, 3791.6 ± 3496.64 and 431.50 ± 327.45 pg/ml respectively)

As regard serum **VEGF** level, it was significantly higher in ischaemic obese group than non ischaemic obese group ***(P1=0.001)*** and it was significantly higher in obese ischaemic group than control group (***P3 =0.001***). But, there was no significant difference between non ischaemic obese group in comparison with control group ***(P2= 0.109)***. Mean VEGF levels in all groups were (836.63 ± 370.45, 2487.3 ± 1581.27 and 438.63 ± 283.84 ng/l respectively).

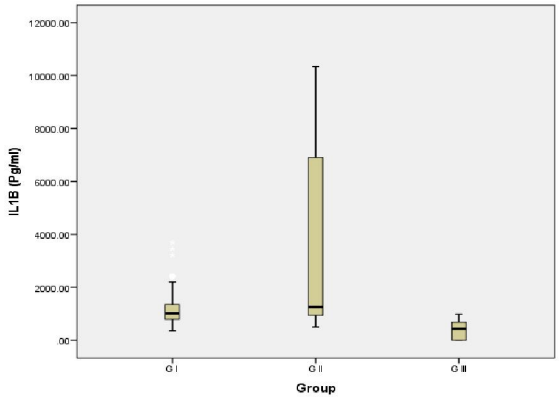

Figure (3): Serum IL1β in different studied groups

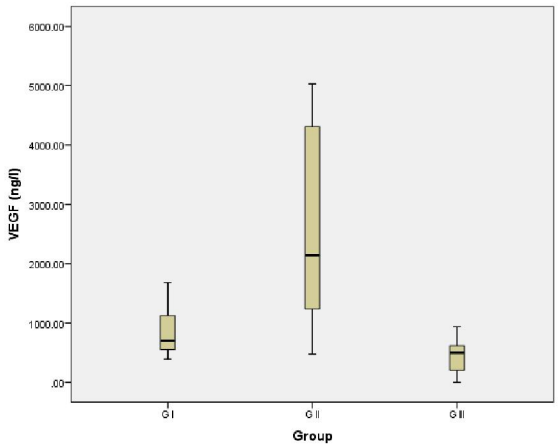

Figure (4): Serum VEGF levels in different studied groups.

Table (6): Parameters of duplex u/s of carotid arteries in different studied groups.

Data			Non ischemic obese G I	Ischemic obese G II	Control G III	Test	P-value
CIMT (mm)	**Range**		0.7–1.4	1–1.9	0.5–0.8	**F:** 72.232	**0.001***
	Mean		**0.95**	**1.32**	**0.69**		
	S. D		0.25	0.24	0.08		
Atheromatous plaque	**Yes**	**N %**	3 **(10.0%)**	14 **(46.7%)**	0 **(0 %)**	**X^2**: 23.642	**0.001***
	No	**N %**	27 **(90.0%)**	16 **(53.3%)**	30**(100%)**		

P: probability. **SD** standard deviation CIMT, Carotid intima media thickness.

Statistically significant increase of CIMT was also observed in patients of ischaemic obese compared with that of non ischaemic obese and control groups (1.32 ± 0.24, 0.95 ± 0.25 and 0.69 ± 0.08 mm, respectively] ***(P= 0.001)***.

Also, there was significant increase in presence of atheromatous plaque in ischaemic obese group patients as 14 patients (46.7%) had atheromatous plaque in ischaemic obese group and only 3 patients (10%) had atheromatous plaque in non ischaemic obese group ***(P =0.001)***.

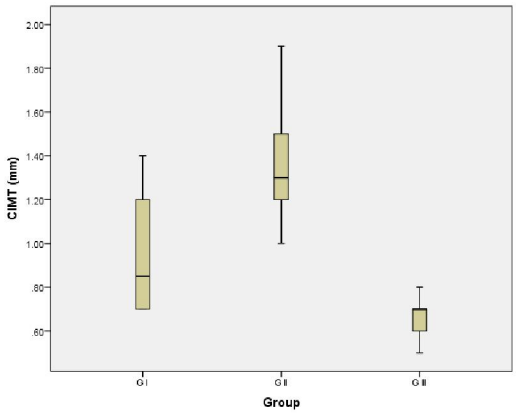

Figure (5): CIMT in different studied groups.

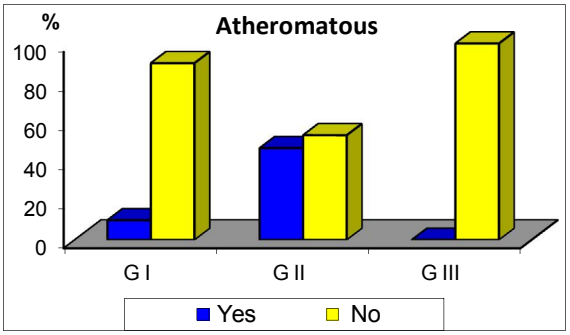

Figure (6): Percentage of atheromatous plague in different groups.

4. Discussion

The World Health Organization (WHO) has estimated that one in three global deaths are as a result of CVD-related events such as myocardial infarction (MI) and stroke (19).

Common carotid Intima-Media Thickness (CIMT) is accepted as a marker to detect early atherosclerosis (20). Also, patients with CAD also have increased circulating and expression levels of various inflammatory mediators and/or markers, such as IL-1α, IL-4, IL-8, IL-10, VEGF (21), IL-1β, IL-2, IL-6, TNF-α, MCP-1, IFN-γ (22), IL-23A, IL-27, IL-37 (23), and PAI-1 (24). These results suggest that systemic inflammation is present in these patients with increased CIMT or CAD. Inflammatory markers can predict incident cardiovascular disease and are associated with the presence of subclinical atherosclerosis. The relations between multiple inflammatory markers and direct measures of atherosclerosis are less well established.

Atherosclerosis is a chronic inflammatory disease and inflammation is one of the primary mechanisms in the pathogenesis of it (25). The extent of inflammation as measured by specific biomarkers probably reflects the activity of the disease and therefore may predict the individual's risk for progression of atherosclerosis. In our study, there was significant increase of IL 1β, VEGF levels in ischaemic obese patients than non ischaemic obese and control group ***(P=0.001)*** and CIMT was positively correlated with serum IL1β, VEGF and Eotaxin levels ***(r=0.321 p=0.018, r=0.256 p=0.038 and 0.512 p=0.001 respectively).***

In agreement with our data ***Rueda-clausen CF et al 2009*** higher levels of IL-1β in patients with CAD, than in patients without CAD. (26) Also, ***Mirhafez SR et al 2015*** found that significant increase of VEGF in CAD patients than control (21)**.**

As regard, hsCRP, there was no significant difference between ischaemic obese and non ischaemic obese groups ***(P=0.280)*** but it was significantly higher in ischaemic obese patients than control ***(P=0.001).*** In agreement with us was, ***Rueda-clausen CF et al 2009*** reported that higher levels of hsCRP in patients with CAD than patients without CAD (26). Also, ***Jenny et al 2016*** reported that CRP was found to identify asymptomatic individuals at higher risk of a CVD event than predicted by traditional risk-screening guidelines. In addition, CRP was found to be an independent predictor of myocardial functional deterioration in asymptomatic individuals with no history of heart disease and a nontraditional marker for CVD risk with clinical utility in screening (27). On the other hand, **Wang et al 2017** reported that there was no association between hs CRP and progression of CIMT (28).

It is important to understand the link between inflammation and atherosclerosis and how obesity accelerates this process. Obese individuals have a higher propensity toward inflammation compared to non obese individuals. Patients with visceral obesity have been found to have higher levels of proinflammatory adipokines including TNF-alpha, IL-6, MCP-1, resistin, and leptin. CRP is a nonspecific acute phase protein synthesized by hepatocytes, arterial smooth muscle cells, and adipocytes in response to inflammatory cytokines such as IL-6 (27). The higher level of inflammation has been correlated with observations from several large scale prospective studies that demonstrated elevated levels of CRP in obese patients (29); (30) and that may explain our results with a significant increase of CRP in ischaemic obese patients than control and. no significant difference between both obese groups.

As regard serum Eotaxin, it was significantly higher in ishaemic obese patients than non ischaemic obese and control groups ***(P=0.001).*** It was positively correlated with IL1β (proatherogenic cytokine) and VEGF (angiogenic factor) included in pathogenesis of atherosclerosis ***(r=0.552 p=0.001 and r=0.510 p=0.001 respectively).***

Studies of Eotaxin in patients with atherosclerosis reported conflicting results. Some

studies had reported elevated serum levels of Eotaxin in patients with CAD as ***Wyss et al 2010*** who found serum Eotaxin present in higher concentration in local blood samples during ACS (31). Also**,** ***Ardigo D et al. 2007, Emanuele E et al. 2006; Raaz-Schrauder D et al. 2012 and Kaehler J et al. 2006)*** who reported that Eotaxin concentrations were higher in patients with CAD than in control patients(8); (32); (33).

On the other hand, some studies had reported no association between serum level of serum Eotaxin and coronary artery disease as ***Mosedale DE et al. 2005; Canouï-Poitrine F et al. 2011***(34)**;**(35).

This association can be supported by some studies reported an association between Eotaxin and atherosclerosis as ***Haley KJ et al. 2000*** who found that Eotaxin is locally overexpressed during arterial wall inflammation (36). Besides its leukocyte attracting properties, ***Kodali RB et al. 2004*** reported that it stimulates the migration of smooth muscle cell from media to intima of the injured arterial wall (37). This process is thought to have a crucial role in the development of atherosclerotic plaque and restenosis (38).

In genetic studies about Eotaxin gene polymorphism, ***Machal J et al 2012 and Kincli V et al 2015*** reported that there was an association between genetic polymorphism in Eotaxin (CCL 11) gene and severity and course of coronary atherosclerosis (29); (35), also ***Wang Y et al 2010*** found that there was an effect of Eotaxin gene polymorphisms on cardiac events in diabetic patients (39).

In our study also serum Eotaxin level was found to be positively correlated with CIMT ***(r=0.512 p=0.0012)***, and its level was significantly higher in obese patients with a potential atherosclerotic disease (CIMT > 0.9 mm) ***(P= 0.001).*** ROC curve analysis of CIMT and serum Eotaxin levels in obese groups showed higher sensitivity **(97%)** and specificity **(93%)** in detection and prediction of atherosclerosis in obese patients. Also by univariate and multivariate regression analysis, serum Eotaxin level can predict CIMT ***(P=0.001 and 0.005 respectively).***

In agreement with us ***Tarantino G et al 2014*** in two different studies reported that early atherosclerosis evidenced as increased CIMT was strongly predicted by circulating Eotaxin(40).

The link between serum Eotaxin levels with established and emerging risk factors for progression of atherosclerosis had therefore been investigated in this current study. Putatively, a number of such risk factors, including BMI, WC, smoking, HOMA IR, and NAFLD fibrosis score, hepatic steatosis and intramuscular fat and presence of metabolic syndrome.

Serum Eotaxin levels were significantly higher in obese patients than control group ***(P=0.001)*** and was positively correlated with BMI, WC and W/H ratio ***(r=0.368 p=0.012, r =0.258 p=0.039 and r=0.395 p=0.013 respectively)***. Also there was positive correlation between serum Eotaxin levels and HOMA for insulin resistance in obese patients ***(r=0.459, p = 0.001).***

In agreement with our results ***Choi K et al 2007*** reported elevated levels of serum Eotaxin in patients with central obesity and was significantly associated with WC (41). Also, ***Vasudevan AR et al 2006*** found that Eotaxin mRNA levels were 4.7-fold higher in visceral adipose tissue than in adipose tissue (42) and ***Huber J et al 2008*** reported that gene expression of CCL11 (Eotaxin) has been found higher in visceral than in subcutaneous adipose tissue (43).

This can be explained as obesity induces a complex remodeling of adipose tissue, which expands to accommodate the excessive caloric intake and markedly changes its structure and cellular composition. It is widely accepted that this obesity-associated remodeling generates a systemic proinflammatory state, which is mediated by an imbalanced production of adipocyte-derived cytokines (adipokines) that directly and indirectly affect the cardiovascular system (44). In addition to macrophages, other myeloid cells, such as neutrophils and mast cells, contribute to adipose tissue dysfunction in obesity. Similarly, mast cells have been reported to accumulate in obese adipose tissue that may be linked to increased Eotaxin in obese patients (44).

Higher levels of serum Eotaxin were found in smokers than non smoker obese patients ***(p=0.001)*** that was in agreement with ***Shiels MS et al 2014*** reported that serum Eotaxin level was increased in current smokers than never or former smoker (45). Also, ***Bade G et al 2014*** suggested that smoking partially contributes to the increase in Eotaxin level observed in the sera of smokers in his study population (46).

This can be explained by Cigarette smoking is known to cause several pulmonary and systemic immune alterations pertaining to both the number of immune cells, such as increases in macrophages, neutrophils, eosinophils, and mast cells and functionality of various immune cells (47). In addition, smoking-induced inflammation and immune modulation are emerging as potentially important mechanisms in the development of cancer and other systemic chronic diseases such as coronary artery disease and stroke. (45)

In our study we investigated the relation between serum Eotaxin and CIMT and presence of MetS in obese patients. Both CIMT and serum Eotaxin levels were significantly higher in obese patients with MetS than obese patients without MetS ***(P=0.001 and 0.013***

respectively). This can be explained as MetS has also been independently linked with increased oxidative stress and inflammatory burden and our results showed that serum Eotaxin levels were positively correlated with BMI, WC and HOMA for insulin resistance and other inflammatory cytokines which are main components of MetS and this explains its higher levels in obese patients with MetS.

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Conflict of interest:

The authors have no conflict of interest.

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