STUDY OF PLASMA SOLUBLE VASCULAR CELL ADHESION MOLECULE -1 (sVCAM-1) IN RELATION TO INSULIN STATUS IN OBESE FEMALES

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of

Master of Science

in

Chemical Pathology

By

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صدق الله العظيم

(البقرة : ٣٢)

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Presented by

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In

Chemical Pathology

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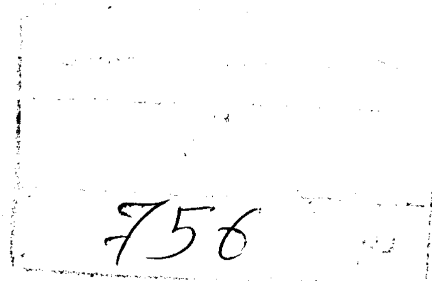

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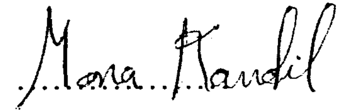

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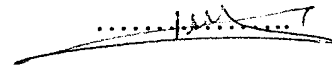

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LIST OF ABBREVIATIONS

- ΔA/min — Change in absorbance per minute
- ACRP30 — Adipocyte complement related protein
- ADP — Adenosine diphosphate
- ALT — Alanine aminotransferase
- AP-1 — Activator protein-1
- AST — Aspartate aminotransferase
- ATP — Adenosine triphosphate
- BMI — Body mass index
- BP — Blood Pressure
- C — Constant
- CAD — Coronary artery disease
- CAMs — Cell Adhesion molecules
- CD36 — Cluster of differentiation 36
- Chol — Cholesterol
- CIMT — Carotid intima media thickness
- CLIA — Chemiluminescent enzyme immunometric assay
- Cs — Concentration of the standard
- CSF — Colony stimulating factor
- CVD — Cardiovascular disease
- ECD — Endothelial cell dysfunction
- ECG — Electrocardiogram
- ECs — Endothelial cells
- Egr-1 — Early growth response-1
- ELISA — Enzyme linked immunosorbant assay
- E-selectin — Endothelial selectin
- ET-1 — Endothelin-1
- FFA — Free fatty acid
- FPG — Fasting plasma glucose
- FPI — Fasting plasma insulin
- GAD — Glutamic acid decarboxylase
- HC — Hip circumference
- HDL-C — High density lipoprotein- cholesterol
- HOMA — Homeostasis model assessment
- HRP — Horse radish peroxidase
- ICAM-1 — Intercellular adhesion molecule-1
- IFN-γ — Interferon-γ
- Ig — Immunoglobulin
- IgSF — Immunoglobulin superfamily
- IL-1 — Interleukin-1.
- IL-6 — Interleukin-6.
- IR — Insulin resistance
- IRS-1 — Insulin receptor substrate-1
- LD — Lactate dehydrogenase
- LDL-C — Low density lipoprotein cholesterol
- LFA-1 — Lymphocyte function- related antigen-1
- L-selectin — Leukocyte-selectin
- MAC — Mid arm circumference
- Mac-1 — Macrophage-1

- MCP-1 Monocyte Chemoattractant Protein-1
- M-CSF Monocyte-CSF
- MMPs Matrix Metalloproteinases
- NAD Nicotineamide adenine dinucleotide
- NADH Reduced nicotineamide adenine dinucleotide
- NADPH Reduced nicotineamide adenine dinucleotide phosphate
- NF-kB Nuclear factor кappa B
- NO Nitric oxide
- PAI-1 plasminogen activator inhibitor-1;
- PECAM-1 Platelet endothelial adhesion molecule-1
- PI 3K Phosphatidyl inositol 3-kinase
- PMT Photomultiplier tube
- PPG Post prandial glucose
- P-Selectin platelet-Selectin
- ROS Reactive oxygen species
- rpm Revolutions per minute
- S Standard
- S.D Standard deviation
- T Test
- TG Triglycerides
- TMB Tetramethyl benzidine
- TNF- α. Tumor necrosis factor-α.
- TSFT Triceps skin fold thickness
- V Variable
- VCAM-1 Vascular cell adhesion molecule
- VLA-4 Very late antigen-4
- VLDL-C Very low density lipoprotein cholesterol
- VSMCs Vascular smooth muscle cells
- WC Waist circumference
- WHR Waist to hip ratio

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INTRODUCTION

Obesity

Definition:

Obesity is the deposition of excessive amounts of adipose tissue in the body. The World Health Organization [1] has defined obesity by a body mass index (BMI) of over 30 kg/m^2 while overweight by a BMI of 25–30 kg/m^2.

Prevalence:

In industrialized countries, the prevalence of obesity is 20% of the adult population. [2] In addition, the prevalence of obesity is rapidly increasing even among children. [3]

Measurement of obesity:

The most commonly used indices for determination of obesity are the body mass index and waist to hip ratio. The latter is more preferable by some as it can differentiate between upper and lower types of adiposity. Other less important measures adopted include skin fold thickness measurements. Furthermore, in western societies tables of average weights by heights and age based on periodically conducted National health and Nutritional examination Surveys (NHANES) are used (table I).[4]

Table I: Measurements of overweight and obesity [4]
• Tables of average weights by heights and age. • Tables of desirable weights for height. • Body mass index (BMI) = weight (Kg) /height $(meter)^2$ [5] • Waist to hip ratio(WHR) = waist circumference/hip circumference[6] • Skin fold measurements: by measuring skin thickness at various sites as biceps, triceps, subscapular and suprailiac regions using standard skin calipers.

Risks and outcomes of obesity:

Several complications develop as a consequence of obesity.[7-9] (table II) Obesity has been found to be one of the criteria of metabolic syndrome. Furthermore, about one-third of obese people will develop diabetes.[2] Obesity is strongly associated with atherosclerosis and is an important risk factor for the development of coronary artery disease (CAD).[10-13]

The risk of coronary artery disease increases with the severity of obesity especially for those with abdominal (visceral or central) obesity. The risk exists when the waist to hip ratio is >0.90 in males and >0.85 in females. Abdominal obesity has been demonstrated to be an independent predictor of silent myocardial ischaemia in otherwise healthy asymptomatic subjects. [14]

In a study, it was found that men with a waist circumference of >93cm have a 31% greater increase in the mean intima media thickness of the common carotid artery (CIMT) when compared to men with a waist circumference of <85cm, indicating that abdominal obesity is associated with accelerated progression of atherosclerosis. [15]

Potential links of obesity with increased risk for atherosclerosis:

The potential links between obesity and increased risk for atherosclerosis are glucose intolerance, hyperinsulinaemia, hyperleptinaemia, disturbed lipid pattern [reduction of high-density lipoprotein (HDL) and elevation of triglycerides] and hypertension. Hyperinsulinaemia and hyperleptinaemia characterize obese subjects already at the early phase of obesity.

Table II: Complications of obesity[7-9]

1. Metabolic and hormonal complications
   Metabolic syndrome: which comprises :
   - Type 2 diabetes.
   - Hyperinsulinemia and insulin resistance
   - Dyslipidemia:
     (Hypertriglyceridemia, low HDL–cholesterol)
   - Hypertension.
   - Hypercoagulable state.
2. Vascular complications
   - Cerebrovascular disease.
   - Congestive heart failure.
   - Coronary heat disease.
   - Hypertension.
   - Thromboembolic disease.
3. Other related complications
   - Respiratory problems (obstructive sleep apnea).
   - Gastrointestinal diseases.
   - Joint diseases (Osteo / gouty arthritis).
   - Cancer.
   - Skin disease.
   - Psychological complications.

Insulin resistance and obesity

Insulin, a 51 amino acid polypeptide synthesized by the β cells of pancreatic islets, is the only hormone responsible for regulating tissue uptake of glucose and the control of blood glucose level. Insulin is important in the major steps of glucose metabolism (glycolysis and energy production), as well as glycogenesis and lipogenesis. [16]

Insulin resistance and hyperinsulinemia

Definition of insulin resistance:

Insulin resistance (IR) is a state in which normal or even elevated amounts of insulin produce a subnormal biological response in the form of an impaired glucose tolerance. [17,18]

Causes of insulin resistance:

Insulin resistance can be the outcome of different etiologies [19,20] (table III), yet the commonest cause of insulin resistance is obesity in which resistance may be mild to moderate.[21]

Degree of obesity and site of distribution of adiposity as determinants of insulin resistance:

Obesity has been clearly demonstrated to be associated with insulin resistance.[22] The degree of insulin resistance increases in a linear fashion with BMI at an age- and sex-adjusted rates (at BMI of 30–35 kg/m^2, the prevalence of insulin resistance is 34% and at BMI of >35 kg/m^2 it is 41%).[23]

In the evaluation of obesity, it has become apparent that not only the magnitude of increase in fat mass, but also the site of distribution is an important determinant of the development of insulin resistance. Intra abdominal fat was found to be a more important determinant of insulin sensitivity than subcutaneous fat. [24]

The accumulation of central fat and presence of insulin resistance have both been associated with the dyslipidemia seen in the metabolic syndrome.[25] Therefore, an increase in the waist to hip ratio (WHR) has been found to be associated with small dense Low density lipoprotein (LDL) particles [26], as has been an increase in intra-abdomiral fat mass. This pattern of central fat distribution has also been associated with an increase in very low density lipoprotein (VLDL) and intermediate- density lipoprotein (IDL) cholesterol fractions, and a decrease in HDL-cholesterol.[27, 28]

Table III: Causes of insulin resistance :[20,21]

1. **Insulin antagonists:**
   - Exogenously administered drugs
   - Counter-insulin hormones
2. **Antibodies that antagonize insulin secretion and action:**
   - Islet cell antibodies
   - Anti glutamic acid decarboxylase (GAD) antibodies
   - Insulin receptor antibodies
   - Antibodies to exogenous insulin
   - Autoantibodies to endogenous insulin
3. **Insulin post-receptor (signaling pathway) defect:**
   eg: obesity.

Mechanisms of insulin resistance and hyperinsulinemia in obesity:

Subjects with insulin resistance are characterized by reduced insulin-mediated glucose uptake [29] and increased serum insulin concentration. [30] Insulin resistance in obesity has been attributed mainly to a post receptor defect in insulin action, at the insulin-stimulated insulin receptor substrate-1 (IRS-1) association with phosphatidylinositol 3-kinase (PI 3K), which seems to be a key step in insulin resistance to glucose uptake. [31] Furthermore, adipocytokines, such as tumor necrosis factor alpha (TNF-α), interact with their cellular receptors, resulting in the activation of the stress-related protein kinases that promote insulin resistance.[32] Hyperinsulinaemia, caused by over-secretion of insulin by the pancreas, is a compensatory mechanism for this post receptor defect.

Outcomes of hyperinsulinemia and insulin resistance:

Besides its metabolic actions, insulin plays a role in the normal functioning of the vasculature. It induces a time- and dose- dependent vasodilatation mainly via the endothelium-dependent nitric oxide (NO) pathway.[33]

Insulin also plays an important role in vascular pathophysiology.[34] In contrast to its acute beneficial effects, chronically high serum insulin concentration appears to have a harmful effect on endothelial function as it stimulates the release of endothelin-1 (ET-1).[35,36]The imbalance between the release of ET-1 and NO may blunt the insulin-induced vasodilatation in subjects with insulin resistance.

In addition, insulin may also promote vascular smooth muscle cell (VSMC) proliferation and cholesteryl ester accumulation in the arterial wall.[37] Furthermore, insulin has been found to induce sympathetic activity in a dose dependent manner via the central nervous system.[38,39] Insulin-induced sympathetic activity might lead to augmented coronary vasoconstriction in subjects with endothelial cell dysfunction (ECD).[40] It may also predispose endothelial cells to high-shear-stress-induced damage.[41]

Thus, hyperinsulinaemia in obese subjects has been suggested to be the link between adiposity, and cardiovascular complications,[42] being identified by epidemiological studies as an independent risk factor for the development of coronary artery disease (CAD), where a link between hyperinsulinaemia and cardiovascular mortality has been suggested.[43-46] (figure 1).

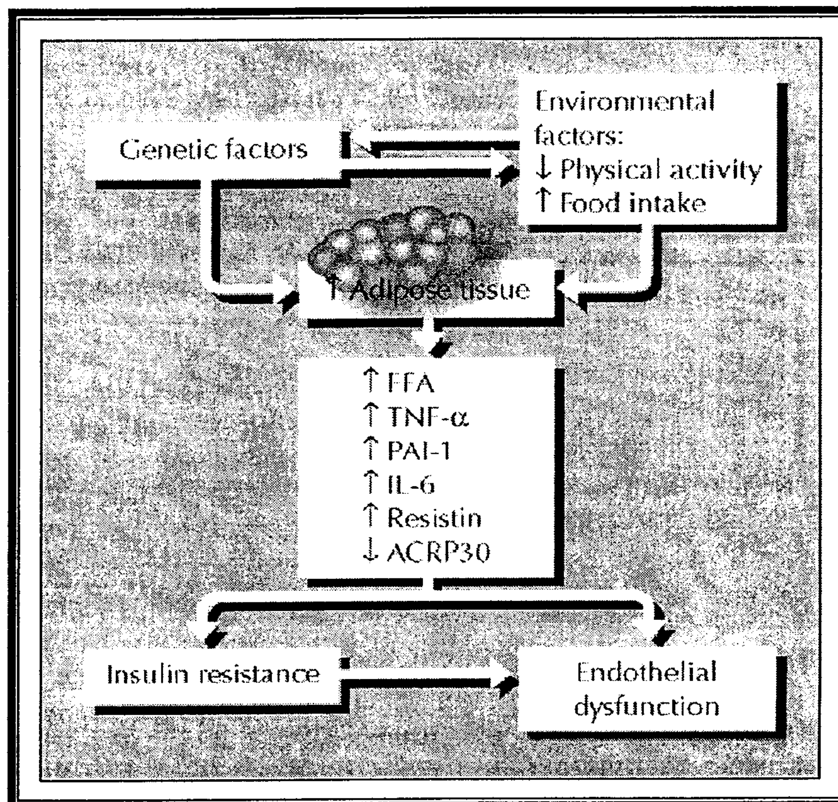


Figure 1: Links between adipose tissue mass and both insulin resistance and endothelial dysfunction. [42]

Abbreviations:

ACRP30: adipocyte complement related protein; FFA: free fatty acid; IL-6: interleukin-6; PAI-1: plasminogen activator inhibitor-1; TNF-α: tumor necrosis factor-α.

Atherosclerosis and endothelial cell dysfunction in obesity

In obesity there is an increase in the expression and release of adipocytokines, some of which correlate with inflammation, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6. The level of TNF gene expression differs among different fat depots. It has been reported that visceral adipose tissue produces more TNF-α than peripheral subcutaneous adipose tissue. [47]

The pro-inflammatory cytokines released from adipose tissue are involved in the process of insulin resistance as well as their contribution to the development of obesity induced atherosclerosis.[48]

Atherosclerosis is now regarded as an inflammatory disease of the vessel wall. Hypercholesterolemia, hypertension, hyperglycemia, smoking, lack of estrogen and obesity are probably the pro-inflammatory triggers for initiating the atherogenic process. These metabolic disturbances cause chronic inflammation in contrast to endotoxin and bacterial infections, which cause acute inflammation. Endotoxin and pro-inflammatory cytokines induce a cascade of events that eventually result in the increased expression of adhesion molecules on the endothelium and their respective ligands on the leukocytes. [49]

In acute inflammatory processes, polymorphonuclear leukocytes adhere to the endothelium, whereas in chronic inflammatory processes, such as atherosclerosis, the mononuclear cells are the dominant adherent cells. The initiation of this chronic inflammatory process is through the adhesion of monocytes on the endothelial surface via the adhesion molecules expressed on the endothelium, resulting in the so called process of endothelial cell dysfunction (ECD).[50]

Endothelial cell dysfunction is defined as a change towards an injurious process. It contributes to vasospasm, excessive thrombosis and abnormal VSMCs proliferation. [51]

The normal resting endothelium expresses adhesion molecules in limited amounts. In inflammatory processes, both the total expression and the turnover of cell adhesion molecules (CAMs) is markedly increased.[52,53]

Activated monocytes and endothelium also increase the secretion of chemokines, like monocyte chemoattractant protein-1 (MCP-1), which attracts monocytes to the inflammation site. Furthermore, activated monocytes generate the superoxide free radical production, which induces the oxidative damage of the low-density lipoprotein (LDL) particles. Oxidized LDL is then internalized by scavenger receptors expressed on the monocyte surface. Persistent loading of the monocyte/macrophage results in the formation of the foam cell, clusters of which collectively form the fatty streak, the initial macroscopic lesion of atherosclerosis [52-57] (Figure 2).

Recent work has elucidated the molecular mechanisms underlying inflammatory processes and the expression of pro-inflammatory molecules. Key proteins called

transcription factors bind to specific gene promoters to induce the transcription of mRNA from these target genes.[55]

Nuclear factor κB (NF-κB) is the cardinal pro-inflammatory transcription factor. It induces the transcription of pro-inflammatory cytokines like TNF-α and IL-6, chemokines like MCP-1 and IL-8, adhesion molecules like ICAM-1 and VCAM-1, and protein subunits of reactive oxygen species (ROS)–generating enzymes like NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase.[58-61]

Similarly, another transcription factor, activator protein-1 (AP-1), modulates the transcription of matrix metalloproteinases (MMPs) and adhesion molecules.[62] MMPs cause lysis of collagen and other matrix proteins, which form the fibrous cap of the atherosclerotic plaque resulting in plaque thinning and rupture. NF-κB and AP-1 are both redox sensitive and activated by ROS.[63-65]

A third transcription factor, early growth response-1 (Egr-1), modulates the transcription of tissue factor and plasminogen activator inhibitor (PAI)-1. Tissue factor activates the extrinsic pathway of coagulation, which results in the conversion of prothrombin to thrombin. Therefore, tissue factor modulates both platelet activation and coagulation. PAI-1 inhibits fibrinolysis and reduces thrombolysis following thrombus formation.[66]

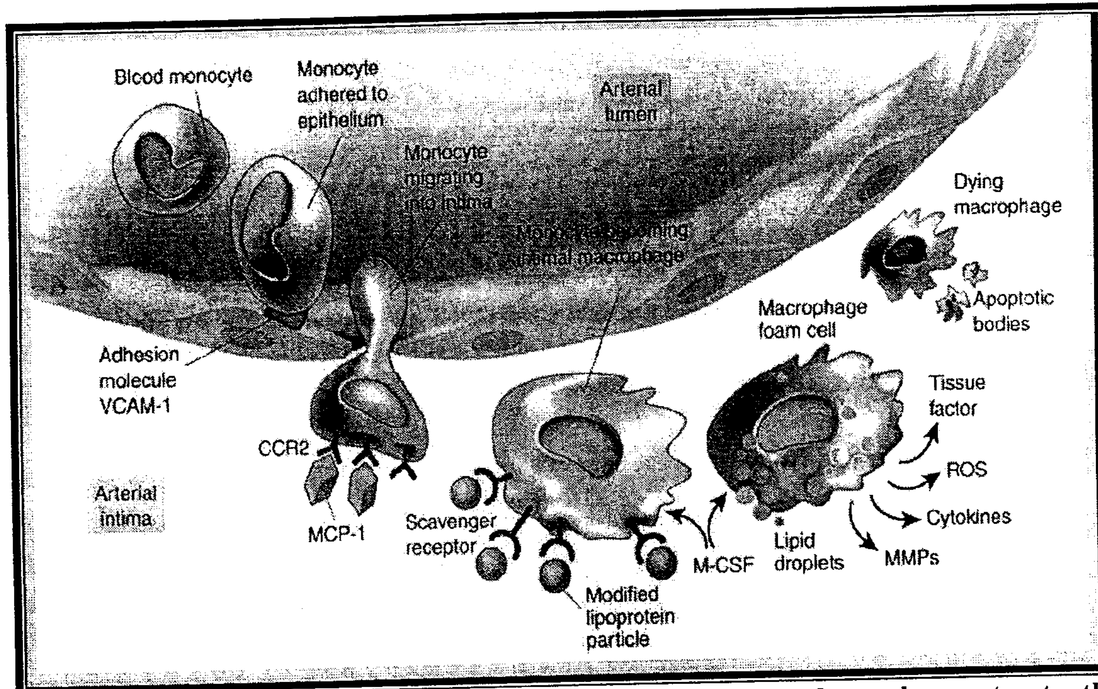


Figure 2: Schematic steps in the recruitment of mononuclear phagocytes to the nascent atherosclerotic plaque and some of the functions of these cells in the mature atheroma.[42]

(abbreviations: VCAM-1: Vascular Cell Adhesion Molecule-1, MCP-1: Monocyte Chemoattractant Protein-1, and its receptor CCR-2, M-CSF: Monocyte Colony Stimulating Factor, MMPs: Matrix Metalloproteinases, ROS: Reactive Oxygen Species)

Cell Adhesion molecules (CAMs) and their role in obesity induced atherosclerosis and insulin resistance

Definition of CAMs:

Cell adhesion molecules (CAMs) are transmembrane glycoproteins that are involved in the binding of cells, usually leukocytes, to each other, to endothelial cells or to extracellular matrix, thus mediating cell–cell and cell–matrix interactions.[67-69] Some interactions are relatively static, and maintain the three-dimensional integrity of an organism, while others, such as those involved in the recruitment of leucocytes to sites of inflammation, may be transient over very short periods of time.

General structure of CAMs:

Cell adhesion molecules share a basic structure of an extracellular component with binding domains that interact with other adhesion molecules expressed on cell surface or the extracellular matrix, a hydrophobic transmembrane component and an intracytoplasmic component, which mediates interactions with the cell cytoskeleton via intracellular signalling pathways[68,70] (figure 3).[71]

Classification of CAMs family:

Cell adhesion molecules may be classified into four families based on their structure and function. They include the selectins, integrins, immunoglobulin superfamily and the cadherins (Table IV).[67-69] The ones that have been implicated in the pathogenesis of atherosclerosis were intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), e-(endothelial) selectin and p-(platelet) selectin.[72]

Sites of CAMs expression:

Adhesion molecules are distributed on a wide range of cells.[67-69,72] Those that have been implicated in the process of atherogenesis are found largely on the endothelium and on circulating leukocytes and platelets.[67-69,73]CAMs are poorly expressed by the resting endothelium, but their expression is upregulated in activated endothelial cells.[74] In addition to being membrane-bound, adhesion molecules may also be shed from the cell surface and can be found in soluble forms in the plasma, however, little is known about the mechanism by which this occurs.[75]

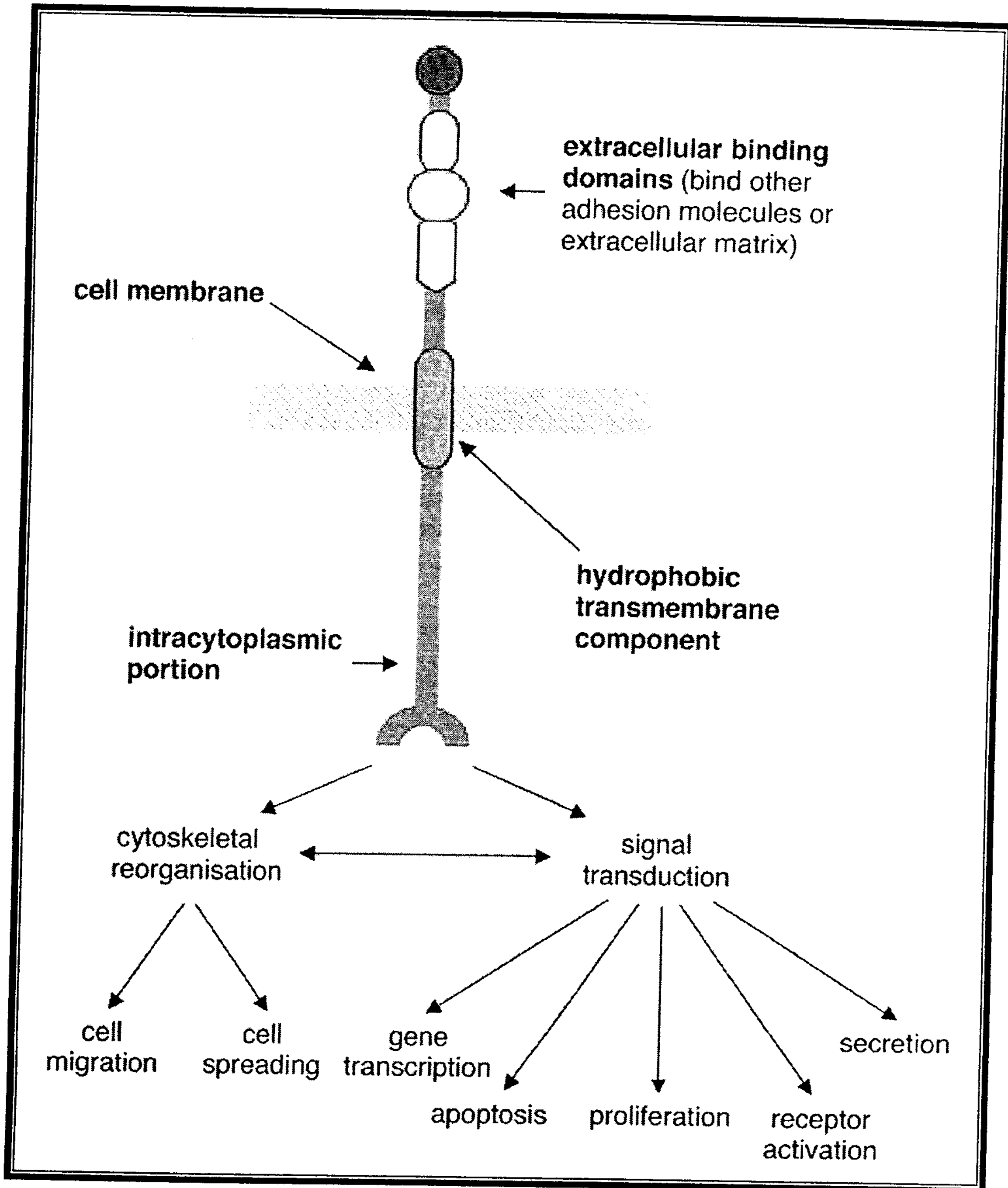


Figure 3: general structure of adhesion molecules.(71)

Regulators of CAMs expression:

Cytokines

Interleukin (IL)-1β and TNF-α have been implicated in the expression of P-& E-selectins as well as ICAM-1 and VCAM-1, mostly through Nuclear factor- Kappa Beta (NF-κB) mediated pathway. [67,76]

Nitric oxide

The protective effects of nitric oxide against endothelial CAMs expression are possibly mediated through effects on the NF-κB pathway, by maintaining NF-κB in an inactive form and thereby reducing the effect of cytokines in promoting adhesion molecule gene transcription.[67,76]

Biomechanical influences

Variations in flow conditions may cause changes in endothelial CAMs expression and therefore the propensity for the development of atherosclerotic lesions.[71]

Functions of CAMs:

With a critical role in many processes, including embryogenesis, wound healing, angiogenesis, immune response, inflammation, and malignancy, CAMs are central to both the maintenance of health and the development of disease.[77]

Role of CAMs in the atherogenic process:

Damage and inflammation result in an increased expression of CAMs that mediate leucocyte adhesion to the endothelium and subsequent migration across the endothelium into the intima.

The first stage of leucocyte recruitment to an inflammatory lesion involves tethering and rolling of the leucocyte to the endothelium. This is mediated largely by members of the selectin family of adhesion molecules, particularly P-and E-selectins, where the P-selectin binds monocytes weakly and transiently allowing them to roll slowly on the endothelium. Firm adhesion and transmigration of leucocytes across the endothelium follows rolling. The ICAM-1 binds the slowed monocytes firmly and also activates the monocyte through this binding, whereas VCAM-1 mediates the movement of the monocyte into the subendothelium through intercellular spaces in the endothelium created by matrix metalloproteinase (MMP) action. [71]

The endothelium overlying atherosclerotic lesions expresses P-selectin and the shoulder regions express VCAM-1 and ICAM-1. The latter is also expressed on endothelium in regions not prone to plaque development (Figure 4).[71]

Table IV: Classes of adhesion molecules of the selectin family, integrin family, and immunoglobulin superfamily, their distribution, and their functions.[71]

Adhesion molecule	Distribution	Function
Integrins		
GPIIb/IIIa	Platelets	Formation of platelet thrombus
VLA-4	Leucocytes (not neutrophils)	Binds VCAM-1
LFA-1	Leucocytes	Binds ICAM-1
Mac-1	Monocytes, granulocytes	Binds ICAM-1
Selectins		
L-selectin	Leucocytes	Leucocyte rolling
E-selectin	Endothelium	Leucocyte rolling
P-selectin	Platelets, endothelium	Platelet/endothelial interaction, leucocyte rolling
Immunoglobulin superfamily		
ICAM-1	Endothelium, leucocytes, fibroblasts, atherosclerotic lesion smooth muscle cells	Adherence and transmigration of leucocytes
VCAM-1	Endothelium, atherosclerotic lesion smooth muscle cells	Adherence of leucocytes
PECAM	Endothelium, leucocytes, platelets	Platelet/endothelial interaction, adherence and transmigration of leucocytes

ICAM-1, intercellular adhesion molecule-1; LFA, lymphocyte function-related antigen-1; PECAM, platelet-endothelial cell adhesion molecule; VCAM-1, vascular adhesion molecule-1; VLA-4, very late activation-4.

Abbreviations: Mac, macrophage; Gp, glycoprotein.

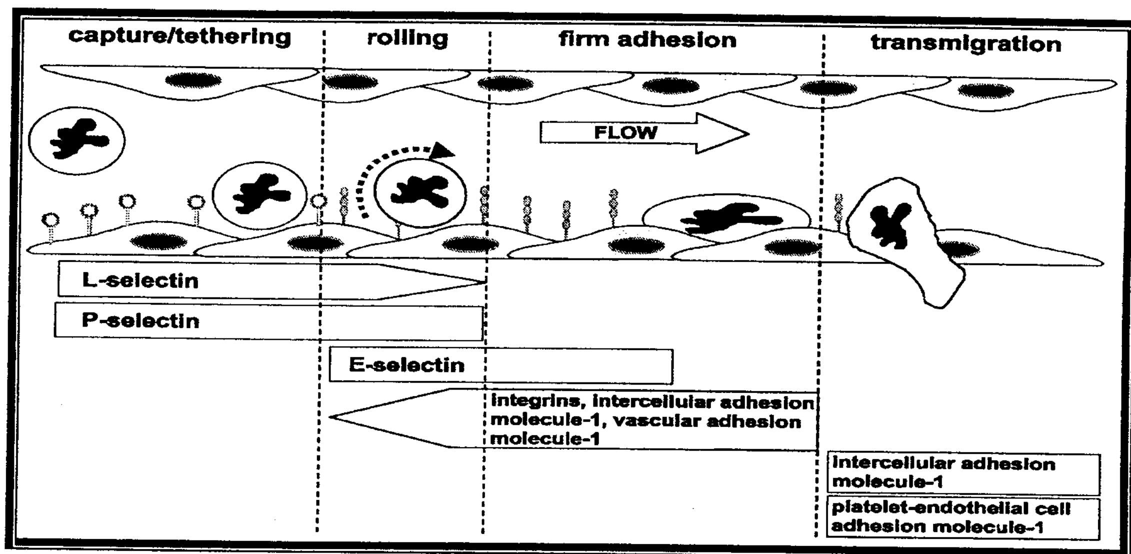


Figure (4): Schematic representation of leucocyte rolling, adhesion, and transmigration across the endothelium, as well as the cellular adhesion molecules involved in these processes.[71]

Choice of VCAM-1 as a candidate marker for inflammation:

The endothelial leukocyte adhesion molecule VCAM-1 has emerged as a particularly attractive candidate for the early adhesion of mononuclear leukocytes to arterial endothelium at sites of atheroma initiation. This was for the following reasons: [71]

1. Vascular cell adhesion molecule-1 (VCAM-1) binds particularly those classes of leukocytes found in nascent atheroma: the monocyte and the T lymphocyte.

2. Endothelial cells express VCAM-1 in response to cholesterol feeding selectively in areas prone to lesion formation.

3. VCAM-1 rises before leukocyte recruitment begins in both rabbit and mouse models of cholesterol-induced lesion formation.

Vascular cell adhesion molecule-1 (VCAM-1)

VCAM-1 structure:

VCAM-1 is 100-110 KDa calcium independent cell surface glycoprotein that belongs to the immunoglobulin supefamily (IgSF) which is the largest group of adhesion molecules. VCAM-1 consists of six extracellular immunoglobulin (Ig)-like domains (6D VCAM-1). The 6D VCAM-1 arises from alternative splicing of a seven domain form of VCAM-1 (7D VCAM-1), the form that is predominantly expressed on activated human endothelial cells.[78] The additional domain in 7D VCAM-1 may have a functional role in leukocyte adhesion to the endothelium. The Ig domain consists of 90 to 100 amino acids arranged as two sheets of β strands stabilized by a disulfide bond in its center.[79] The Ig domains are classified as V (variable) like or C (constant) likes on the basis of closest homology to either IgV or IgC domains. The V domains are formed from a longer polypeptide than C domain and contain an extra pair of β strands within the β-sheet sandwich.[80]

Sites and regulators of VCAM-1 expression:

VCAM-1 is expressed on the surface of activated endothelial cells (ECs), bone marrow fibroblasts, smooth muscles, perineural cells, tissue macrophages, dendritic cells, and myoblasts. The basal expression of VCAM-1 can be stimulated by some mediators such as interleukin IL-1 & 4, TNF-α and endotoxin but not by IFN-γ.[81] Endothelial expression of VCAM-1 in vivo is evident at sites of chronic inflammation and at atherosclerotic plaques.[82]

In vitro studies demonstrated that VCAM-1 expression peaks 6 to 10 hours after cytokine treatment and is sustained in some cases for as long as 72 hours.[81]

A soluble form of VCAM-1 (sVCAM-1) originates from proteolytic cleavage of the membrane bound molecule. It may serve as a marker of endothelial cell activation and inflammation.[83]

Mechanism of VCAM-1 induction and its action in inflammation:

The mechanism of VCAM-1 induction probably depends on inflammation triggered by modified lipoprotein particles accumulating in the arterial intima. Constituents of modified lipoprotein particles, among them certain oxidized phospholipids and short-chain aldehydes arising from lipoprotein oxidation, can induce transcriptional activation of the VCAM-1 gene mediated in part by nuclear factor-κB (NF-κB).[84] Pro-inflammatory cytokines, present in atherosclerotic lesions, such as interleukin (IL)-1β or tumour-necrosis factor-α (TNF-α) induce VCAM-1 expression in endothelial cells by this pathway. Thus, pro-inflammatory cytokines may link hypercholesterolaemia to VCAM-1 expression. The VCAM-1 expressed on activated ECs binds to the very late antigen-4 (VLA-4) of the integrin family that is expressed on the surface of T-lymphocytes, monocytes, and eosinophils, thus mediating adhesion and transmigration of leucocytes across the endothelium. [85]

Value of soluble CAMs in the atherogenic process:

Much of the data linking adhesion molecules to the pathogenesis of atherosclerosis in humans relates to the measurement of soluble adhesion molecules, although the relationship between plasma levels and cell surface activity is unclear and unpredictable.[75] Indeed, even the relationship between cellular adhesion molecule protein synthesis and cell surface activity may not be easily predicted, as the latter is also influenced by the rate of surface shedding, yet it has been suggested that elevated plasma levels of some CAMs may be an index of endothelial activation or even a molecular marker of early atherosclerosis. In fact, serum levels of soluble P-selectin, ICAM-1 and VCAM-1 were reported to be elevated in patients with angina pectoris or peripheral atherosclerotic disease. These data suggest that P-selectin, VLA-4 and VCAM-1 are the most important adhesion molecules involved in monocyte recruitment to atherosclerotic lesions.[86]

Thus, the increased release of adipo-cytokines (TNF-α and IL-6) from adipose tissue with the resulting activation of NF-KB pathway, will induce mild chronic inflammation and IR on one hand and an initial endothelial cell dysfunction (ECD) on the other hand, thus linking adiposity with insulin resistance and increased plasma levels of adhesion molecules. [87,88]

Methods of determination of CAMs:

Cellular adhesion molecules are measured by monoclonal antibody techniques. They may be measured:

1. On the surface of vascular cells, either in culture or in pathological specimens, using immunohistochemical techniques.[89]

2. On the surface of circulating leucocytes and platelets from whole blood samples, using fluorescin-labeled monoclonal antibodies and flow cytometry.[90,91]

3. In plasma, as soluble forms of adhesion molecules, by enzyme-linked immunosorbent assays.[92]

AIM OF THE WORK

AIM OF THE WORK

The study aims at evaluating plasma soluble vascular cell adhesion molecule-1 (sVCAM -1) in relation to insulin status in obese females.

SUBJECTS AND METHODS

SUBJECTS

Sixty subjects were included in the present study, divided as follows:

- Obese females group:

It consisted of forty five adult premenopausal females with varying degrees of obesity, defined by a body mass index (BMI) (kg/m^2) of more than 30.

- Control group:

It consisted of fifteen apparently healthy females of comparable age, and socioeconomic state to the obese group, with BMI(kg/m^2) less than 25, and free from diabetes mellitus, coronary heart disease, liver and renal diseases.

- Remark:

All the subjects involved in this study were free from any allergic condition, recent infectious or inflammatory diseases, malignancy as well as endocrinological disorders.

METHODS

To all the studied subjects, the following was done:

1. **Full clinical examination:** including:
   - Thorough history taking.
   - Complete physical examination: including the determination of:
     - Body mass index (BMI): which was calculated as follows:

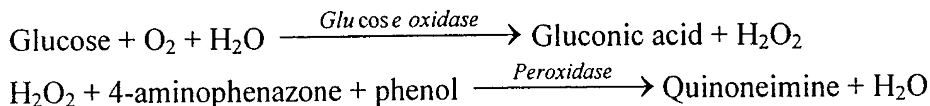
$$BMI = \frac{Body\ weight\ (Kg)}{Square\ of\ the\ body\ height\ (meters)}$$

     - Triceps skin fold thickness (TSF): which was measured using a skin caliper. [4]
     - Midarm, waist, and hip circumferences: were measured using a measuring tape and waist to hip ratio (WHR) was calculated. [4]
2. **Doppler echocardiographic examination of the carotid intima media thickness (CIMT):** using a B mode ultrasound.[93]
3. **Twelve leads standard electrocardiogram.**
4. **Laboratory investigations:**
   Following a twelve hours fasting period, five milliliters whole blood were obtained from each patient using heparin as an anticoagulant. After centrifugation, the plasma was immediately separated and divided into three aliquots, one for the determination of the concentrations and activities of the routine analytes, the second aliquot was used for insulin determination, while the last aliquot was stored in eppendorf tubes at – 20°C till the time of sVCAM-1 analysis. Two hours following meal, three milliliters whole blood sample were obtained from each individual for determination of plasma glucose.

The concentrations and activities of the following analytes were determined :

1- Plasma glucose concentration:[94]

Glucose was determined without deproteinization using an enzymatic method based on the following reaction:



$$Glucose + O_2 + H_2O \xrightarrow{Glucose\ oxidase} Gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4\text{-aminophenazone} + phenol \xrightarrow{Peroxidase} Quinoneimine + H_2O$$

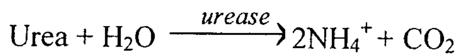
The oxidized rose coloured product, was proportionate to the concentration of glucose in the sample (T). It was read spectrophotometrically at wave length (λ) 546nm, and compared to a standard glucose solution (S) of a known concentration (Cs) similarly treated. Serum Glucose (C_T) was calculated as follows:

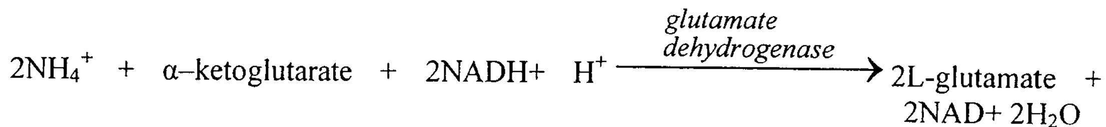
$$C_T\,(mg/dL) = \frac{A_T}{A_S} \times C_S\,(mg/dL)$$

$$\text{mmol glucose/L} = \text{mg/dL} \times 0.055$$

2-**Plasma urea concentration:**[95]

Urea was determined kinetically without deproteinization according to the following reaction:





After a 30 seconds delay, the mean decrease in sample (T) absorbance ($\Delta A/min$) due to $NADH+H^+$ oxidation was monitored spectrophotometrically at λ 340 nm for 60 seconds, and compared to a standard urea solution (S) of a known concentration (Cs) similarly treated. The concentration of urea in the sample was calculated as follows

$$\text{mg/dL urea} = \frac{\Delta A_T}{\Delta A_S} \times C_S\,(mg/dL)$$

$$\text{mmol/L urea} = \text{mg/dL urea} \times 0.166$$

3-**Plasma creatinine concentration:**[96]

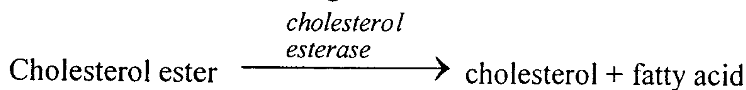
Creatinine was determined without deproteinization using Jaffé reaction in a kinetic manner. After a 20 seconds delay, the rate of increase in absorbance due to complex formation between creatinine in the sample (T) and alkaline picrate reagent was monitored kinetically over a period of 1 minute at λ 546 nm, and compared to a standard creatinine solution (S) of a known concentration (Cs) similarly treated. Creatinine concentration in the sample (C_T) was determined as follows:

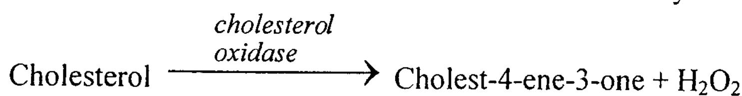
$$\text{mg/dL creatinine } (C_T) = \frac{\Delta A_T}{\Delta A_S} \times C_S\,(mg/dL)$$

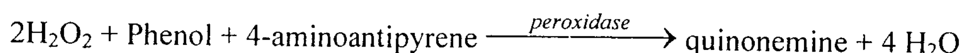
$$\text{mmol creatinine/L} = \text{mg/dl} \times 0.0884$$

4-**Plasma total cholesterol (TC) concentration:**[97]

Total cholesterol (TC) was determined enzymatically without deproteinization according to the following reactions:






$2H_2O_2$ + Phenol + 4-aminoantipyrene $\xrightarrow{peroxidase}$ quinonemine + 4 H_2O

The oxidized rose coloured chromogen product was proportionate to cholesterol concentration in the sample (T). It was measured at λ 546 nm, and compared to the colour of a standard of a known cholesterol concentration (mg/dL) similarly treated (S). The concentration of cholesterol was obtained by the following equation:

$$\text{mg cholesterol / dL} = {}^{A_T}\!/_{A_S} \times Cs \text{ (mg/dL)}$$

$$\text{mmol cholesterol / L} = \text{mg / dL} \times 0.026$$

5-**Plasma high density lipoprotein cholesterol (HDL-C)concentration:**[98]

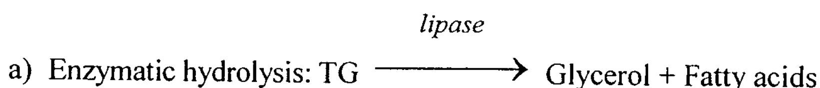
HDL-C was determined by precipitating Apo-B 100 containing lipoproteins (LDL and VLDL) with phosphotungestic acid in the presence of magnesium ions. Following centrifugation, the cholesterol concentration in the supernatant (HDL-C fraction) was determined enzymatically in the same manner as total cholesterol. The final result was multiplied by a factor of 3 to correct for the sample (1 part) to precipitating mixture (2 parts) ratio.

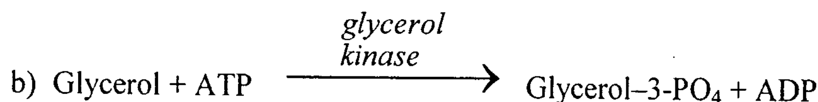
6-**Plasma low density lipoprotein cholesterol (LDL-C) concentration:**[99]

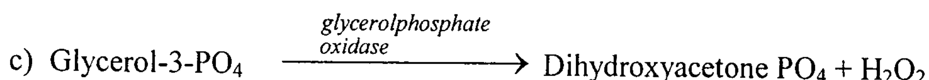
LDL-C was determined in the sample by precipitation with polyvinyl sulphate. The supernatant obtained by centrifugation (HDL-C and VLDL-C) was analysed enzymatically for cholesterol. LDL-C concentration was calculated from the difference between total cholesterol and cholesterol measured in the supernatant (HDL-C and VLDL-C). The final result was multiplied by a factor of 11 to correct for the sample (one part) to reagent (ten parts) ratio.

7-**Plasma triglycerides (TG) concentration:**[100]

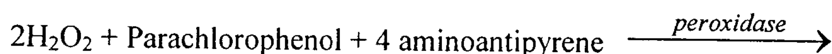
Triglycerides were determined enzymatically without deproteinization according to the following reactions:


a) Enzymatic hydrolysis: TG $\xrightarrow{lipase}$ Glycerol + Fatty acids


b) Glycerol + ATP $\xrightarrow{glycerol\ kinase}$ Glycerol–3-PO_4 + ADP


c) Glycerol-3-PO_4 $\xrightarrow{glycerolphosphate\ oxidase}$ Dihydroxyacetone PO_4 + H_2O_2

d) Colour development:


$2H_2O_2$ + Parachlorophenol + 4 aminoantipyrene $\xrightarrow{peroxidase}$

quoneimine (rose coloured) + 4 H_2O

The rose coloured chromogen, proportionate to TG concentration in the sample (T), was measured spectrophotometrically at λ 546 nm and compared to a standard of known TG concentration (mg/dL) similarly treated (S). TG level was obtained by the following equation:

$$\text{mg TG / dL} = \frac{A_T}{A_S} \times Cs \text{ (mg/dL)}$$

$$\text{mmol triglycerides / L} = \text{mg / dL x } 0.01145$$

8-**Plasma albumin**:(101)

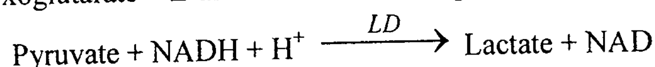
Albumin was determined using bromocresol green dye that gave a green coloured product with albumin. The resulting colour was read spectrophotometrically at λ 600 nm (T), and compared to a standard albumin solution (S) of a known concentration (Cs) similarly treated. The albumin concentration (C_T) was calculated as follows:

$$C_T \text{ (gm/dL)} = \frac{A_T}{A_S} \times Cs \text{ (gm/dL)}$$

$$\text{gm albumin/L} = \text{gm/dl} \times 10$$

9-**Plasma alanine aminotransferase (ALT) activity**:(102)

ALT activity was determined as follows:

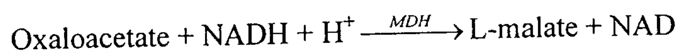


(LD: Lactate dehydrogenase)

The decrease in absorbance per minute (ΔA/min) at λ340 nm (due to NADH + H^+ oxidation) was monitored kinetically for 3 minutes. The enzyme activity – expressed in units/L – was calculated as a function of ΔA.

10-**Plasma aspartate aminotransferase (AST) activity**:(103)

AST activity was determined as follows:



(MDH = malate dehydrogenase)

The decrease in absorbance per minute (ΔA/min) at 340 nm (due to NADH + H^+oxidation) was monitored kinetically for 3 minutes. The enzyme activity – expressed in units/L – was calculated as a function of ΔA.

11- **Plasma insulin concentration**:(104)

Plasma insulin was measured using a two site, solid phase chemiluminescent enzyme immunometric assay (CLIA) by the Immulite 2000 Automated Analyzer (Diagnostic Products Corporation). The solid phase consisted of polystyrene beads, coated with a monoclonal murine anti-insulin antibody. Both the bead and the sample (or calibrator) was dispensed into a specially designed reaction tube.

(N.B: The reaction tube served as a vessel for incubation, wash, and signal development).

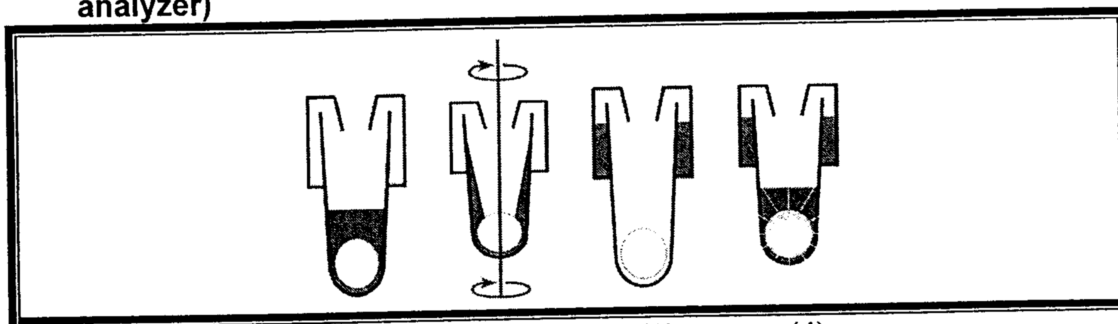
The sample (or calibrator) was incubated at 37°C with both the alkaline phosphatase conjugated to polyclonal sheep anti-insulin antibody and the monoclonal murine anti-insulin antibody in the reaction tube. The insulin present in the sample (or calibrator) bound to both anti-insulin antibodies forming a sandwich complex.

The reaction mixture was separated from the bead by spinning the reaction tube at a high speed along its vertical axis, followed by transferring the fluid (reaction mixture) to a coaxial sump chamber, which is integral to the bead / tube wash station.

Unbound conjugate in the reaction tube was removed using four discrete wash cycles within seconds, leaving the beads in the reaction tubes with no residual unbound label. A luminogenic substrate was then added to the reaction tube. The reaction tube was then transferred to the luminometer chain, where the light generated by the luminometric reaction was detected by the photomultiplier tube (PMT).

In the luminogenic reaction, the substrate (adamantyl dioxetane phosphate) was dephosphorylated into an unstable anion (unstable intermediate dioxetane) by the alkaline phosphatase conjugate captured on the bead. The unstable intermediary emitted photons upon decomposition, directly proportional to the amount of bound enzyme, and therefore directly proportional to the concentration of insulin in the plasma sample.

(Schematic representation of the insulin assay on the Immulite 2000 analyzer)



(1) (2) (3) (4)

(1) Sample (or calibrator), polystyrene coated bead, and alkaline phosphatase conjugate reagent were automatically pipetted into the reaction vessel, which was then incubated at 37°C with intermittent agitation.
(2) Following incubation, the reaction vessel was spun at high speed about its vertical axis. [Reaction fluid is forced up and completely captured in the sump chamber].
(3) A series of washes efficiently removed unbound material from the bead and inner tube.
(4) Chemiluminescent substrate was added to the reaction tube. Light emission was read with a high sensitivity photon counter.

Assessment of insulin status was done by homeostasis model assessment **(HOMA – IR) score :**[105]

$$\frac{\text{fasting plasma insulin } (\mu U/ml) \times \text{fasting plasma glucose } (mmol/l)}{22.5}$$

12- **Plasma soluble vascular cell adhesion molecule -1 (sVCAM-1)** :[106]

Principle:

Plasma sVCAM-1 was quantitatively determined using an enzyme linked immunosorbant assay (ELISA) from Bender Medsystems GmbH (serial number BMS232), (Vienna, Austria).

In this assay, the pretreated sample (or control serum), as well as the serially diluted standards, were incubated with a mixture of biotin conjugated monoclonal anti-sVCAM-1 antibody and streptavidin-HRP in microwells coated with anti-sVCAM-1 monoclonal antibodies. The sVCAM-1 in the sample, provided kit control and serially diluted standards were bound to the antibodies coating the microwells, and the biotinylated anti-sVCAM-1 was bound to the sVCAM-1 captured by the antibody coating the tube. The streptavidin-HRP was bound to the bound biotinylated sVCAM-1 antibody.

The unbound biotinylated anti-sVCAM-1 antibody and excess streptavidin-HRP were removed by a washing step, followed by the addition of TMB substrate (tetramethylbenzidine / hydrogen peroxide) resulting in the formation of a blue coloured product, directly proportional to the amount of sVCAM-1 present. The reaction was terminated by the addition of an acidic stop solution, and the final yellow colour was measured at λ 450 nm (reference 620 nm).

Specimen collection:

Heparinized plasma samples were used. The samples were stored at –20°C till the assay time. The frozen samples were brought to room temperature slowly, and mixed gently prior to the assay. No grossly haemolysed or lipaemic samples were used, and the samples were not subjected to multiple freeze thaw cycles.

Reagents:

1. **Microwell plate** coated with monoclonal murine antibody to human sVCAM-1.
2. **Assay buffer (5mL bottle – 20 X):** It was formed of PBS (Phophate buffered saline) with 1% tween 20 and 10% BSA (Bovine serum albumin). It was diluted with distilled water in a 1: 20 ratio.
3. **Biotin conjugate / streptavidin-HRP mixture (0.1 mL vial):** It was diluted with the diluted assay buffer in a 1:100 ratio. This was done in a clean disposable Wassermann plastic tube.
4. **Soluble VCAM-1 standard:** The lyophilized standard was reconstituted with 300µL distilled water, followed by swirling and gentle mixing to ensure complete and homogenous solubilization. The resulting concentration was 200 ng/mL. Serial dilutions were made in six clean disposable Wassermann plastic tubes

using the diluted assay buffer, in a 1:2 ratio, resulting in serial concentrations of 100, 50, 25, 12.5, 6.3, and 3.2 ng/mL respectively.

5. **Control:** The lyophilized control was reconstituted with 200µL distilled water, followed by swirling and gentle mixing to ensure complete and homogenous solubilization. It was further treated like a sample in the assay.
6. **TMB substrate solution:** it consisted of two vials (7 mL each) :
   -**Substrate solution I:** Tetramethyl-benzidine.
   -**Substrate solution II:** 0.02% buffered hydrogen peroxide.
   Equal volumes of substrate solutions I & II were dispensed in metal free clean disposable plastic tubes and containers, with gentle swirling to mix both. Both substrate solutions were brought to room temperature few minutes before the wash cycle, and the mixture was prepared immediately following the wash cycle.
7. **Stop solution (1M phosphoric acid, 12 mL):** ready to use.
8. **Wash buffer concentrate (20X – 50 mL bottle):** It consisted of PBS with 1% tween 20. It was diluted with distilled water in a 1: 20 manner using a clean graduated 1L glass cylinder.

Sample (and control) pretreatment :

A 1:50 dilution was done to all samples as well as the supplied reconstituted control, using the diluted assay buffer prior to the assay protocol. The final sVCAM-1 concentration was obtained after multiplying by the dilution factor.

Assay protocol :

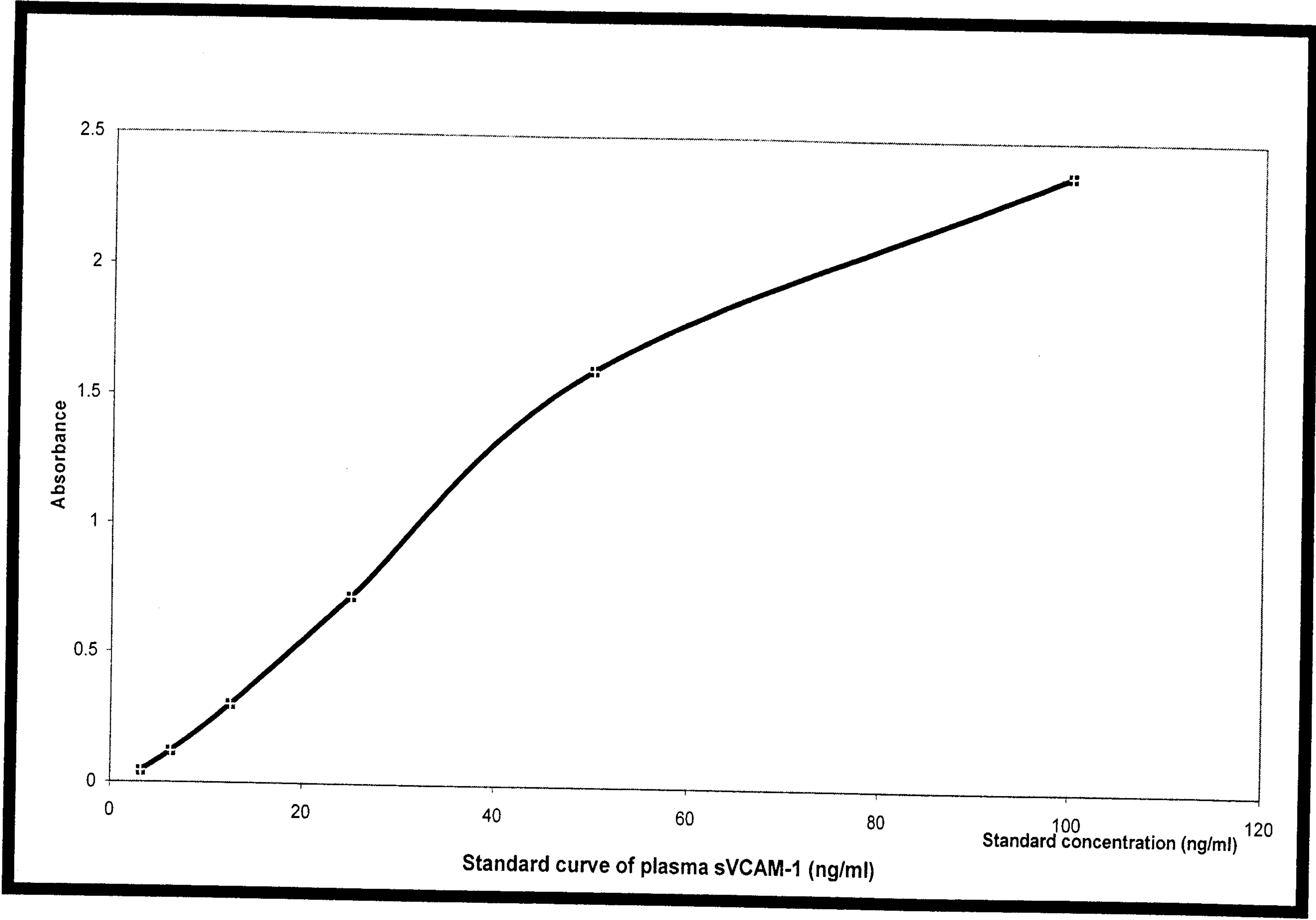
All reagents and samples were brought to room temperature before use. The reagents were gently mixed to avoid foaming.

1. The microwell strips were washed twice with 300 µL wash buffer per well with thorough aspiration of microwell contents between washes. This was done using an automatic microplate washer. Following the last wash the microwell strips were tapped on absorbent paper to remove excess wash buffer, and immediately used after that (wells were not left to dry as instructed).
2. 50 µL of diluted biotin conjugate / streptavidin HRP mixture were dispensed into each well, including the blank wells.
3. In duplicate, 100 µL of prediluted standards, and pretreated control were dispensed, each into its designated well, while 100 µL of assay buffer were dispensed in duplicate into the designated blank wells. 100 µL of pretreated samples were dispensed each into its designated well.
4. The plate was covered with the provided plate cover, incubated at room temperature for two hours on a rotator set at 100 rpm.
5. The plate cover was removed, and plate contents were aspirated. Another three wash cycle was performed (using an automatic microplate washer), followed by tapping on absorbent paper.
6. 100 µL of TMB substrate solution mixture were dispensed into each well, including the blank wells.
7. The microwell strips were incubated at room temperature for 20 minutes on a rotator set at 100 rpm, away from direct light.
8. 100 µL of stop solution were dispensed into each well, including the blank wells.

9. The absorbance of each microwell was read using an ELISA reader set at 450 nm (primary wavelength) and 620 nm (reference wavelength), where blanking of the plate reader was done by the use of the blank wells.

Calculation of results:

The concentrations of sVCAM-1 of both samples and the provided kit control were deduced from a manually plotted best fit standard curve. A linear scale was employed where the X-axis represented the standard concentration in ng/mL, while the Y axis represented the standard absorbance. The deduced results of both samples and the supplied control were multiplied by a factor of 50 in order to correct for the dilution made.



Standard curve of plasma sVCAM-1 (ng/ml)

STATISTICAL ANALYSIS[107]

Statistical analysis was done using the SPSS software package, to obtain the mean, standard deviation, and for comparison between the different groups involved in this study using student "t" test to compare between independent samples. Pearson correlation coefficient (r) was applied to test the hypothesis of linear relation between the studied variables. P<0.05 was considered statistically significant.

1- Arithmetic mean ($\overline{X}$) was calculated as follows:

$$\overline{X} = \frac{\sum X}{n}$$

Where;

$\overline{X}$ = Arithmetic mean

$\sum X$ = Sum of observations

n = number of observations

2- Standard deviation (S.D.) was calculated as follows:

$$S.D. = \sqrt{\frac{\sum (X - \overline{X})^2}{n-1}}$$

Where ;

n = number of cases

X = individual values

$\overline{X}$ = Arithmetic mean of the group

3- Standard error (SE):

$$S.E. = \frac{S.D}{\sqrt{n}}$$

4- Student t-test:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{(S.E_1)^2 + (S.E_2)^2}}$$

Where;

$\overline{X}_1$ = Arithmetic mean of the 1st group

$\overline{X}_2$ = Arithmetic mean of the 2nd group

$S.E_1$ = Standard error of the 1st group

$S.E_2$ = Standard error of the 2nd group

The probability "P" values of less than 0.05 are considered statistically significant, where degrees of freedom were taken as $(n_1 + n_2) - 2$ for student "t" test.

5- Coefficient of correlation:

A measure of the strength of the association between 2 variables is calculated by Pearson's product-moment coefficient of correlation "r".

This measure reports the strength of the relationship between dependent and independent variables. For two variables, "r" can have any value from -1.00 to + 1.00. The strength of the relationship is not dependent on the direction of the relationship. It is obtained by:

$$r = \frac{n(\Sigma XY) - (\Sigma X)(\Sigma Y)}{\sqrt{[n(\Sigma X^2) - (\Sigma X)^2][n(\Sigma Y^2) - (\Sigma Y)^2]}}$$

Where ;

n = The number of paired observations

$\sum X$ = The sum of the X variable

$\sum Y$ = The sum of the Y variable

$\sum X^2$ = The X – variable squared and the squares summed.

$(\sum X)^2$ = The X-variable summed and the sum squared.

$\sum Y^2$ = The Y-variable squared and the squares summed.

$(\sum Y)^2$ = The Y-variable summed and the sum squared.

RESULTS

RESULTS

1. Clinical findings:

Control group individuals were subjected to thorough clinical examination. They were clinically free with normal ECG findings.

- **Table (Va):** shows age (years), blood pressure (systolic, diastolic, and mean pressure) and carotid intima media thickness values (CIMT) (mm) in the control group.
- **Table (Vb):** shows age (years), blood pressure (systolic, diastolic, and mean pressure), ECG-findings and CIMT (mm) values in obese females group.
- **Table (Vc):** shows the mean, standard deviation and p-value of age (years), blood pressure (systolic, diastolic, and mean pressure), and CIMT (mm) between the two studied groups.

(P-value <0.05 was considered statistically significant).

Table (Va): Clinical data of the control group:

Case number	Age (years)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	CIMT (mm)
1	34	110	70	83.3	0.70
2	25	120	80	93.3	0.65
3	33	110	70	83.3	0.69
4	26	120	80	93.3	0.80
5	41	110	70	83.3	0.69
6	33	120	70	86.7	0.87
7	37	110	70	83.3	0.90
8	28	120	70	86.7	0.94
9	29	120	80	93.3	0.72
10	28	110	80	90	0.68
11	30	110	70	83.3	0.91
12	27	110	70	83.3	0.82
13	34	120	80	93.3	0.67
14	44	110	80	90	0.71
15	40	120	80	93.3	0.69
Mean	32.60	114.67	74.67	87.98	0.76
±SD	5.82	5.16	5.16	4.51	0.10

Table (Vb): Clinical data of obese females group:

Case number	Age (years)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	ECG Finding	CIMT (mm)
1	37	110	70	83.3	N	0.92
2	41	130	80	96.7	N	0.87
3	40	120	80	93.3	N	0.85
4	40	140	90	103.3	N	0.88
5	38	110	80	90.0	N	0.89
6	39	135	85	96.7	N	0.93
7	30	130	80	96.7	N	1.15
8	40	110	70	83.3	N	0.94
9	26	130	85	96.0	Low amp	0.88
10	44	110	80	90.0	N	0.95
11	42	120	80	93.3	N	0.79
12	40	110	80	90.0	Low amp	0.92
13	40	120	70	86.7	N	1.10
14	39	110	80	90.0	N	0.98
15	40	120	80	93.3	N	0.93
16	37	110	70	83.3	N	1.00
17	38	110	80	90.0	N	1.12
18	44	110	80	90.0	N	1.15
19	39	110	70	83.3	N	0.89
20	43	120	80	93.3	Low amp	0.82
21	45	110	70	83.3	N	0.97
22	36	120	80	93.3	Low amp	0.89
23	25	110	70	83.3	N	0.94

Amp = amplitude

Table (Vb) (Continued): Clinical data of obese females group

Case number	Age (years)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	ECG Finding	CIMT (mm)
24	27	110	80	90.0	N	0.93
25	38	125	80	93.3	Low amp	0.88
26	25	120	70	86.7	N	0.93
27	40	130	80	96.7	Low amp	0.92
28	41	110	70	83.3	N	0.97
29	44	140	90	103.3	N	1.23
30	40	130	80	96.7	N	0.94
31	44	110	70	83.3	N	1.24
32	45	130	85	96.0	N	0.97
33	38	120	80	93.3	N	0.88
34	36	130	80	96.7	N	0.87
35	34	110	80	90.0	N	0.94
36	33	110	80	90.0	N	1.04
37	40	140	95	103.3	N	0.87
38	33	120	70	86.7	N	0.96
39	32	110	80	90.0	N	0.99
40	30	110	70	83.3	low amp	0.73
41	33	120	80	93.3	N	0.89
42	25	110	80	90.0	Low amp	0.94
43	39	130	80	96.7	N	1.23
44	28	130	70	83.3	N	0.81
45	40	110	80	96.7	N	0.94
Mean	37.07	119.11	78.22	91.22	-	0.95
±SD	5.73	10.02	6.14	5.77	-	0.11
P	0.012	0.106	0.049	0.052	-	0.000

Table (Vc): The mean, S.D. and p-values of age, blood pressure (systolic, diastolic, and mean BP) and CIMT between the two studied groups.

Items \ Groups	Control group (n=15)	Obese group (n=45)	p-Value
Age (years)	32.60 ±5.82	37.07 ±5.73	0.012*
Systolic BP (mm Hg)	114.67 ±5.16	119.11 ±10.02	0.106
Diastolic BP (mm Hg)	74.67 ±5.16	78.22 ±6.14	0.049*
Mean BP (mm Hg)	87.98 ±4.51	91.22 ±5.77	0.052
CIMT (mm)	0.76 ±0.10	0.95 ±0.11	0.000*

P value <0.05 was considered statistically significant.

II. Anthropometric measurements in the studied groups (Table VIa, b and c)

1. Body weight(Kg):

- **Control group:** the body weight in the control group ranged from 50 to70 Kg with a mean of 62.07± 6.92 Kg.
- **Obese females group:** the body weight in this group ranged from 70 to 140 Kg with a mean of 97.48 ± 16.57 Kg, showing a statistically significant higher value than that of the control group (P = 0.000).

2. Height (meters):

- **Control group:** the height in the control group ranged from 1.53 to 1.8m with a mean of 1.65 ±0.08m.
- **Obese females group:** the height ranged from 1.50 to 1.85m with a mean of 1.63± 0.08m.

3. Body mass index (BMI)(Kg/m^2):

- **Control group:** BMI ranged from 20.0 to 25.0 Kg/m^2 with a mean of 22.73± 1.53 Kg/m^2.
- **Obese females group:** the BMI ranged from 30 to 51.9 Kg/m^2 with a mean of 36.724 ± 5.58 Kg/m^2, showing a statistically significant higher value than that of the control group (p = 0.000).

4. Triceps skin folds (TSF) (mm):

- **Control group:** TSF ranged from 25 to 40mm with a mean of 27.2 ±3.65mm.
- **Obese females group:** TSF level ranged from 29 to 50mm with a mean of 37.24 ± 4.10 mm, showing a statistically significant higher value than that of the control group (P =0.000).

5. Mid arm circumference (MAC) (cm):

- **Control group:** MAC ranged from 23 to 30 cm with a mean of 25.33 ± 2.13 cm.
- **Obese females group:** MAC ranged from 27 to 48 cm with a mean of 35.82 ± 5.43 cm. showing a statistically significant higher value than that of the control group (P =0.000).

6. Waist circumference (WC) (cm):

- **Control group:** WC ranged from 69 to 86 cm with a mean of 73 ±4.47 cm.

- **Obese females group:** WC ranged from 91 to 138 cm with a mean of 113.47 ± 11.51 cm. showing a statistically significant higher value than that of the control group (P =0.000).

7. Hip circumference (HC) (cm):

- **Control group:** HC ranged from 88 to 108 cm with a mean of 91.93 ±5.28 cm

- **Obese females group:** HC ranged from 108 to 151 cm with a mean of 122.60 ± 11.33 cm. Showing a statistically significant higher value than that of the control group (P =0.000).

8. Waist to hip ratio (WHR):

- **Control group:** WHR ranged from 0.78 to 0.80 with a mean of 0.79 ± 0.01.

- **Obese females group:** WHR ranged from 0.84 to 1.01 with a mean of 0.92 ± 0.04. Showing a statistically significant higher value than that of the control group (P =0.000).

Table (VIa): Anthropometric measurements in the control group:

Case number	Weight (Kg)	Height (m)	BMI (Kg/m^2)	TSF (mm)	MAC (cm)	WC (cm)	HC (cm)	WHR
1	55	1.53	23.5	27	26	76	90	0.79
2	50	1.58	20.4	26	25	69	88	0.78
3	69	1.70	24.0	28	28	75	95	0.79
4	70	1.65	25.0	40	30	86	108	0.80
5	66	1.70	22.8	25	23	70	88	0.80
6	59	1.60	23.4	27	26	72	91	0.79
7	65	1.80	20.3	26	26	71	90	0.79
8	60	1.66	21.8	25	24	70	88	0.79
9	64	1.70	22.0	26	23	69	88	0.78
10	50	1.58	20.0	26	25	69	89	0.78
11	66	1.70	22.8	25	23	72	91	0.79
12	55	1.53	23.5	27	26	76	95	0.80
13	63	1.62	24.2	27	28	75	95	0.79
14	70	1.73	23.1	27	24	70	88	0.80
15	69	1.69	24.1	26	23	75	95	0.79
Mean	62.07	1.65	22.73	27.20	25.33	73.00	91.93	0.79
±SD	6.92	0.08	1.53	3.65	2.13	4.47	5.28	0.007

Table (VIb): Anthropometric measurements in the obese females group.

Case number	Weight (Kg)	Height (m)	BMI Kg/m^2	TSF (mm)	MAC (cm)	WC (cm)	HC (cm)	WHR
1	90	1.65	33.1	33	33	99	112	0.88
2	100	1.60	39.1	37	36	131	146	0.90
3	105	1.64	39.0	40	43	125	131	0.95
4	100	1.68	35.4	40	42	103	119	0.87
5	124	1.60	47.6	40	42	138	150	0.92
6	92	1.64	34.2	41	33	114	115	0.99
7	85	1.56	34.9	29	27	104	111	0.94
8	125	1.60	47.6	40	41	137	151	0.92
9	90	1.63	33.3	34	32	113	116	0.98
10	104	1.50	46.2	39	35	127	133	0.95
11	128	1.57	51.9	40	39	130	137	0.94
12	86	1.56	35.3	36	37	104	110	0.95
13	80	1.57	32.5	30	28	103	113	0.91
14	95	1.62	36.2	41	32	113	128	0.90
15	91	1.73	30.4	36	31	112	120	0.93
16	88	1.65	32.3	30	28	103	113	0.91
17	85	1.58	34.1	30	28	105	110	0.93
18	74	1.50	33.6	38	29	103	108	0.94
19	110	1.80	34.4	36	37	113	120	0.90
20	120	1.61	46.3	50	48	113	120	0.94
21	98	1.58	39.2	41	38	102	113	0.90
22	130	1.85	38.2	40	43	125	118	1.0
23	90	1.62	34.3	36	37	113	120	0.90
24	95	1.73	31.7	36	33	114	125	0.95
25	125	1.64	46.5	39	46	123	131	0.94

Table (VIb) (Continued): Anthropometric measurements in the obese females group

Case number	Weight (Kg)	Height (m)	BMI (Kg/m^2)	TSF (mm)	MAC (cm)	WC (cm)	HC (cm)	WHR
26	70	1.53	30.4	35	35	100	118	0.85
27	105	1.64	39.0	40	43	125	131	0.95
28	95	1.55	39.6	42	36	102	113	0.90
29	99	1.73	33.0	37	34	114	123	0.93
30	84	1.64	31.2	35	35	100	118	0.85
31	93	1.61	35.9	39	30	116	115	1.01
32	80	1.61	30.8	38	30	113	122	0.93
33	90	1.52	39.0	34	36	114	125	0.95
34	90	1.65	33.1	31	31	99	112	0.88
35	94	1.67	34.0	41	33	113	114	0.99
36	105	1.64	39.0	40	43	125	131	0.95
37	75	1.58	30.0	36	31	112	120	0.93
38	95	1.73	31.7	35	35	114	123	0.92
39	105	1.65	38.6	41	42	123	135	0.91
40	121	1.62	46.2	38	45	124	132	0.93
41	75	1.58	30.0	31	30	91	108	0.84
42	140	1.85	40.9	39	42	123	134	0.91
43	93	1.61	35.9	37	33	102	113	0.90
44	75	1.58	30.0	33	34	97	114	0.88
45	93	1.58	37.0	42	36	132	146	0.90
Mean	97.48	1.63	36.72	37.24	35.82	113.47	122.60	0.92
±SD	16.57	0.08	5.58	4.10	5.43	11.51	11.33	0.04
p	0.000	0.329	0.000	0.000	0.000	0.000	0.000	0.000

($p<0.05$ was considered statistically significant).

Table (VIc): The mean, S.D. and p-values of weight, height, BMI, MAC, WC, HC, WHR, and TSF between the two studied groups:

Items \ Groups	Control group (n=15)	Obese group (n=45)	p-Value
Weight (kg)	62.07 ±6.92	97.48 ±16.57	0.000*
Height (m)	1.65 ±0.08	1.63 ±0.08	0.329
BMI (Kg/m^2)	22.73 ±1.53	36.72 ±5.58	0.000*
MAC (cm)	25.33 ±2.13	35.82 ±5.43	0.000*
WC (cm)	73.00 ±4.47	113.47 ±11.51	0.000*
HC (cm)	91.93 ±5.28	122.60 ±11.33	0.000*
WHR	0.79 ±0.01	0.92 ±0.04	0.000*
TSF (mm)	27.20 ±3.65	37.24 ±4.10	0.000*

(* denotes a p-value <0.05 that was considered statistically significant)

III- Laboratory investigations:

❖ **Fasting Plasma levels of glucose (FPG), urea, creatinine (Cr), albumin, alanine aminotransferase activity (ALT activity), aspartate aminotransferase activity (AST activity) as well as post-prandial plasma glucose (PPG).**

1. Glucose:
Fasting plasma glucose (FPG) (Table VIIa, b and c):

- **Control group:** The fasting plasma glucose ranged from 70 to 99 mg/dl with a mean value of 86.73 ± 8.33 mg/dl.

- **Obese females group:** FPG ranged from 78 to 113 mg/dl with a mean value of 93.36 ± 9.93 mg/dl, showing a statistically significant higher value than that of the control group (P = 0.024)

- **Post prandial plasma glucose level (PPG) (Table VIIa, b and c):**

- **Control group:** The post prandial plasma glucose level ranged from 82 to 102 mg/dl with a mean value of 94.07 ± 6.41 mg/dl.

- **Obese females group:** PPG ranged from 89 to 120 mg/dl with a mean value of 105.00±7.60 mg/dl. Showing a statistically significant higher value than that of the control group (P = 0.000).

2. Urea (Table VII a, b and c):

- **Control group:** Urea level ranged from 13 mg/dl to 27 mg/dl with a mean value of 17.93 ±5.26 mg/dl.

- **Obese females group:** Urea level ranged from 16 mg/dl to 38 mg/dl with a mean value of 23.00 ± 6.45 mg/dl, showing a statistically significant higher value than that of the control group (P = 0.008).

3. Creatinine (Table VII a, b and c):

- **Control group:** The creatinine level ranged from 0.70 to 1.00 with a mean value of 0.85 ±0.09 mg/dl.

- **Obese females group:** creatinine ranged from 0.70 to 1.20 mg/dl with a mean value of 0.99 ± 0.14 mg/dl, showing a statistically significant higher value than that of the control group (P = 0.000).

4. Alanine aminotransferase (ALT) activity(Table VII a, b and c):

- **Control group:** ALT activity ranged from 10 to 23 U/L with a mean value of 17.20 ± 4.31 U/L.

- **Obese females group:** ALT activity ranged from 10 to 38 U/L with a mean value of 19.76 ± 8.29 U/L

5. Aspartate aminotransferase (AST) activity (Table VII a, b and c):

- **Control group:** AST activity ranged from 10 to 24 U/L with a mean value of 16.60 ± 4.24 U/L

- **Obese females group:** AST activity ranged from 11 to 30U/L with a mean value of 18.67±5.02 U/L.

6. Albumin (Table VII a, b and c):

- **Control group:** Albumin level ranged from 3.8 to 4.4 g/dl with a mean value of 4.05 ±0.16 g/dl

- **Obese females group:** Albumin level ranged from 3.7 to 4.6 g/dl with a mean value of 4.10 ± 0.24 g/dl

Table (VIIa): Fasting Plasma levels of glucose (FPG), urea, creatinine (Cr), alanine aminotransferase activity (ALT activity) aspartate aminotransferase activity (AST activity), and albumin as well as post-prandial plasma glucose (PPG) in the control group.

Case number	FPG (mg/dl)	PPG (mg/dl)	Urea (mg/dl)	Cr (mg/dl)	ALT (U/L)	AST (U/L)	Albumin (g/dl)
1	82	90	18	0.8	13	12	4.1
2	70	82	17	1.0	13	14	4.1
3	73	86	14	0.8	14	13	3.8
4	84	90	19	0.9	23	20	4.0
5	91	102	27	0.9	22	24	4.1
6	99	100	13	0.8	20	22	4.2
7	91	101	13	0.7	10	13	4.1
8	98	100	13	0.8	12	10	4.0
9	84	90	19	0.9	23	12	4.0
10	90	95	13	0.7	18	20	4.2
11	91	102	27	0.9	20	18	4.1
12	82	90	18	0.8	13	15	3.9
13	87	100	27	1.0	18	21	3.8
14	96	90	18	0.8	20	18	3.9
15	83	93	13	0.9	19	17	4.4
Mean	86.73	94.07	17.93	0.85	17.20	16.60	4.05
±SD	8.33	6.41	5.26	0.09	4.31	4.24	0.16

Table (VIIb): Fasting Plasma levels of glucose (FPG), urea, creatinine (Cr), alanine aminotransferase activity (ALT activity), aspartate aminotransferase activity (AST activity) and albumin as well as post-prandial plasma glucose (PPG) in the obese females group.

Case number	FPG (mg/dl)	PPG (mg/dl)	Urea (mg/dl)	Cr (mg/dl)	ALT (U/L)	AST (U/L)	Albumin (g/dl)
1	110	120	34	0.9	12	14	3.9
2	93	98	16	0.9	21	19	4.2
3	110	117	19	0.8	15	18	4.3
4	100	106	21	0.9	35	22	4.5
5	102	112	26	0.9	12	16	4.1
6	102	100	30	1.2	30	27	4.3
7	83	110	26	1.0	18	13	4.5
8	80	105	20	0.9	25	19	3.7
9	83	109	19	0.8	14	16	4.2
10	100	101	18	0.8	24	18	4.2
11	82	89	34	1.1	15	20	4.1
12	83	89	21	0.9	18	22	4.0
13	97	109	38	1.2	20	19	3.9
14	102	106	28	0.8	19	18	4.1
15	90	109	34	1.1	37	29	3.8
16	90	113	16	0.9	15	18	3.8
17	80	109	17	1.1	22	20	4.1
18	97	100	21	0.9	38	29	4.6
19	87	107	17	0.9	21	19	3.7
20	92	100	24	1.1	25	22	3.8
21	88	97	19	1.1	27	20	4.0

Table (VIIb) (Continued): Fasting Plasma levels of glucose (FPG), urea, creatinine (Cr), alanine aminotransferase activity (ALT activity), aspartate aminotransferase activity (AST activity), and albumin as well as post-prandial plasma glucose (PPG) in the obese females group.

Case number	FPG (mg/dl)	PPG (mg/dl)	Urea (mg/dl)	Cr (mg/dl)	ALT (U/L)	AST (U/L)	Albumin (g/dl)
22	80	95	20	1.1	30	25	4.2
23	110	120	20	0.9	15	14	3.9
24	81	100	19	1.0	10	13	4.1
25	78	97	25	0.9	13	12	3.7
26	84	99	19	1.0	18	15	3.8
27	97	100	25	0.9	34	30	4.2
28	100	110	15	0.9	21	18	4.3
29	102	104	16	1.0	13	17	4.2
30	91	105	25	1.2	10	13	4.2
31	94	108	26	1.1	14	15	3.7
32	105	108	25	1.1	12	11	4.3
33	92	105	18	1.2	16	18	4.1
34	104	107	28	1.2	10	16	3.9
35	79	91	38	1.2	16	19	3.8
36	97	100	25	0.8	27	22	4.1
37	83	99	36	1.2	15	18	4.2
38	95	107	16	0.9	36	27	4.3
39	87	111	18	1.1	15	12	4.2

Table (VIIb) (Continued): fasting Plasma levels of glucose (FPG), urea, creatinine (Cr), alanine aminotransferase activity (ALT activity), aspartate aminotransferase activity (AST activity), and albumin as well as post-prandial plasma glucose (PPG) in the obese females group.

Case number	FPG (mg/dl)	PPG (mg/dl)	Urea (mg/dl)	Cr (mg/dl)	ALT (U/L)	AST (U/L)	Albumin (g/dl)
40	83	105	25	1.0	13	16	4.6
41	94	107	28	1.0	35	29	4.2
42	113	120	19	1.1	15	18	4.2
43	97	103	13	1.0	10	11	4.1
44	112	118	19	0.9	18	19	4.4
45	92	100	19	0.7	10	14	4.1
Mean	93.36	105.00	23.00	0.99	19.76	18.67	4.10
±SD	9.93	7.60	6.45	0.14	8.285	5.02	0.24
p-value	0.024	0.000	0.008	0.000	0.259	0.130	0.404

P<0.05 was considered statistically significant.

Table (VIIc): The mean, S.D., and p-value of FPG(mg/dl), PPG(mg/dl), Urea(mg/dl), Creatinine(mg/dl), ALT activity(U/L), AST activity(U/L) and Albumin(g/dl) between the two studied groups.

Items \ Groups	Control group (n=15)	Obese group (n=45)	p-Value
FPG (mg/dl)	86.73 ±8.33	93.36 ±9.93	0.024*
PPG (mg/dl)	94.07 ±6.41	105.00 ±7.60	0.000*
Urea (mg/dl)	17.93 ±5.26	23.00 ±6.45	0.008*
Creatinine (mg/dl)	0.85 ±0.09	0.99 ±0.14	0.000*
ALT (U/L)	17.20 ±4.31	19.76 ±8.29	0.259
AST (U/L)	16.60 ±4.24	18.67 ±5.02	0.130
Albumin (gm/dl)	4.05 ±0.16	4.10 ±0.24	0.404

(* denotes a p-value <0.05 that was considered statistically significant)

❖ Fasting Plasma levels of total cholesterol (chol), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (T.G):

1. Total cholesterol (Table VIII a, b and c)

- **Control group:** cholesterol level ranged from 140 to 170 mg/dl with a mean value of 154.13 ±12.01 mg/dl.

- **Obese females group:** cholesterol level ranged from 140 to 265 mg/dl with a mean value of 185.87 ±31.93 mg/dl, Showing a statistically significant higher mean value than that of the control group (p = 0.000).

2. High density lipoprotein cholesterol (HDL-C) (Table VIII a, b and c):

- **Control group:** HDL-C ranged from 39 to 55 mg/dl with a mean value of 44.73 ±5.09 mg/dl.

- **Obese females group:** HDL-C level ranged from 27 to 64 mg/dl with a mean value of 41.73 ±7.14 mg/dl.

3. Low density lipoprotein cholesterol (LDL-C) (Table VIII a, b and c):

- **Control group:** LDL-C level ranged from 80 to 110 mg/dl with a mean value of 93.35 ± 12.11 mg/dl.

- **Obese females group:** LDL-C level ranged from 60 to 204 mg/dl with a mean value of 121.10 ± 30.03 mg/dl, showing a statistically significant higher mean value than that of the control group (p = 0.001).

4. Triglycerides (TG) (Table VIII a, b and c):

- **Control group:** TG level ranged from 55 to 116 mg/dl with a mean value of 80.33 ± 20.90 mg/dl.

- **Obese females group:** TG level ranged from 60 to 247 mg/dl with a mean value of 114.80 ± 46.14 mg/dl, showing a statistically significant higher mean value than that of the control group (p = 0.007).

Table VIIIa: fasting Plasma levels of total cholesterol (chol), high-density lipoprotein cholesterol (HDL-C), low -density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in the control group.

Case number	Chol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	T.G (mg/dl)
1	141	49	80	60
2	160	39	107	69
3	142	50	81	55
4	143	47	84	62
5	170	39	109	110
6	143	39	81	116
7	150	44	92	70
8	140	43	85	60
9	143	42	83	90
10	170	40	108	110
11	160	47	93	100
12	169	49	104	80
13	161	39	110	60
14	150	55	80	73
15	170	49	103	90
Mean	154.13	44.73	93.35	80.33
±SD	12.01	5.09	12.11	20.90

Table VIIIb: fasting Plasma levels of total cholesterol (chol.), high-density lipoprotein cholesterol (HDL-C), low -density lipoprotein cholesterol (LDL-C) and triglycerides (T.G) in the obese females group.

Case number	Chol. (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	T.G (mg/dl)
1	241	37	163	207
2	152	41	99	60
3	191	46	124	104
4	140	64	60	81
5	175	41	87	235
6	209	41	140	140
7	232	36	164	158
8	154	45	97	62
9	140	39	88	64
10	160	38	102	100
11	265	40	204	105
12	169	38	93	192
13	249	51	181	85
14	204	46	134	122
15	157	37	91	144
16	150	27	105	90
17	191	51	104	178

Table (VIIIb) (Continued): fasting Plasma levels of total cholesterol (chol.), high-density lipoprotein cholesterol (HDL-C), low -density lipoprotein cholesterol (LDL-C) and triglycerides (T.G) in the obese females group.

Case number	Chol. (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	T.G (mg/dl)
18	198	39	139	100
19	195	35	138	109
20	218	45	142	155
21	183	51	114	89
22	174	39	108	133
23	180	39	117	118
24	142	45	79	90
25	169	42	111	80
26	164	41	109	68
27	160	52	88	98
28	211	41	147	117
29	221	40	132	247
30	168	37	112	97
31	200	34	135	157
32	180	35	124	105
33	178	46	115	83
34	151	43	93	77
35	175	42	117	78

Table (VIIIb) (Continued): fasting Plasma levels of total cholesterol (chol.), high-density lipoprotein cholesterol (HDL-C), low –density lipoprotein cholesterol (LDL-C) and triglycerides (T.G) in the obese females group.

Case number	Chol. (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	T.G (mg/dl)
36	160	52	88	98
37	158	31	115	61
38	175	31	126	89
39	197	34	143	99
40	214	41	154	93
41	156	41	100	74
42	185	38	109	188
43	220	54	146	98
44	191	55	120	78
45	262	37	193	160
Mean	185.87	41.73	121.10	114.80
±SD	31.93	7.14	30.03	46.14
P	0.000	0.139	0.001	0.007

$p<0.05$ was considered statistically significant.

Table (VIIIc): the mean, S.D. and P value of fasting Plasma levels of total cholesterol (chol.), high-density lipoprotein cholesterol (HDL-C), low -density lipoprotein cholesterol (LDL-C) and triglycerides (T.G) between the two studied groups.

Items \ Groups	Control group (n=15)	Obese group (n=45)	p-Value
Total cholesterol (mg/dl)	154.13 ±12.01	185.87 ±31.93	0.000*
HDL-Cholesterol (mg/dl)	44.73 ±5.09	41.73 ±7.14	0.139
LDL-Cholesterol (mg/dl)	93.35 ±12.11	121.10 ±30.03	0.001*
Triglycerides (mg/dl)	80.33 ±20.90	114.80 ±46.14	0.007*

* denotes a p-value <0.05 which was considered statistically significant.

❖ Fasting plasma glucose (mmol/L), insulin (μIU/mL), and HOMA-IR.

1. Fasting Plasma insulin (FPI) (Table IXa, b and c):

- **Control group:** FPI ranged from 3.01 to 9.01μIU/ml with a mean value of 5.29 ± 1.65μIU/ml.
- **Obese females group:** FPI ranged from 8.91 to 41.30 μIU/ml. with a mean value of 22.87 ± 11.40μIU/ml, showing a statistically significant higher value than that of the control group (p = 0.000), but still within the reference interval for FPI

2. HOMA-IR of the studied groups (Table IXa, b and c):

- **Control group**: HOMA-IR values ranged from 0.52 to 1.81 with a mean value of 1.13 ± 0.35
- **Obese females group:** HOMA values ranged from 1.83 to 10.94 with a mean value of 5.32±2.84, showing a statistically significant higher mean value than that of the control group (p = 0.000).

Table (IXa): Fasting plasma levels of glucose (mmol/L), insulin (μIU/mL), and HOMA-IR in the control group.

Case number	FPG (mmol/l)	Insulin (μIU/ml)	HOMA-IR value
1	4.56	4.63	0.94
2	3.89	3.01	0.52
3	4.06	9.01	1.62
4	4.67	4.52	0.94
5	5.06	8.06	1.81
6	5.50	5.21	1.27
7	5.06	6.31	1.42
8	5.44	5.99	1.45
9	4.67	3.07	0.64
10	4.10	4.31	0.96
11	5.06	4.62	1.04
12	4.56	5.01	1.01
13	4.83	5.03	1.08
14	5.33	4.20	0.10
15	4.61	6.33	1.30
Mean	4.76	5.29	1.13
± SD	0.49	1.65	0.35

Table (IXb): Fasting plasma levels of glucose (mmol/L), insulin (µIU/mL), and HOMA-IR value in the obese females group.

Case number	FPG (mmol/L)	Insulin (µIU/ml)	HOMA-IR
1	6.11	40.10	10.89
2	5.17	41.30	9.48
3	6.11	33.10	8.99
4	5.56	12.80	3.16
5	5.67	39.30	9.90
6	5.67	18.80	4.73
7	4.61	18.20	3.73
8	4.44	25.60	5.06
9	4.61	8.91	1.83
10	5.56	38.20	9.43
11	4.56	26.30	5.33
12	4.61	11.30	2.32
13	5.39	19.20	4.60
14	5.67	9.04	2.28
15	4.10	29.10	6.47
16	4.10	25.20	5.60
17	4.44	10.60	2.09
18	5.39	9.02	2.16

Table (IXb) (Continued): Fasting plasma levels of glucose (mmol/L), insulin (µIU/mL), and HOMA-IR value in the obese females group.

Case number	FPG (mmol/L)	Insulin (µIU/ml)	HOMA-IR
19	4.83	35.90	7.71
20	5.11	31.10	7.07
21	4.89	15.99	3.47
22	4.44	16.30	3.22
23	6.11	19.90	5.41
24	4.50	9.23	1.85
25	4.33	35.01	6.74
26	4.67	40.70	8.44
27	5.39	9.01	2.16
28	5.56	16.60	4.10
29	5.67	18.30	4.61
30	5.06	28.60	6.43
31	5.22	18.50	4.29
32	5.83	9.60	2.49
33	5.11	26.03	5.91
34	5.78	10.40	2.67
35	4.39	40.10	7.82
36	5.39	39.40	9.44
37	4.61	9.51	1.95

Table (IXb) (Continued): Fasting plasma levels of glucose (mmol/L), insulin (μIU/mL), and HOMA-IR value in the obese females group.

Case number	FPG(mmol/L)	Insulin (μIU/ml)	HOMA-IR
38	5.28	12.40	2.91
39	4.83	14.40	3.09
40	4.61	15.28	3.13
41	5.22	10.20	2.37
42	6.28	39.20	10.94
43	5.39	34.30	8.22
44	6.22	37.10	10.26
45	5.11	20.07	4.56
Mean	5.15	22.87	5.32
SD	0.60	11.40	2.84
P	0.024	0.000	0.000

$P<0.05$ was considered statistically significant.

Table (IXc): The mean, S.D. and p-value of fasting plasma levels of glucose, insulin, HOMA-IR between the two studied groups.

Groups / Items	Control group (n=15)	Obese females (n=45)	p-Value
Insulin (μIU/ml)	5.29 ±1.65	22.87 ±11.40	0.000*
Glucose (fasting) (mmol/L)	4.76 ±0.49	5.15 ±0.60	0.024*
HOMA-IR value	1.13 ±0.35	5.32 ±2.84	0.000*

* denotes a p-value <0.05 which was considered statistically significant.

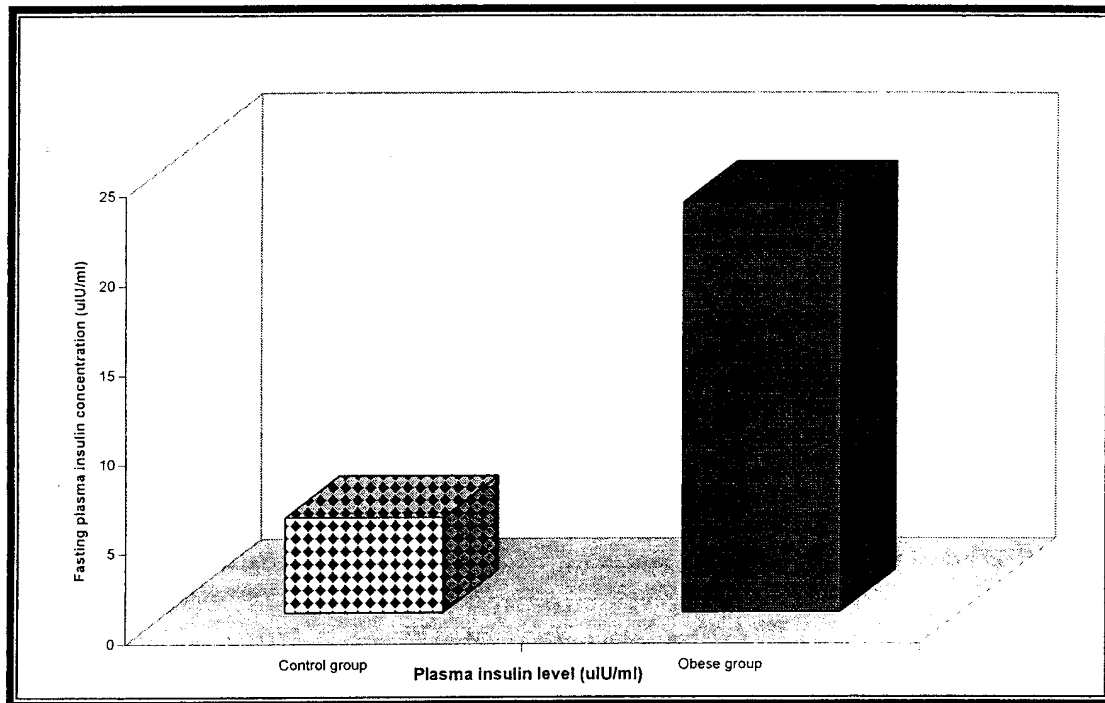


Figure (6): Fasting plasma insulin in the studied groups

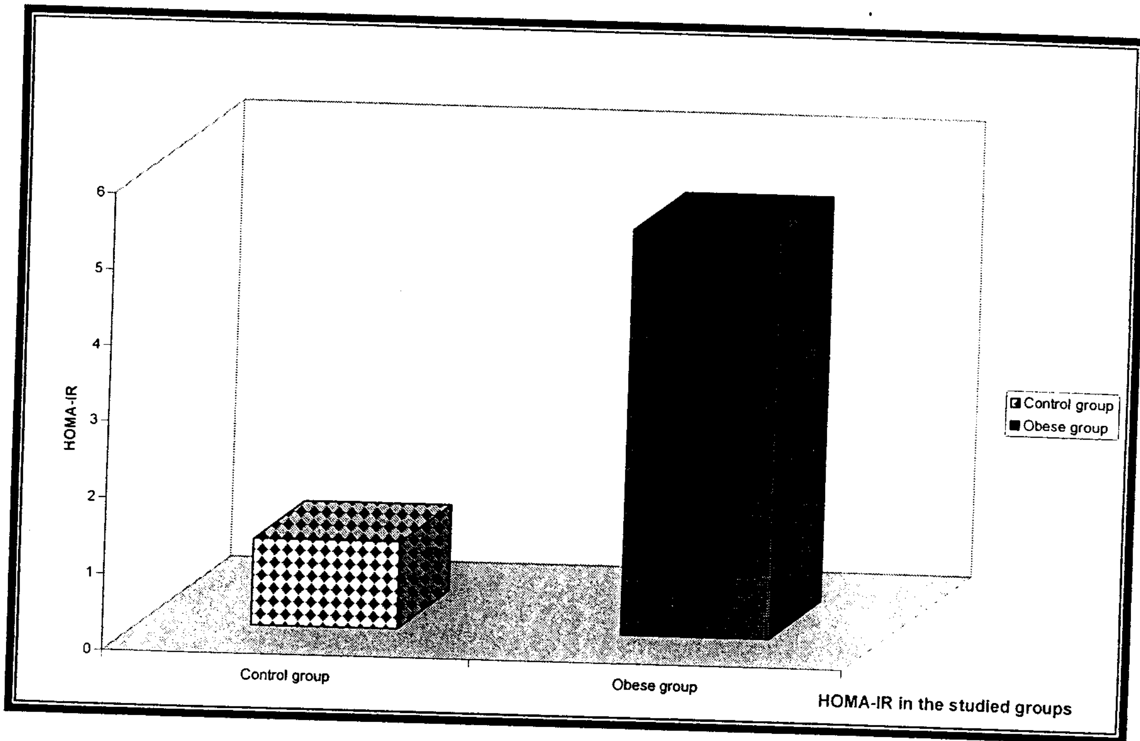


Figure (7): HOMA-IR in the studied groups

Plasma soluble VCAM-1 in the studied groups

1. **Plasma sVCAM-1 (Table Xa, b):**

- **Control group:** Plasma level of sVCAM-1 ranged from 663.94 to 1582.45ng/ml with a mean value of 1090.96 ±263.98ng/ml.
- **Obese females group:** Plasma sVCAM-1 level ranged from 550.26 to 1987.10 ng/ml with a mean value of 1008.70 ± 320.95 ng/ml.

Table (Xa): plasma levels of sVCAM-1(ng/ml) in the control group.

Case number	sVCAM-1 (ng/ml)
1	966.66
2	818.52
3	969.39
4	1073.33
5	1038.30
6	1048.49
7	1263.31
8	799.58
9	--------
10	1549.97
11	1582.45
12	1132.29
13	1064.24
14	663.94
15	1302.96
Mean	1090.96
±SD	263.98

Table (Xb): Plasma levels of sVCAM-1(ng/ml) in the obese females group.

Case number	sVCAM-1 (ng/ml)
1	1937.20
2	1012.24
3	1078.07
4	906.70
5	1987.10
6	663.09
7	616.19
8	550.26
9	551.634
10	641.99
11	1303.88
12	651.80
13	1097.95
14	928.37
15	1278.04
16	-------------
17	927.83
18	1058.01

Table (Xb) (Continued): plasma levels of sVCAM-1(ng/ml) in the obese females group.

Case number	sVCAM-1 (ng/ml)
19	780.82
20	1155.603
21	791.16
22	985.13
23	1408.89
24	570.62
25	819.24
26	1274.03
27	722.87
28	783.87
29	862.56
30	1041.36
31	1256.89
32	670.50
33	1195.85
34	847.96
35	1247.47

Table (Xb) (Continued): plasma levels of sVCAM-1(ng/ml) in the obese females group.

Case number	sVCAM-1 (ng/ml)
36	1285.07
37	963.01
38	1144.14
39	888.65
40	903.30
41	1089.08
42	1245.54
43	1098.23
44	1416.41
45	744.31
Mean	1008.70
± SD	320.95
P	0.389

P-value <0.05 was considered statistically significant.

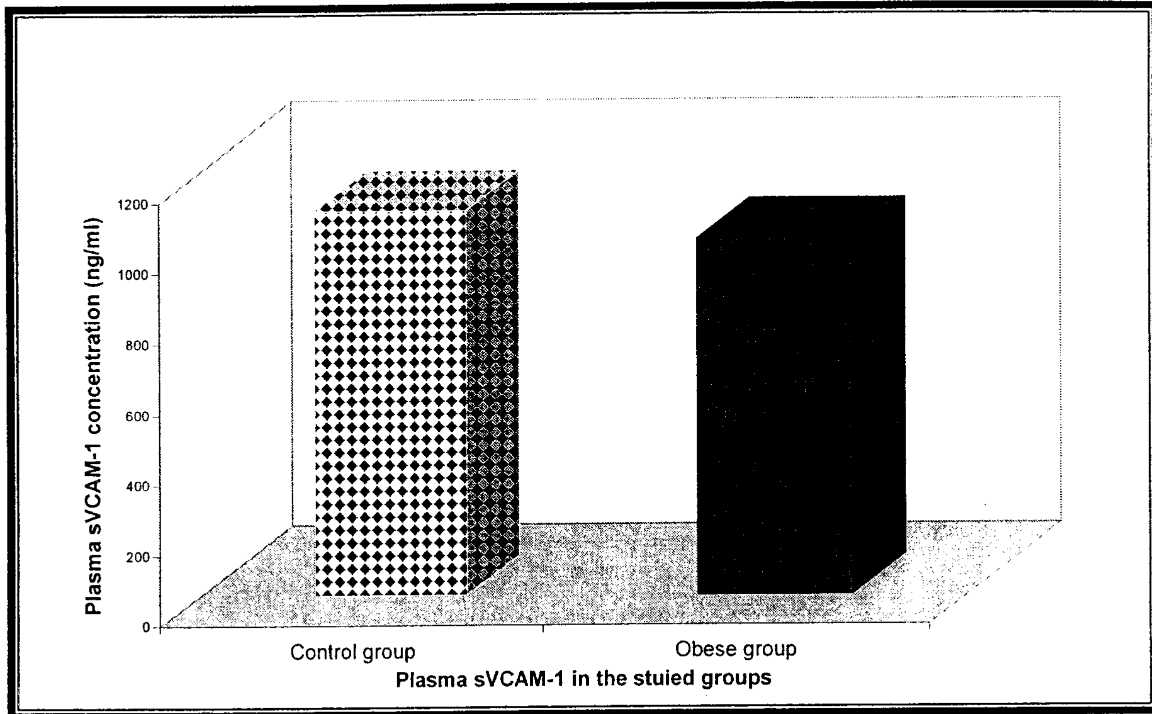


Figure (8): Plasma sVCAM-1 in the studied groups

The mean, S.D. and p-value of plasma levels of sVCAM-1 in obese females with fasting plasma insulin < 26 μIU/ml and obese females with fasting plasma insulin ≥26μIU/ml (Table XI):

Upon dividing obese females according to their fasting plasma insulin (FPI) into two subgroups those with FPI more than ≥26 μIU/ml and those with FPI< 26 μIU/ml, it was found that:

1. **Obese females with FPI < 26 μIU/ml** had a plasma sVCAM-1 mean value of 880.76 ± 225.71 ng/ml.
2. **Obese females with FPI ≥ 26 μIU/ml** had a plasma sVCAM-1 mean value of 1211.90 ± 350.09 ng/ml.

The plasma sVCAM-1 mean value was significantly higher in the group with FPI ≥ 26 μIU/ml than the corresponding group with FPI < 26 μIU/ml.

Table (XI): The mean, S.D. and P value of Plasma levels of sVCAM-1 in obese females of fasting plasma insulin < 26 μIU/ml and obese females of fasting plasma insulin ≥ 26μIU/ml.

Groups / Item	FPI< 26 μIU/ml (n = 28)	FPI ≥ 26 μIU/ml (n = 17)	p-value
sVCAM-1 (ng/ml)	880.76 ±225.71	1211.90 ±350.09	0.000*

* denotes a p-value <0.05 which was considered statistically significant.

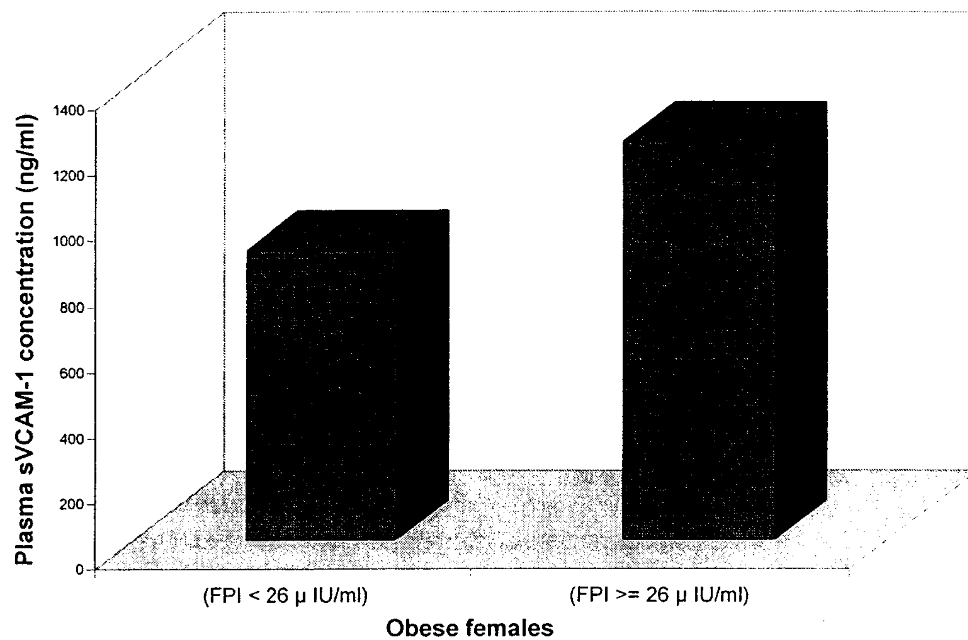


Figure (9): Plasma sVCAM-1 in obese females with FPI < 26 μIU/ml and FPI ≥ 26 μIU/ml.

Table (XII): shows the significant correlations of some studied items in the obese females group.

- CIMT positively correlates with WHR (r=0.580) (p=0.000)
- sVCAM-1 positively correlates with FPG ,PPG, T.G, FPI and HOMA-IR (r= 0.382, 0.350, 0.302, 0.528 and 0.589 respectively) (p= 0.010, 0.020, 0.047, 0.000 and 0.000 respectively).

Table (XII): Significant correlations of some studied items in the obese females group.

Items in obese females	r-value	p-value
CIMT with:		
WHR	0.580	0.000
sVCAM-1 with:		
FPG	0.382	0.010
PPG	0.350	0.020
T.G	0.302	0.047
FPI	0.528	0.000
HOMA-IR	0.589	0.000

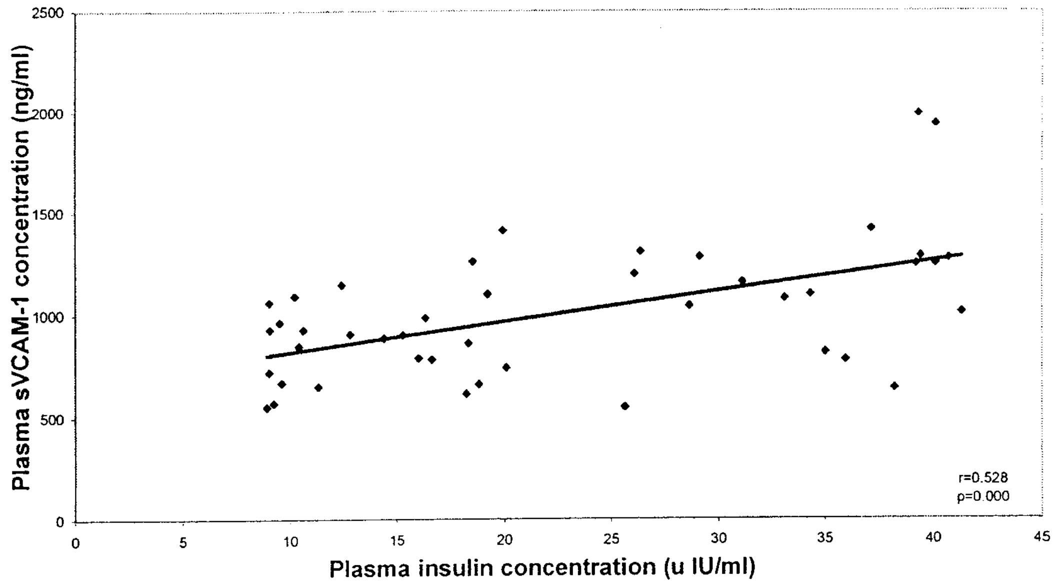


Figure (10): correlation between Plasma sVCAM-1 and insulin

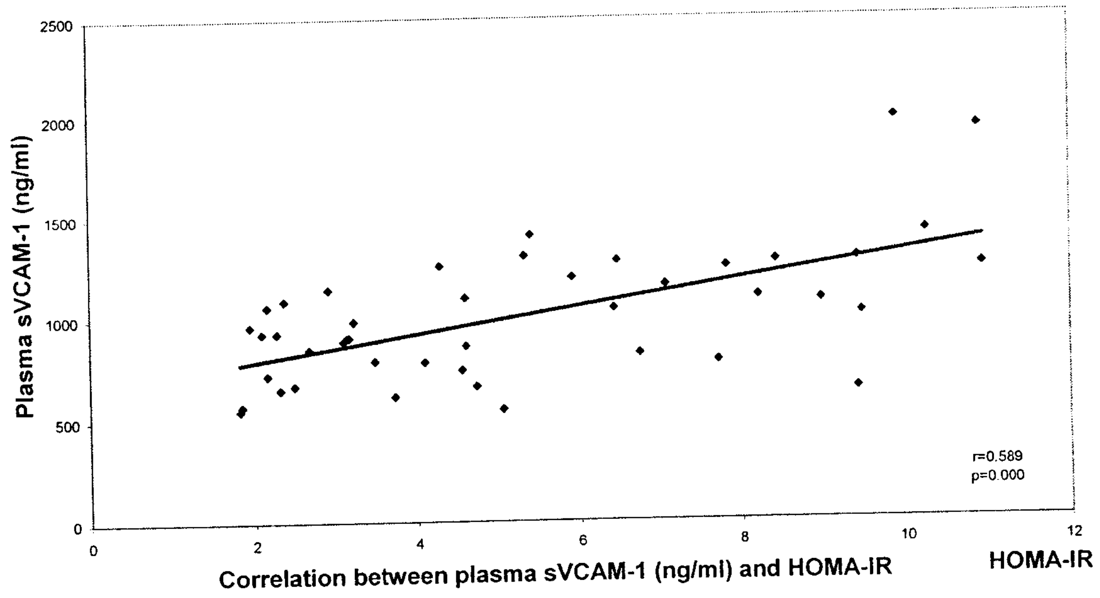


Figure (11): correlation between Plasma sVCAM-1 and HOMA-IR

DISCUSSION

DISCUSSION

Obesity is a common metabolic disorder of multiple etiologies. It affects around 20% of the adult population.[2] In addition, its prevalence is rapidly increasing even in children.[3]

Obesity is a part of a wide spectrum of co-morbidities collectively known as the metabolic syndrome that include dyslipidemia (atherogenic lipoprotein profile), hypertension, type 2 DM, pro-coagulant and pro-thrombotic states. The underlying cause for most of these conditions is insulin resistance. [108, 109]

Insulin resistance is defined when a normal or elevated insulin level produces an impaired glucose tolerance.[110] This refers to impaired sensitivity to insulin mediated glucose disposal. [111]

There is a strong and close association between obesity and insulin resistance (IR). It has been found that the risk of developing IR increases with the increase in body fat mass. Furthermore, obesity has been found to be a major contributor to atherosclerotic cardiovascular complications by inducing an initial low grade type of inflammatory response.[4]

In obesity and insulin resistance (IR), the initial trigger is attributed to the increased release of adipo-cytokines (TNF-α and IL-6) from adipose tissue with the resulting activation of NF-KB pathway.[87,88] Such an adiposity induced mild chronic inflammation would induce IR on one hand and an initial endothelial cell dysfunction (ECD) on the other hand, thus linking adiposity with insulin resistance and increased plasma levels of cell adhesion molecules (CAMs). [87,88]

The up-regulated expression of CAMs of the selectin family, integrin family and immunoglobulin superfamily, that mediate leucocyte endothelial cell (ECs) interaction, represents an initiating event in the process of atherogenesis.[112,113]

The upregulation of CAMs expression is accompanied by the release of their soluble fractions in the circulation. As CAMs are poorly expressed by resting healthy ECs, so the values of their plasma soluble forms are low in the healthy population.[114] Plasma soluble CAMs levels were taken as the most feasible index of increased CAMs expression by intact vascular ECs in vivo.

Vascular cell adhesion molecule-1 (VCAM-1) is a transmembrane protein and a member of the immunoglobulin superfamily (IgSF), expressed on the surface of endothelium and follicular dendritic cells.[79]

Endothelial expression of VCAM-1 in vivo is evident at sites of chronic inflammation and at atherosclerotic plaques.[82]

VCAM-1 promotes the adhesions of eosinophils, lymphocytes, monocytes and basophils to the vascular endothelium.

The present study was carried out to evaluate plasma soluble VCAM-1 in relation to plasma insulin status in adult obese females.

The study was conducted on sixty pre-menopausal adult female subjects divided according to the body mass index (BMI) into forty five obese females (BMI >30 kg/m^2) and fifteen non obese (BMI <25 kg/m^2) apparently healthy female volunteers of comparable age and socioeconomic status.

Anthropometric measurements and CIMT in the studied groups:

In the present work, obesity was assessed by means of TSF, MAC, WC, and HC, as well as calculation of BMI and WHR. All the above mentioned measures showed a statistically significant increase in the obese female group than the corresponding control group (p=0.000) (Table VIb).

A BMI greater than 28 kg/m^2 has been found to be associated three to four times with higher risk of morbidity from coronary artery disease (CAD) or stroke than the risk in the general population. [13]

In spite of the fact that BMI was chosen in this study as a discriminative measure for defining obesity, yet BMI has certain limitations as a measure of adiposity, being unable to distinguish upper from lower body adiposity.[4]

Many studies have shown that upper body adiposity is more related to cardiovascular diseases and death than the lower body adiposity.[5] Moreover, central (intra-abdominal) adiposity is more strongly linked to insulin resistance.[115] That is why the WHR was resorted to in the evaluation of central adiposity.

Obesity has been found to be associated with the early development of coronary atherosclerosis in adolescent and young adult men.[116]

As CIMT increased thickness is a sign of atherosclerotic changes, its elevation in obese females group denotes that they are prone to atherosclerotic changes.

In the present study, ultrasonographic study of common carotid arteries showed that the mean common carotid artery intima media thickness (CIMT) value in the obese females group was significantly higher than its corresponding mean value in the control group (p =0.000)(Table Vb).

In the present study, the WHR positively correlated with CIMT (r=0.58, p=0.000) in obese females, denoting that central obesity is a predisposing factor for atherosclerosis and so with endothelial activation and dysfunction compared to other measurements of adiposity.[5]

Fasting plasma urea, creatinine, albumin, AST and ALT in the studied groups:

These analytes were chosen as indices of normal renal and hepatic functions to exclude possible sources of an elevated sVCAM-1 level.[117,118]

In the present study all cases in both control group and obese group had normal values of urea, creatinine, albumin, AST and ALT.

Fasting plasma lipid profile in the studied groups:

A well established atherogenic lipoprotein profile in obesity has been documented by several studies. It ranged from hypercholesterolemia, low serum HDL-C, high serum LDL-C, and hypertriglyceridemia, to an abnormal LDL phenotype (small dense LDL).[4]

In the present work, the total plasma cholesterol mean value was 154.13 ± 12.01 mg/dl in the control group (Table VIIIa) ,while its mean value in the obese females was 185.87 ± 31.93 mg/dl, showing a statistically significant higher mean value than that of the corresponding control group (p=0.000) (Table VIIIb).

As regards the LDL-C in this study, its mean value in the control group was 93.35 ± 12.11 mg/dl (Table VIIIa), while its mean value in the obese females was 121.10 ±30.03 mg/dl, showing a significantly higher mean value from the corresponding control group (p=0.000) (Table VIIIb).

The mean plasma HDL-C value in the control group (44.73± 5.09 mg/dl) was slightly higher than its corresponding mean value in the obese females group (41.73 ± 7.14 mg/dl).

As for TG, its mean value was 80.33 ± 20.90 mg/dl in the control group (Table VIIIa), while in the obese females its mean value was 114.80 ± 46.14 mg/dl, with a statistically significant higher mean value in obese females than the corresponding control group (p = 0.007) (Table VIIIb).

It has been found that the source of high plasma total cholesterol, particularly its LDL fraction has been the adipose tissue[119], while hypertriglyceridemia has been found to be the result of increased hepatic production which is augmented by the IR state of the individual.[4]

In the present study, no significant correlation existed between plasma sVCAM-1 and plasma LDL-C level. However, in another study plasma concentration of soluble VCAM-1 was significantly correlated with plasma LDL-C level.[120]

It is noteworthy to state that, in obese females, the highest plasma total cholesterol was 265 mg/dl, which exceeded the permissible value recommended by the WHO for a healthy lipid profile while LDL-C fraction and TG were within the permissible values.

Fasting plasma glucose level, insulin level and HOMA-IR in the studied groups:

Fasting plasma insulin is a crude, simple, practical and inexpensive measure to diagnose insulin resistance.[121]

In obese people, hyperinsulinemia, IR and impaired glucose tolerance are expected to occur with the increase in BMI. In our study, this was partly true for the part dealing with IR using the HOMA-IR.

Although the mean values of both fasting plasma glucose (FPG) and insulin (FPI) values were within the safely reported reference intervals (FPG 93.36 ± 9.93 Vs 86.73 ± 8.33 mg/dl, and FPI 22.87 ± 11.40 Vs 5.29 ± 1.65 μIU/ml for obese females & control group respectively), yet statistically significant higher mean values of both fasting plasma glucose and insulin were noted in obese females than their corresponding control group (p=0.024, and p=0.000 respectively). Also no correlation was found between fasting plasma insulin and HDL and LDL cholesterol fractions.

Similarly, plasma insulin level has repeatedly been shown by several workers to be higher in obese females as BMI increased. Furthermore, it was not found to correlate with HDL and LDL cholesterol fractions.[122,123]

The normal fasting plasma insulin level reported by Sacks (1999) ranged from 2–25 μIU/ml [124], and according to the criteria of Kraft (1975) its level ranged from 0–30 μIU/ml.[125]

As regards the Homeostasis Model Assessment (HOMA) value, it was developed by Mathews et al (1985) to estimate insulin resistance from the mathematical modeling of fasting plasma glucose and insulin concentrations.[105] HOMA was found to be a reliable measure of the degree of IR in humans.[126]

In the present study, the obese females showed a mean HOMA value of 5.32±2.84, which was significantly higher (p=0.000) than the corresponding control value that had a mean HOMA value of 1.13±0.35 (Table IX)

Subjects with insulin resistance are characterized by reduced insulin-mediated glucose uptake, and increased serum insulin concentration.[29,30] Insulin resistance increases in a linear fashion with BMI at age- and sex-adjusted rates, where subjects with a BMI of 30–35 kg/m^2, have a 34% prevalence of insulin resistance, and subjects with a BMI >35 kg/m^2 have a 41% prevalence of IR.[23] However, no significant correlation was found between HOMA-IR & BMI in the present study.

Fasting plasma sVCAM-1 levels in the studied groups:

In the present study, the plasma sVCAM-1 level in the control group ranged from 663.94 to 1582.45 ng/ml with a mean value of 1090.96 ± 263.98 ng/ml (Table Xa). In three previous studies, the mean plasma values of sVCAM-1 in normal healthy adult subjects were reported to be 608 ± 71.50 ng/ml 551 ± 109 ng/ml, and 822.1 ± 197 ng/ml respectively.[88,127,128]

In the present study, the plasma sVCAM-1 level in obese females ranged from 550.26 to 1987.10 with a mean value of 1008.70 ±320.95 ng/ml (Table Xb), which did not show significant difference than its mean value in the corresponding control group.

Adhesion of leukocytes to endothelial cells via adhesion molecules (CAMs) is thought to be vital in the initiation of cardiovascular diseases as atherosclerosis.[129]

In contrast to some studies reporting a statistically significant higher plasma value of sVCAM-1 between obese persons and healthy volunteers, our study showed no statistically significant difference between both groups as regards the mean plasma sVCAM-1 value (p=0.680). The lack of a statistically significant difference in mean sVCAM-1 value between premenopausal obese females included in the present work and the corresponding control group may be in part explained by the possibility of a protective effect of female sex hormones against the development of CVD. This may suggest that female sex hormones may modulate the expression of cellular adhesion molecules. Whether this may add to the protective role of female sex hormones against the progression of atherosclerosis remains contentious.

The choice of females in this study was to nullify the effect of sex on the expression of CAMs in general and sVCAM-1 in particular. It has been reported that there might be a direct inflammatory effect of male sex hormones, since exposure to androgens has been demonstrated to increase endothelial cell surface expression of VCAM-1.[130]

Although several studies have reported associations between plasma levels of some adhesion molecules (sICAM-1 and sE-selectin) and BMI or the presence of clinical obesity, the findings are variable, and no association has been demonstrated with plasma sVCAM-1.[131,132,133,134] This agrees with our study, where no statistically significant correlation was noted between sVCAM-1 plasma value and BMI.

Furthermore, the lack of a significant correlation between sVCAM-1 plasma value and CIMT (r=0.073, p=0.638) in the obese females involved in this study, does not nullify the fact that CAMs expression is enhanced in obesity induced atherosclerotic lesions, as reported by many studies.[135,136]

In the present study, there were no statistically significant correlations between sVCAM-1 plasma value and plasma values of total cholesterol and its fractions (HDL-C and LDL-C). However, a statistically significant correlation existed between sVCAM-1 plasma value and triglycerides mean plasma value in the obese females group (r=0.302, p=0.047). This finding was found to be partially agreeable with some tudies that reported no association between sVCAM-1 levels and each of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides plasma levels.[131,132,133] Total cholesterol alone was associated with sVCAM-1 plasma level in the remaining large population based study.[137] Some smaller studies reported similar findings,[138,139]but again other studies did not.[140,141]

In our study, statistically significant positive correlations existed between plasma sVCAM-1 value and each of FPG & PPG (r=0.38 & 0.35, p=0.01 & 0.02 respectively), FPI (r=0.528) (p=0.000) and HOMA-IR (r=0.589) (p=0.000) in the obese females group, denoting that IR could contribute to the process of ECD found in obese patients & leading to atherosclerosis. This is in accordance with other workers where the degree of insulin resistance and hyperinsulinemia were significantly correlated with the concentration of plasma sVCAM-1. [142,143]

The obese females group was further subdivided into two groups according to a cut off value for fasting plasma insulin (FPI) of 26μIU/ml. The group with FPI value <26 μIU/ml (n=28) had a mean sVCAM-1 value of 880.76 ± 225.71 ng/ml while that with FPI ≥26μIU/ml (n=17) had a mean sVCAM-1 value of 1211.90 ± 350.09 ng/ml. A statistically significant difference was noted between the two subgroups regarding the plasma sVCAM-1 mean value (p=0.000) (Table IX). Furthermore, in the group with FPI of ≥26 μIU/ml (n=17), the sVCAM-1 mean plasma value showed a statistically significant correlation with plasma triglycerides mean value (r=0.670, p=0.003), while this correlation was absent in those with FPI value <26 μIU/ml, implying that disturbance in lipid metabolism as a result of obesity induced hyperinsulinemia could affect endothelial cell function, resulting in an increased VCAM-1 expression and release from activated ECs.

It is noteworthy to state that a fall in the levels of plasma cell adhesion molecules, namely sICAM-1, sVCAM-1, sE-selectin and sP-selectin, has been reported to be associated with weight reduction. In addition, weight reduction was also associated with improvement in other risk factors, including glucose and insulin levels.[120,144]

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Vascular cell adhesion molecule-1 (VCAM-1) is a cell surface glycoprotein that belongs to the immunoglobulin superfamily (IgSF). It is expressed on the surface of activated endothelial cells (ECs), bone marrow, fibroblasts, smooth muscles, perineural cells, tissue macrophages, dendritic cells and myoblasts.

The primary function of VCAM-1 is adhesion of T-cells, monocytes and eosinophils to endothelium activating inflammation. It can be stimulated by some mediators such as interleukin (IL)-1, IL-4, TNF-α and endotoxin.

In obesity there is an increased expression and release of adipocytokines, some of which correlate with inflammation, such as tumor necrosis factor alpha (TNF-α) and interleukin-6.

The pro-inflammatory cytokines released from adipose tissue are involved in the process of insulin resistance as well as their contribution to the development of obesity induced atherosclerosis. Endothelial cell dysfunction plays an important part in the initiation of atherogenesis, where the role of cell adhesion molecules exists.
The VCAM-1 has emerged as a particularly attractive candidate for the early adhesion of mononuclear leukocytes to arterial endothelium at sites of atheroma initiation.

The aim of this study was to evaluate the plasma level of sVCAM-1 in obese premenopausal females in a trial to relate its level to the occurrence of obesity related insulin resistance and endothelial dysfunction.

The study was conducted on sixty adult premenopausal females divided into two groups; the first group (Obese group) consisted of forty five obese females with a mean body mass index (BMI) value of 36.72 ± 5.5 Kg/m^2. The second group (Control group) consisted of fifteen apparently healthy female volunteers with a mean body mass index (BMI) value of 22.73 ± 1.53 Kg/m^2. All were selected to be free from recent infectious or inflammatory conditions, malignancy, endocrinological disorders, renal and hepatic diseases.

To all studied female subjects, a full clinical examination was done. Anthropometric measurements were performed, together with the calculation of BMI and WHR. In addition, a 12 leads standard ECG, as well as common carotid artery intima media thickness (CIMT) were also performed.

Laboratory investigations included estimation of plasma levels of fasting glucose (and glucose 2 hours after meal), fasting plasma insulin, urea, creatinine, alanine and aspartate aminotransferases (ALT & AST) activity, albumin, cholesterol (total, low and high density lipoprotein fractions), triglycerides, and sVCAM-1 levels. The HOMA-IR value was calculated.

The results of the present study showed that there was no significant difference in the mean plasma value of sVCAM-1 in obese females from its corresponding value in the control group, but upon dividing the obese females group according to a cut off value for fasting plasma insulin (FPI) value of 26 μIU/ml into two subgroups, the group with FPI ≥26 μIU/ml showed a statistically significant higher sVCAM-1 value when compared with the group with FPI< 26 μIU/ml.

Statistically significant positive correlations were found between plasma sVCAM-1 and triglycerides, insulin, as well as HOMA-IR in the whole group of obese females.

From the present study the following could be concluded:

- The lack of a statistically significant difference in the mean sVCAM-1 plasma value between obese females and the corresponding control group may be in part explained by the possibility of a protective effect of female sex hormones against the development of atherosclerotic CVD. This agrees with some studies suggesting that female sex hormones may modulate the expression of cellular adhesion molecules. Whether this may be protective or not against the progression of atherosclerosis remains unclear.

- Inspite of this lack of difference in sVCAM-1 level between the whole obese group and the controls, sVCAM-1 plasma value in obese females with a FPI of ≥26μIU/ml was significantly higher than the group with FPI of < 26μIU/ml. This could point out to the effect of hyperinsulinemia on increasing sVCAM-1 expression and release from activated endothelial cells. Furthermore the significant positive correlation found between sVCAM-1 & insulin level as well as HOMA-IR emphasizes this interrelationship.

To sum up, the possible mechanisms implicated in obesity related endothelial activation and dysfunction include insulin resistance and compensatory hyperinsulinemia which is accompanied by an increased expression and release of sVCAM-1 (found in the hyperinsulinemic group), together with disturbed lipid pattern.

RECOMMENDATIONS

RECOMMENDATIONS

- Determination of VCAM-1 on the surface of vascular cells may add more information to its role in endothelial dysfunction.
- Determination of small dense LDL particles associated with central obesity & study of their correlation with VCAM-1 level is also recommended.
- The implication of genetic predisposition to endothelial dysfunction in obese subjects can be further studied.

REFERENCES

REFERENCES

1. WHO. Prevention and Management of the Global Epidemic of Obesity. Report of the WHO, Consultation on Obesity (Geneva, June 3-5,1997) 1997.

2. Bjorntorp P. Obesity. Lancet 1997; 350: 423–6.

3. Howdle S, Wilkin T. Type 2 diabetes in children. Nurs Stand 2001; 15: 38–42.

4. Pi-Sunyer F. Obesity. In: Cecil Textbook of Medicine, 21st Ed Goldman L, Bennett J (eds). W.B Saunders Company (pub) Philadelphia, London, Toronto, Sydney 2000; pp. 1155-60.

5. Mikhail N, Golub MS, Tuck ML. Obesity and hypertension. Progress in Cardiovascular Diseases 1999; 42:39-58.

6. Vanhala MJ, Pitkajarvi TK, Kumpusalo EA. Obesity type and clustering of insulin resistance-associated cardiovascular risk factors in middle-aged men and women. Int J Obes 1998; 22:369-74.

7. Pi-Sunyer FX. Medical risks of obesity. Obes Surg 2002; 12: 6S – 11S.

8. Stevens J, Evenson KR, Thomas R. Fitness and fatness as predictors of mortality from all causes and from cardiovascular diseases in men and women in the lipid research clinics study. Am J Epidemiol 2002; 156: 832-41.

9. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, JAMA 2003; 289: 76-9.

10. Hubert HB, Feinleib M, McNamara PM, Castelli WP.Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983; 67: 968–77.

11. Van Itallie TB. Health implications of overweight and obesity in the United States. Ann Intern Med 1985; 103: 983–8.

12. Casassus P, Fontbonne A, Thibult N. Upper-body fat distribution: a hyperinsulinemia-independent predictor of coronary heart disease mortality. The Paris Prospective Study. Arterioscler Thromb 1992; 12: 1387–92.

13. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation 1998; 97: 2099–100.

14. Katzel LI, Sorkin KD, Colman E. Risk factors for exercise-induced silent myocardial ischemia in healthy volunteers. Am J Cardiol 1994; 74: 869–74.

15. Lakka TA, Lakka HM, Salonen R, Kaplan GA, Salonen JT. Abdominal obesity is associated with accelerated progression of carotid atherosclerosis in men. Atherosclerosis 2001; 154: 497–504.

16. Denton RM, Tavaré JM. Molecular basis of insulin action on intracellular metabolism. In: Alberti KGMM, Zimmet P, Defronzo RA, Keen H (eds). International Textbook of Diabetes Mellitus (2nd ed) John Wiley & Sons, New York; 1997 pp. 469–88.

17. Hunter SJ, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. Am J Med 1998; 105: 331–45.

18. Cefalu WT. Insulin resistance: cellular and clinical concepts. Exp Biol Med2001; 22:13–26.

19. Karam JH. Pancreatic Hormones and Diabetes Mellitus. In: Greenspan FS, Strewler GJ, (eds). Basic and Clinical Endocrinology. Appleton & Lange, Stamford CT USA. 1997; pp.601–2.

20. Wheatcroft SB, Williams IL, Shah AM, Kearney MT. Pathophysiological implications of insulin resistance on vascular endothelial function. Diabet Med2003; 20: 255–68.

21. Perseghin G, Petersen K, Shulman GI. Cellular mechanism of insulin resistance: potential links with inflammation. Int J Obes Relat Metab Disord 2003; 27: S6–11.

22. Lillioja S, Bogardus C. Obesity and insulin resistance: lessons learned from the Pima Indians. Diabetes Metab. 1988; 4: 517–40.

23. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Invest 1997; 100: 1166–73.

24. Nieves D, Cnop M, Retzlaff B, Walden C, Brunzell J, Knopp R, Kahn S. The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. Diabetes 2003; 52: 172–9.

25. Expert panel on detection evaluation and treatment of high blood cholesterol in adults: Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285: 2486 –97.

26. Terry RB, Wood PD, Haskell WL, Stefanick ML, Krauss RM: Regional adiposity patterns in relation to lipids, lipoprotein cholesterol, and lipoprotein subfraction mass in men. J Clin Endocrinol Metab 1989; 68: 191–9.

27. Williams PT, Haskell WL, Vranizan KM, Krauss RM. The associations of high-density lipoprotein subclasses with insulin and glucose levels, physical activity, resting heart rate, and regional adiposity in men with coronary artery disease: the Stanford Coronary Risk Intervention Project baseline survey. Metabolism 1995; 44: 106 –14.

28. Abbasi F, McLaughlin T, Lamendola C, Yeni-Komshian H, Tanaka A, Wang T, Nakajima K, Reaven GM. Fasting remnant lipoprotein cholesterol and triglyceride concentrations are elevated in nondiabetic, insulin-resistant, female volunteers. J Clin Endocrinol Metab 1999; 84: 3903-6.

29. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am. 2004; 33: 283–303.

30. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for Studying Insulin Resistance (EGIR). Diabet Med 1999; 16: 442–3.

31. Cusi K, Maezono K, Osman A. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase mediated signaling in human muscle. J Clin Invest 2000; 105: 311–20.

32. Diehl A. Tumor necrosis factor and its potential role in insulin resistance and non alcoholic fatty liver disease. Clin Liver Dis. 2004; 8: 619–38.

33. Sobrevia L, Nadal A, Yudilevich DL, Mann GE. Activation of L-arginine transport system and nitric oxide synthase by elevated glucose and insulin in human endothelial cells. J Physiol 1996; 490: 775–81.

34. Mather K, Anderson TJ, Verma S. Insulin action in the vasculature: physiology and pathophysiology. J Vasc Res 2001; 38: 415–22.

35. Piatti PM, Monti LD, Galli L. Relationship between endothelin-1 concentration and metabolic alterations typical of the insulin resistance syndrome. Metabolism 2000; 49: 748–52.

36. Bierman EL, George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Arterioscler Thromb 1992; 12: 647–56.

37. Wheatcroft SB, Williams IL, Shah AM, Kearney MT. Pathophysiological implications of insulin resistance on vascular endothelial function. Diabet Med 2003; 20: 255–68.

38. Lembo G, Napoli R, Capaldo B. Abnormal sympathetic overactivity evoked by insulin in the skeletal muscle of patients with essential hypertension. J Clin Invest 1992; 90: 24–9.

39. Sundell J, Nuutila P, Laine H. Dose-dependent vasodilating effects of insulin on adenosine-stimulated myocardial blood flow. Diabetes 2002; 51: 1125–30.

40. Vita JA, Treasure CB, Yeung AC. Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation 1992; 85: 1390–7.

41. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arteriosclerosis 1985; 5: 293–302.

42. Sundell J. Obesity and diabetes as risk factors for coronary artery disease: from the epidemiological aspect to the initial vascular mechanisms. Diabetes, Obesity and Metabolism. 2005; 7: 9–20.

43. Fontbonne A, Charles MA, Thibult N. Hyperinsulinaemia as a predictor of coronary heart disease mortality in a healthy population: the Paris Prospective Study, 15-year follow-up. Diabetologia 1991; 34: 356–61.

44. Scherrer U. Insulin and the regulation of cardiovascular system: role of the L-arginine nitric oxide pathway and the sympathetic nervous system. In: Lu¨ scher TE, ed._The Endothelium in Cardiovascular Disease. Heidelberg: Springer Verlag, 1995: pp.108–28.

45. Despres JP, Lamarche B, Mauriege P. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996; 334: 952–7.

46. Pyorala M, Miettinen H, Laakso M, Pyorala K. Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. Circulation 1998; 98: 398–404.

47. Matsuzawa Y. Therapy Insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 2006; 3: 35–42.

48. Vettor R, Milan G, Rossato M, Federspil G. Adipocytokines and insulin resistance. Aliment Pharmacol Ther 2005; 22: 3–10.

49. Jara LJ, Medina G, Vera-Lastra O, Amigo MC. Accelerated atherosclerosis, immune response and autoimmune rheumatic diseases. Autoimmune Rev 2005; 5: 195–201

50.Bacon PA. Endothelial cell dysfunction in systemic vasculitis: new developments and therapeutic prospects Curr Opin Rheumatol. 2005;17(1):49-55.

51.Celemajer DS. Endothelial dysfunction: Does it matter? Is it reversible? J Am Coll Cardiol 1997; 30: 325–33.

52.Faruqi RM, DiCorleto PE. Mechanisms of monocyte recruitment and accumulation. Br Heart J 1993; 69: S19–S29.

53.Segrest JP, Anantharamaiah GM. Pathogenesis of atherosclerosis. Curr Opin Cardiol 1994; 9: 404–10.

54.Yla-Herttuala S. Biochemistry of the arterial wall in developing atherosclerosis. Ann N Y Acad Sci 1991; 623: 40–59.

55.DiCorleto PE. Cellular mechanisms of atherogenesis. Am J Hypertens 1993; 6: 314S–8S.

56.Plenz G, Robenek H. Monocytes/macrophages in atherosclerosis. Eur Cytokine Netw 1998; 9: 701–3.

57.Libby P. Inflammation in atherosclerosis. Nature 2002; 420: 19–26.

58.Baeuerle PA, Baltimore D. NF-kappa B: ten years after. Cell 1996; 87: 13–20.

59.Barnes PJ, Karin M. Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336: 1066–71.

60.Neurath MF, Becker C, Barbulescu K. Role of NF-kappaB in immune and inflammatory responses in the gut. Gut 1998; 43: 856–60.

61.De Martin R, Hoeth M, Hofer-Warbinek R, Schmid JA. The transcription factor NF-kappa B and the regulation of vascular cell function. Arterioscler Thromb Vasc Biol 2000; 20: E83–E8.

62.Ray A, Bal BS, Ray BK.Transcriptional induction of matrix metalloproteinase-9 in the chondrocyte and synoviocyte cells is regulated via a novel mechanism: evidence for functional cooperation between serum amyloid A-activating factor-1 and AP-1. J Immunol 2005; 175: 4039-48.

63.Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994; 94: 2493–503.

64.Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995; 92: 657–71.

65. Southgate KM, Fisher M, Banning AP. Upregulation of basement membrane-degrading metalloproteinase secretion after balloon injury of pig carotid arteries. Circ Res 1996; 79: 1177–87.

66. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. J Clin Endocrinol Metab 2002; 87: 1419–22.

67. Petruzzelli L, Takami M, Humes HD. Structure and function of cell adhesion molecules. Am J Med 1999; 106: 467–76.

68. Price DT, Loscalzo J. Cellular adhesion molecules and atherogenesis. Am J Med 1999; 107: 85–97.

69. Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. Am J Hypertens 2001; 14: 44S–54S.

70. Frenette PS and Wagner DD. Adhesion molecules – Part 1. N Engl J Med 1996; 334: 1526–9.

71. Hope SA and Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part I. Their expression and role in atherogenesis. Int Med J 2003; 33: 380–6.

72. Jang Y, Lincoff AM, Plow EF, Topol EJ. Cell adhesion molecules in coronary artery disease. J Am Coll Cardiol 1994; 24:1591–601.

73. Braun M, Pietsch P, Schror K, Baumann G, Felix SB. Cellular adhesion molecules on vascular smooth muscle cells. Cardiovasc Res 1999; 41: 395–401.

74. Cronstein BN, Weissmann C. The adhesion molecules of inflammation. Arthritis Rheum 1993;36: 147–9.

75. Ridker PM. Role of inflammatory biomarkers in prediction of coronary heart disease. Lancet 2001; 358: 946–8.

76. Jiang MZ, Tsukahara H, Hayakawa K, Todoroki Y, Tamura S, Ohshima Y, Hiraoka M, Mayumi M. Effects of antioxidants and NO on TNF-alpha-induced adhesion molecule expression in human pulmonary microvascular endothelial cells.Respir Med 2005;99:580-91.

77. Huang YW, Baluna R, Vitetta ES. Adhesion molecules as targets for cancer therapy. Histol Histopathol 1997; 12 :467-77.

78.Abbas AK, Lichtman AH, Pobers JS. T lymphocyte antigen recognition and activation. In: Cellular and Molecular Immunology. WB Saunders Company, Philadelphia, London: 1999: pp. 139-70.

79.Carlos TM, Barlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994; 84: 2068-101.

80.Menger MD, Vollmar B. Adhesion molecules as determinants of disease: From molecular biology to surgical research. Br J Surg 1996; 83: 588-601.

81.Briscoe DM, Cotran RS, Pober JS. Effects of tumour necrosis factor, lipopolysaccaride and IL-4 on the expression of vascular cell adhesion molecule-1 in vivo, correlation with $CD3^+$ T cell infiltration. J Immunol 1992; 149: 2954-60.

82. Williams TJ, Hellewell PG. Adhesion molecules in the microvascular inflammatory response. Am Rev Respir Dis 1992; 146: S45-S50.

83.Pigott R,Dillon LP, Hemingway IH and Gearing AJ. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Bichem Biophs Res Commun. 1992; 187: 584-9.

84.Kota RS, Ramana CV, Tenorio FAEnelow RI, Rutledge JC. Differential effects of lipoprotein lipase on tumor necrosis factor- alpha and interferon-gamma-mediated gene expression in human endothelial cells.J Boil chem..2005; 280: 31076-84.

85.Wilczynski JR, Glowacka E, Nowak M Szpakowski A Serum concentration of soluble vascular-cellular adhesion molecule-1 (VCAM-1) and expression of its receptor VLA-4 on the surface of peripheral blood and decidual lymphocytes of preeclamptic women Ginekol Pol. 2003; 74: 1335-42.

86.Gurbel PA, Serebruany V. Soluble vascular cell adhesion molecule-1 and E-selectin in patients with acute myocardial infarction treated with thrombolytic agents. Am J Cardiol 1998; 81: 772-5.

87.Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 1999; 148: 209–14.

88.Giovanni T, Riccardo C, Maria A, Marina B, Michele M. Relation between Soluble Adhesion Molecules and Insulin Sensitivity in Type2 Diabetic Individuals. Diabetes Care 2001; 24: 1961–6.

89.Pober JS, Gimbrone MA Jr, Lapierre LA, Mendrick DL, Fiers W, Rothlein R. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 1986; 137: 1893–6.

90. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995; 15: 1987–94.
91. Hollander JE, Muttreja MR, Dalesandro MR, Shofer FS. Risk stratification of emergency department patients with acute coronary syndromes using P-selectin. J Am Coll Cardiol 1999; 34: 95–105.

92. Kurz RW, Graf B, Gremmel F, Wurnig C, Stockenhuber F. Increased serum concentrations of adhesion molecules after coronary angioplasty. Clin Sci (Lond) 1994; 87: 627–33.

93. Yildiz A, Tepe S, Olfaz H, Yazici H, Ark E, Erzengin F. Carotid atherosclerosis is a predictor of coronary calcification in chronic hemodialysis patients. Nephrol Dial Transplant 2004; 19: 885-91.

94. Trinder P. Ann Clin Biochem 1969; 6: 24 (Quoted from Varley H, Gowenlock AH and Bell M (ed). Practical clinical biochemistry. 5th ed. Heinmann Medical Books Ltd. London 1980; 1: 405).

95. Sampson EJ, Baird MA, Burtis CA, Smith EM, Wilte DL, Bayse DD. A coupled enzyme equilibrium for measuring urea in serum: optimization and evaluation of the AACC study on urea: Candidate reference method. Clin Chem 1980; 26: 816–26.

96. Ward P, Ewen M, Pomeroy J and Leung F. Kinetic creatinine determination. Clin Biochem 1976; 9: 225-8.

97. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total cholesterol. Clin Chem 1974; 20: 470–5.

98. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem 1977; 25: 882 – 4.
99. Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H. LDL-C cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulphate. Clin Chim Acta 1984; 140: 77 – 83.

100. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem 1983; 29: 538–42.

101. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 1997; 258: 21–30.

102. Berg Meyer HU and Horder M. IFCC method for alanine aminotransferase. Clin Chem Acta 1980; 105: 147–72.

103. Rej R and Horder M. Aspartate aminotransferase. In: Methods of Enzymatic Analysis (3rd ed). Berg Meyer H (ed). Verlag Chemie (pub). Basel. 1983, pp.416–33.

104. PM Clark. Assays for insulin, pro-insulin(s) and C-peptide. Ann Clin Biochem 1999; 36: 541– 64.

105. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF. Homeostasis model assessment: insulin resistance and B- cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985; 28: 412–9.

106. Jilma B, Dallinger S, Hergovich N, Eichler H-G, Richter V, and Wagner O. Effects of hyperinsulinemia on plasma levels of circulating adhesion molecules.J Clin Endocrinol Metab 2000; 85: 1748–51.

107. Leslie E, Geoffrey J, James M. Statistical analysis. In: Interpretation and uses of medical statistics. 4th Ed. Scientific Publications. Oxford 1991, pp. 411-6.

108. Unger R H and Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. FASEB J 2001: 15: 312-21.

109. Wellen K E and Hotamisligil G S. Obesity-induced nflammatory changes in adipose tissue. J Clin Invest 2003; 112: 1785-8.

110. Cefalu WT. Insulin resistance: cellular and clinical concepts. Exp Biol Med (Maywood). 2001; 226: 13–26.

111. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004; 33: 283–303.

112. Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993; 14: 506–12.

113. Blann AD, McCollum CN. Increased levels of soluble adhesion molecules in atherosclerosis. Thromb Haemost 1994; 72: 151–4.

114. Frenette PN, Wagner DD. Adhesion molecules. Part II. Blood vessels and blood cells. N Eng J Med 1996; 335: 43–5.

115. Soliman LA, Grosvenor MB. Nutrition: Science and Applications, 2nd ed. 1994, pp. 182–220.

116. McGill HC Jr, McMahan CA, Herderick EE, Obesity accelerates the progression of coronary atherosclerosis in young men. Circulation 2002; 105: 2712–8.

117. Suliman ME, Qureshi AR, Heimburger O, Lindholm B, Stenvinkel P. Soluble adhesion molecules in end-stage renal disease: a predictor of outcome.Nephrol Dail Transplant. 2006;51:187–91.

118. Bruno CM, Sciacca C, Cilio D, Bertino G, Marchese E, Politi G, Chinnice L, Pellicano R. Chronic anaemia and adhesion molecules in patients with liver cirrhosis. A preliminary report.Minerva Gastroenterol Dietol 2005; 40: 177–81.

119. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, 3rd Ed. W.B Saunders Company (Pub) London Toronto 1999; P. 1802t, 1060.

120. Claudio F, Giovambattista D, Marco V, Cesare B, Mehtap P Anna S. Early Upregulation of Endothelial Adhesion Molecules in Obese Hypertensive Men. Hypertension 1999; 34: 568–73.

121. Glueck CJ, Lang JE, Tracy T, Sieve SL, Wang P. Contribution of fasting hyperinsulinemia to prediction of atherosclerotic cardiovascular disease status in 293 hyperlipidemic patients. Metabolism 1999; 48: 1437–44.

122. Lofgren IE, Herron KL, West KL, Zern TL, Brownbill RA, Ilich JZ, Koo SI, Fernandez ML. Weight loss favorably modifies anthropometrics and reverses the metabolic syndrome in premenopausal women. J Am Coll Nutr 2005; 24: 486–93.

123. Ram E, Vishne T, Maayan R, Lerner I, Weizman A, Dreznik Z, Konstantin B, Seror D, Pnina V The relationship between BMI, plasma leptin, insulin and proinsulin before and after laparoscopic adjustable gastric banding. Obes Surg 2005; 15: 1456–62.

124. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, (eds). Tietz Text book of clinical chemistry. 3rd edition. WB Saunders Company, Philadelphia, London, Toronto, 1999.

125. Kraft JR. Detection of Diabetes mellitus in situ "Occult diabetes". Lab med 1975; 6: 10–15.

126. Yamada K, Ywan X, Ishiyama S, Koyama K, Ichikawa F, Koyama W, Nonaka K. Association between Ala 54 Thr substitution of the fatty acid-binding protein 2 gene with insulin resistance and intraabdominal fat thickness in Japanese men. Diabetologia 1997; 40: 706–10.

127. Marfella R, Esposito K, Giunta R, Coppola G, De Angelis L, Farzati B, Paolisso G, Giugliano D. Circulating adhesion molecules in humans role of hyperglycemia and hyperinsulinemia. Circulation 2000; 101: 2247–51.

128. Karasek D, Vaverkova H, Halenka M, Budikova M, Novotny D. Soluble cell adhesion molecules sVCAM-1 and sICAM-1in subjects with familial hyperlipidemia. Biomed Papers 2005; 149: 101-8.

129. Paiker JE, Raal FG, Veller M, von-Arb M, Chetty N and Naran NH. Cell adhesion molecules : Can they be used to predict coronary artery disease in patients with familial hypercholesterolemia? Clin Chim Acta 2000; 293:105-13.

130. McCrohon JA, Jessup W, Handelsman DJ, Celermajer DS. Androgen exposure increases human monocyte adhesion to vascular endothelium and endothelial cell expression of vascular cell adhesion molecule-1. Circulation 1999; 99: 2317–22.

131. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997; 96: 4219–25.

132. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001; 104: 1336–42.

133. Malik I, Danesh J, Whincup P, Bhatia V, Papacosta O, Walker M Soluble. adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. Lancet 2001; 358: 971–6.

134. O'Malley T, Ludlam CA, Riemermsa RA, Fox KA. Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1); potential risk factor for the acute coronary syndromes. Eur Heart J 2001; 22: 1226–34.

135. Moussavi N, Renier G, Roussin A, Mamputu JC, Buithieu J, Serri O. Lack of concordance between plasma markers of cardiovascular risk and intima-media thickness in patients with type 2 diabetes. Diabetes Obes Metab 2004; 6: 69–77.

136. Glowinska B, Urban M, Peczynska J, Florys. Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE-selectin, sP- selectin, sL-selectin) levels in children and adolescents with obesity, hypertension, and diabetes. Metabolism 2005; 54: 10–6.

137. De Lemos JA, Hennekens CH, Ridker PM. Plasma concentration of soluble vascular cell adhesion molecule-1 and subsequent cardiovascular risk. J Am Coll Cardiol 2000; 36: 423–6.

138. Morisaki N, Saito I, Tamura K, Tashiro J, Masuda M, Kanzaki T. New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. Atherosclerosis 1997; 131: 43–8.

139. John S, Jacobi J, Delles C, Schlaich MP, Alter O, Schmieder RE. Plasma soluble adhesion molecules and endothelium-dependent vasodilation in early human atherosclerosis. Clin Sci (Lond) 2000; 98: 521–9.

140. Hackman A, Abe Y, Insull W, Pownall H, Smith L, Dunn. Levels of soluble cell adhesion molecules in patients with dyslipidemia. Circulation 1996; 93: 1334–8.

141. Abe Y, El-Masri B, Kimball KT, Pownall H, Reilly CF, Osmundsen K. Soluble cell adhesion molecules in hypertriglyceridemia and potential significance on monocyte adhesion. Arterioscler Thromb Vasc Biol 1998; 18: 723–31.

142. Reaven GM. Holmes M, Chen NG. Relationship between insulin resistance, soluble adhesion molecules, and mononuclear cell binding in healthy volunteers. J Clin Endocrinol Metab 1999; 84: 3485–9.

143. Suheyl Ezgu F, Hasanoglu A, Tumer L, Ozbay F, Aybay C, Gunduz M. Endothelial activation and inflammation in prepubertal obese Turkish children. Metabolism 2005; 54: 1384–9.

144. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 2002; 105: 804–9.

PROTOCOL

بسم الله الرحمن الرحيم

Study of plasma soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) in relation to insulin status in obese females

دراسة مستوى الجزئ اللاصق لخلايا الأوعية الدموية -١ الذائب فى بلازما الدم وعلاقته بحالة الإنسولين فى السيدات البدينات

Protocol of a thesis submitted to the
Medical Research Institute
Alexandria University
for partial fulfillment of the

خطة بحث مقدمةإلى
معهد البحوث الطبية
جامعةالاسكندرية
ايفاء جزئيا للحصول على

Master degree in
Chemical pathology

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كيمياء الباثولوجيا

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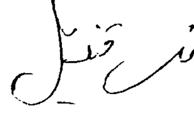

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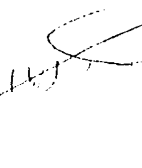

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INTRODUCTION

Obesity and the associated metabolic pathologies are the most common and detrimental metabolic diseases, affecting over 50% of the adult population.[1] The first of such associated diseases to be identified was coronary artery disease. A second group of disorders, including the complications of obesity, as dyslipidemia, insulin resistance, type 2 diabetes mellitus, and hypertension, are often referred to collectively as the "metabolic syndrome" or "insulin resistance syndrome". These conditions are associated with a chronic inflammatory response characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways.[1,2]

Obesity can be graded clinically by body-mass index (BMI). A BMI greater than 28 is associated with a risk of morbidity (eg. stroke, ischemic heart disease, or diabetes mellitus), that is three to four times the risk in the general population. A central distribution of body fat (ratio of waist circumference to hip circumference, 0.90 in women and 1.0 in men) is associated with higher risk of morbidity and mortality than a more peripheral distribution of body fat (waist : hip ratio 0.75 in women and 0.85 in men) and may be a better indicator of the risk of morbidity than absolute fat mass.[3,4]

Inspite of the association of obesity with hypertension, hyperlipidemia, hyperinsulinemia, and physical inactivity, some evidence supports the concept that obesity per se might favour the development of atherosclerosis via inducing early endothelial activation.[5] Upregulation of endothelial adhesins of the selectin family (E-selectin and P-selectin) and of the immunoglobulin superfamily [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] allows the attachment of circulating cells to the endothelium and represents the initiating event in atherogenesis.[6,7] The adhesin genes upregulation leads to the expression of membrane associated adhesins and release of their soluble forms. Thus circulating soluble adhesin levels act as markers of in vivo adhesin expression.[8]

Whereas ICAM-1 is broadly distributed in tissues, VCAM-1 and selectins are mainly and exclusively expressed, respectively, by vascular endothelial cells. Thus, the increased levels of plasma soluble VCAM-1(sVCAM-1) and E-selectin found in obese patients strongly suggest that the vascular endothelium is activated in human obesity.[8-10]

The reasons leading to endothelial adhesin upregulation in human obesity are unclear. A direct relationship between plasma sVCAM-1 and low density lipoprotein (LDL) levels was found in obese patients.[8] Lysophosphatidylcholine, which is a component of oxidized LDL, upregulates VCAM-1 expression in human cultured endothelial cells,[11] suggesting that circulating LDL may upregulate VCAM-1 in obese subjects due to LDL oxidation.[12,13]

Obese subjects can be significantly insulin resistant and hyperinsulinemic. Abnormalities in glucose metabolism might simultaneously induce adipose tissue accumulation and endothelial adhesin upregulation.[8,14,15] Further studies are needed to evaluate the possible intriguing relationships among insulin, glucose, and soluble adhesion molecule levels in obese people.

AIM OF THE WORK

The study aims at evaluating plasma soluble vascular cell adhesion molecule-1 (sVCAM -1) in relation to insulin status in obese females.

SUBJECTS

Sixty subjects will be included in the present study, divided as follows :

- **Patient group** : consisting of forty five adult premenopausal females with varying degrees of obesity, defined by a body mass index (BMI) of more than 30.
- **Control group** : consisting of fifteen apparently healthy females of comparable age, and socioeconomic state to the patient group, with BMI less than 25, and free from diabetes mellitus, coronary heart disease, liver and renal diseases.
- **Remark :**

All the subjects involved in this study will be free from any allergic condition, recent infectious or inflammatory diseases, malignancy as well as endocrinological disorders.

METHODS

To all the studied subjects, the following will be done :

1- Full clinical examination : including :

-Thorough history taking.

-Complete physical examination : including anthropometric measurements in the form of :[15]

-body mass index (BMI).

-triceps skin fold thickness.

-midarm circumference.

-waist circumference, hip circumference and waist to hip ratio.

2- Carotid intima media thickness (CIMT).

3-Twelve leads standard electrocardiogram.

4- Laboratory investigations, including :

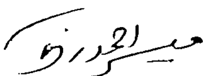
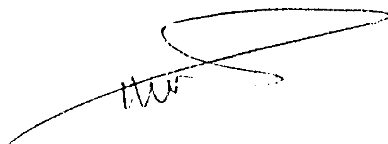
-Determination of fasting plasma concentrations and activities of the following analytes :[16]

glucose (and two hours post prandial plasma glucose), urea, creatinine, total cholesterol, high and low density lipoprotein cholesterol fractions, triglycerides, albumin, insulin hormone, alanine and aspartate aminotransferases.

-Assessment of insulin status by homeostasis model assessment (HOMA) score :[17]

fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/l)/22.5

-Estimation of fasting plasma level of soluble vascular cell adhesion molecule-1 (sVCAM-1) by enzyme immunoassay.[18]

REFERENCES

1-Unger R H and Orci L . Diseases of liporegulation: new perspective on obesity and related disorders. FASEB J 2001; 15: 312–21.

2-Wellen K E and Hotamisligil G S. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 2003; 112: 1785–8.

3-Kissebah AH, Krakower GR. Regional adiposity and morbidity. Physiol Rev 1994; 74: 761–811.

4-Rosenbaum M,. Leibel R and Hirsch J. Obesity. New Engl J Med 1997; 337: 396-407.

5-Barret-Connor EL. Obesity, atherosclerosis and coronary artery disease. Ann Intern Med 1985; 103: 1010 –19.

6-Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993; 14: 506 –12.

7-Blann AD, McCollum CN. Increased levels of soluble adhesion molecules in atherosclerosis. Thromb Haemost 1994 ; 72: 151–4.

8-Ferri C, Desideri G, Valenti M, Bellini C, Pasin M, Santucci A, De Mattia G. Early Upregulation of Endothelial Adhesion Molecules in Obese Hypertensive Men. Hypertension 1999; 34: 568–73.

9-DeCaterina R, Basta G, Lazzerini G. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arterioscl Thromob Vasc Biol 1997; 17: 2646 –54.

10-Peter K, Nawroth P, Conradt C. Circulating vascular cell adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating ICAM-1, E-selectin, P-selectin and thrombomodulin. Arterioscler Thromb Vasc Biol 1997; 17: 505–12

11-Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leucocyte adhesion molecules in cultured human and rabbit endothelial cells. J Clin Invest 1992; 90: 1138 –44.

12-Richardson M, Hadcock SJ, DeReske M, Cybulsky M. Increased expression of in vivo VCAM-1 and E-selectin by the aortic endothelium of normolipemic and hyperlipemic diabetic rabbits. Arterioscler Thromb 1994; 14: 760 –9.

13-Hackman A, Abe Y, Insull W, Pownall H, Smith L, Dunn K, Gotto AM, Ballantyne CM. Levels of soluble cell adhesion molecules in patients with dyslipidemia. Circulation 1996; 93: 1334 –8.

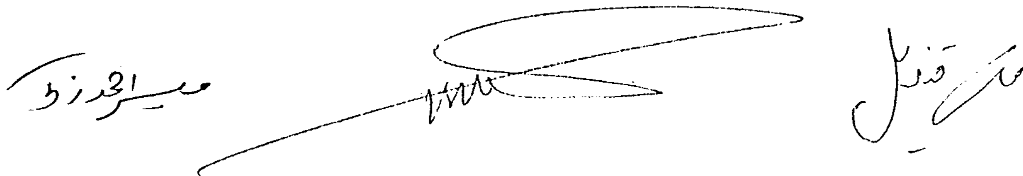
14-Baumgartner-Parzer SM, Wagner L, Pettermann M, Gessl A, Waldhaüsl W. Modulation by high glucose of adhesion molecule expression in cultured endothelial cells. Diabetologia 1995; 38: 1367–70.

15-Pi-Sunyer F. Obesity. In: Cecil Textbook of Medicine (21st ed). Goldmann L, Bennett J (eds). WB Saunders Company (Pub). Philadelphia 2000: pp.1155–60.

16-Burtis CA and Ashwood ER (eds). Tietz Textbook of Clinical Chemistry (3rd ed). WB Saunders Company (Pub). Philadelphia 1999: pp.750–808, 1204–70, 809–61, 477–540, and 617–721 respectively.

17-Bonora E, , Targher G, Alberichi M, Bonadonna RC, Saggiani F, Zenere MB, MonauniT, Muggeo T. Homeostasis Model Assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. Diabetes Care 2000; 23: 57–63.

18-Jilma B, Dallinger S, Hergovich N, Eichler H-G, Richter V, and Wagner O. Effects of hyperinsulinemia on plasma levels of circulating adhesion molecules. J Clin Endocrinol Metab 2000; 85: 1748–51.



ARABIC SUMMARY

الملخص العربى

الملخص العربي

تعد الجزيئات اللاصقة مواد برتينية سكرية عابرة للأغشية توجد على سطح الخلا يا و تعمل على ربطها وبالأخص كرات الدم البيضاء، لبعضها البعض و للخلايا البطانية وللنسيج القالب مما يساعد على تفاعل الخلايا مع بعضها البعض.

و يظهر تعبير الخلايا البطانية للجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب داخل الجسم عند مناطق الالتهاب المزمن ومناطق تصلب الشرايين.

ينتمي الجزيء اللاصق لخلايا الأوعية الدموية-١ لطائفة الجلوبونات المناعية و التي تعد أكبر مجموعة من الجزيئات اللاصقة.

و تنتج الصورة الذائبة من الجزيء اللاصق لخلايا الأوعية الدموية-١ (الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب) من الانقسام الناتج عن تحلل ا لبروتين للجزيء المقيد بالغشاء، و يمكن أن يستخدم كعلامة لنشاط الخلايا البطانية و التهابها.

و لقد وجد أن السمنة تمثل عامل رئيسي في مضاعفات التصلب الشرياني على القلب و الأوعية الدموية عن طريق حث مبدئي لدرجة منخفضة من الالتهاب.

في هذه الحالات، يعتبر الباعث الأول هو زيادة إفراز الحرائك الخلوية (عامل النخر الورمي-ألفا و الانترلوكين-٦) من النسيج الشحمي، مما ينتج عنه تنشيط مسار عامل النواة كابا-بيتا

و يعمل هذا الالتهاب المزمن الضعيف المنبعث من النسيج الشحمى على حث الاستجابة الالتهابية من ناحية و على خلل وظيفي أولي للخلايا البطانية من ناحية أخرى، الأمر الذي يربط البدانة بمقاومة الأنسولين و ارتفاع مستويات الجزيئات اللاصقة في البلازما.

ويهدف هذا البحث إلى دراسة مستوى الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب في بلازما البدينات فيما قبل سن اليأس

ولقد أجريت هذة الدراسة على ستين سيدة تم تقسيمهن إلى مجموعتين كالاتى:

١. المجموعة الأولى(مجموعة البدينات): اشتملت على خمسة و أربعين سيدة بدينة و قد كان معامل كتلة الجسم لهن ٣٦,٧٢ ± ٥,٥٨ كجم/م٢

٢. المجموعة الثانية(المجموعة الضابطة): اشتملت على خمسة عشر سيدة من الأصحاء و قد كان متوسط معامل كتلة الجسم لهن ٢٢,٧٣ ± ١,٥٣ كجم/م٢

ولقد تم اختيارهن متماثلات في العمر و المستوى الاجتماعي للسيدات البدينات.

ولقد روعي عند اختيار السيدات خلوهن من مرض السكري و قصور وظائف الكلى و أمراض الكبد و أمراض الغدد

وقد اشتملت طرق البحث على

١. الفحص الاكلينيكى لجميع السيدات مع قياس ضغط الدم و حساب متوسطة

٢. عمل قياسات أجزاء الجسم وقد اشتملت على كل من الوزن و الطول ومحيط الذراع و محيط الخصر و محيط الأرداف لكل منهن.

٣. حساب كل من: معامل كتلة الجسم و نسبة محيط الخصر إلى محيط الأرداف

٤. عمل رسم قلب وقياس سمك الطبقة الباطنة- الوسطى للشريان السباتى

٥. إجراء الفحوصات المعملية الآتية: قياس مستوى كل من السكر الصائم و بعد الأكل بساعتين في بلازما الدم, نشاط الإنزيم الكبدي المحول للألانين, البولينا, الكرياتينين, الألبومين و قياس الكولسترول(الكلى و المنخفض و العالي الكثافة) و الدهون الثلاثية كما تم أيضا قياس نسبة الأنسولين و الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب و حساب درجة المقاومة لتأثير الأنسولين

و لقد أظهرت النتائج انخفاضا نسبيا في مستوى الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب عنة في الأصحاء.

بعد تقسيم مجموعة البدينات إلى مجموعتين مجموعة ذات نسبة الأنسولين أعلى من أو يساوى ٢٦ ميكرووحدة/ مل و مجموعة ذات نسبة الأنسولين أقل من ٢٦ ميكرووحدة/ مل، أظهرت النتائج ارتفاعا ذو دلالة إحصائية.

في مستوى الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب في مجموعة البدينات ذوات نسبة الأنسولين أعلى من أو يساوى ٢٦ ميكرووحدة/ مل عن مجموعة البدينات ذوا ت نسبة الأنسولين اقل من ٢٦ ميكرووحدة/ مل.

و لقد وجدت علاقة إحصائية موجبة بين مستوى الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب و كل من الدهون الثلاثية والأنسولين و درجة المقاومة لتأثيره.

و نستخلص من هذا البحث ان البدانة من الممكن ان تؤثر على وظائف الطبقة الداخلية للأوعية الدموية و ذلك بسبب المقاومة لعمل الأنسولين المصحوب بزيادة مستواه و كذلك زيادة مستوى الجزيء اللاصق لخلايا الأوعية الدموية-١ الذائب فى البدينات ذوات نسبة الأنسولين المرتفعة والاتى يعانين من اختلال صورة الدهنيات بالدم .

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دراسة مستوى الجزىء اللاصق لخلايا الأوعية الدموية -١ الذائب فى بلازما الدم و علاقته بحالة الأنسولين فى السيدات البدينات

مقدمة من

عبير أحمد محمد على

بكالوريوس الطب والجراحة (٢٠٠٠)

جامعة الأسكندرية

للحصول على درجة الماجستير

في

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التاريخ ٢٠٠٦/٤/١٢

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رسالة

مقدمة لقسم الباثولوجيا الكيميائية

معهد البحوث الطبية

جامعة الإسكندرية

للحصول على درجة

"الماجستير"

في

الكيمياء الباثولوجيا

من

عبير أحمد محمد على

بكالوريوس الطب والجراحة (٢٠٠٠)

جامعة الإسكندرية

أبريل٢٠٠٦