

## III- INSULIN RESISTANCE

### A) Definition:

Insulin resistance (IR) is a physiological condition in which cells fail to respond to the normal actions of the hormone insulin. The body produces insulin, but the cells in the body become resistant to insulin and are unable to use it effectively, leading to hyperglycemia. Beta cells in the pancreas subsequently increase their production of insulin, further contributing to hyperinsulinemia (*Kim et al., 2008*).

The syndromes of insulin resistance actually make up a broad clinical spectrum, which includes obesity, glucose intolerance, diabetes, and the metabolic syndrome, as well as an extreme insulin-resistant state. Many of these disorders are associated with various endocrine, metabolic, and genetic conditions. These syndromes may also be associated with immunological diseases and may exhibit distinct phenotypic characteristics (*Lee et al., 2010*).

It is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. In fat cells, it reduces the effects of insulin and results in elevated hydrolysis of stored triglycerides in the absence of measures which either increase insulin sensitivity or which provide additional insulin (*Moadab et al., 2010*). Increased mobilization of stored lipids in these cells

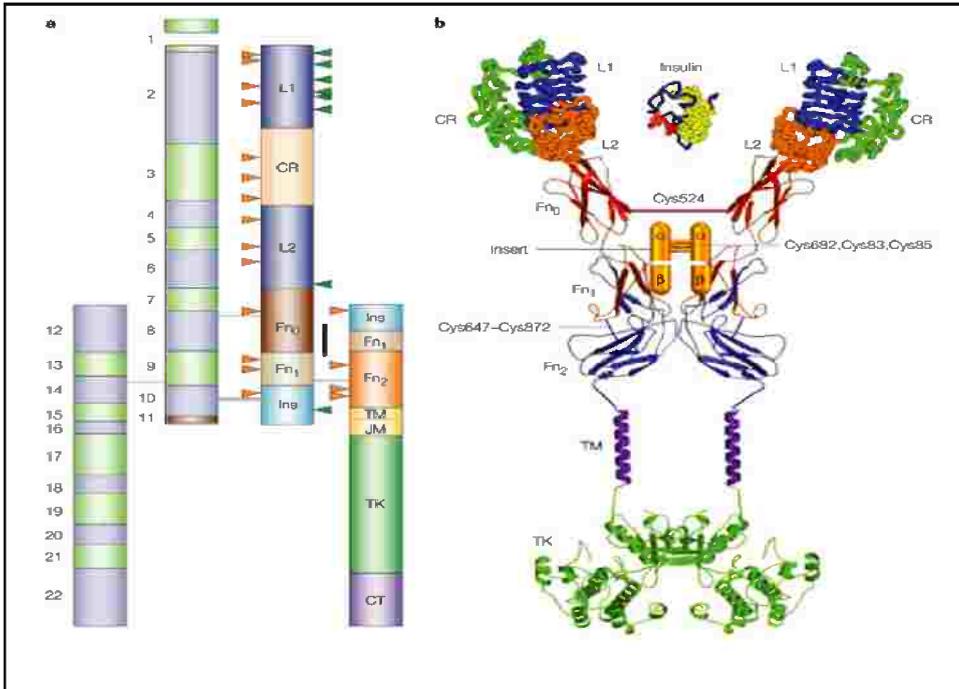
elevates free fatty acids in the blood plasma. In muscle cells, it reduces glucose uptake (and so local storage of glucose as glycogen), whereas insulin resistance in liver cells results in impaired glycogen synthesis and a failure to suppress glucose production (*van Raalte et al., 2011*).

Elevated blood fatty-acid concentrations (associated with insulin resistance and diabetes mellitus type 2), reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose concentration (*Lutsey et al., 2008*).

High plasma levels of insulin and glucose due to insulin resistance are believed to be the origin of metabolic syndrome and type 2 diabetes, including its complications (*Uruska et al., 2010*).

## **B) Structure of Insulin Receptor:**

Insulin receptor, in its native conformation, is a transmembrane glycoprotein composed of two  $\alpha$  subunits (135kd) and two  $\beta$  subunits (95kd) covalently linked through disulfide bonds to form  $\alpha_2 \beta_2$  heterotetramer figure(9) (*Ueki and Khan, 2000*).



**Figure (9):**Structure of insulin receptor(*Meys & Whittaker, 2002*)

The  $\alpha$  subunits is entirely extracellular and contains the sites for insulin binding, whereas the  $\beta$  subunits has a small extracellular portion, a transmembrane domain and an intracellular insulin-regulated tyrosine kinase activity. Binding of insulin to the  $\alpha$  subunits induce a conformational change in the receptor, resulting in activation of tyrosine kinase that catalyzes the phosphorylation of tyrosine residues on a number of specific intracellular proteins (e.g. insulin receptor substrate-1(IRS-1), IRS-2, IRS-3, IRS-4) which mediate insulin signaling (*Sacks, 2006*).

Both  $\alpha$  and  $\beta$  subunits are derived from a single gene via a large proreceptor polypeptide. The proreceptor undergoes glycosylation followed by disulfide bond formation. Final processing occur in Golgi-derived vesicles where cleavage of the proreceptor occurs at a tetrabasic site located at the junction of the  $\alpha$  and  $\beta$  subunits. This is followed by terminal glycosylation and possible fatty acid acylation (*Ueki and Khan, 2000*).

### **C) Physiology of Insulin Action**

Following secretion from the  $\beta$ -cell of the pancreas, insulin binds to its specific cell-surface receptor. Almost all human tissues contain insulin receptor, although their numbers vary greatly, with the highest number being present in insulin-sensitive tissues such as liver, adipocytes and skeletal muscles.

Once insulin binds to its receptors, it triggers a cascade of intracellular events. A primary effect of insulin is to stimulate translocation of a protein called glucose transporter (GLUT4), in skeletal muscle and adipose tissue, from the intracellular pool to the cell surface where it binds and transport glucose into the cell (*Czech et al., 2000*).

The cellular actions of insulin involve a wide variety of effects on post receptor signaling pathways within target cells. The  $\beta$  subunits of the insulin receptor is a tyrosine kinase, which is activated when insulin binds to the  $\alpha$  subunits, to initiate a cascade of phosphorylation and dephosphorylation events, second messenger generation, and protein- protein

interactions that result in the diverse metabolic events of insulin (*Buse et al., 2008*).

#### **D) Insulin Resistance Pathophysiology:**

In a person with normal metabolism, insulin is released from the beta ( $\beta$ ) cells of the Islets of Langerhans located in the pancreas after eating ("postprandial"), and it signals insulin-sensitive tissues in the body (e.g., muscle, adipose) to absorb glucose. This lowers blood glucose levels (*Buse et al., 2008*).

The beta cells reduce their insulin output as blood glucose levels fall, with the result that blood glucose is maintained at approximately 5 mmol/L (mM) (90 mg/dL). In an "insulin-resistant" person, normal levels of insulin do not have the same effect on muscle and adipose cells, with the result that glucose levels stay higher than normal (*Pritchett et al., 2005*).

To compensate for this, the pancreas in an insulin-resistant individual is stimulated to release more insulin(*Herman et al., 2005*).

The most common type of insulin resistance is associated with a collection of symptoms known as metabolic syndrome. Insulin resistance can progress to full Type 2 diabetes mellitus(*Lee et al., 2008*).

This is often seen when hyperglycemia develops after a meal, when pancreatic  $\beta$ -cells are unable to produce sufficient insulin to maintain normal blood sugar levels

(euglycemia) (*Rasouli et al., 2005*). The inability of the  $\beta$ -cells to produce sufficient insulin in a condition of hyperglycemia is what characterizes the transition from insulin resistance to Type 2 diabetes mellitus (*Nissen et al., 2007*).

Various disease states make the body tissues more resistant to the actions of insulin. Examples include infection (mediated by the cytokine TNF $\alpha$ ) and acidosis. Recent research is investigating the roles of adipokines (the cytokines produced by adipose tissue) in insulin resistance. Certain drugs may also be associated with insulin resistance (e.g., glucocorticoids) (*Quinn et al., 2008*).

Insulin itself can lead to insulin resistance; every time a cell is exposed to insulin, the production of GLUT4 (type four glucose receptors) on the cell's membrane is decreased. This leads to a greater need for insulin, which again leads to fewer glucose receptors. Exercise reverses this process in muscle tissue, but if left unchecked, it can spiral into insulin resistance (*Salpeter et al., 2008*).

Elevated blood levels of glucose — regardless of cause — leads to increased glycation of proteins with changes (only a few of which are understood in any detail) in protein function throughout the body (*Jensterle et al., 2008*).

With respect to visceral adiposity, a great deal of evidence suggests two strong links with insulin resistance. First, unlike subcutaneous adipose tissue, visceral adipose cells

---

produce significant amounts of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF $\alpha$ ), and Interleukins-1 and-6(*Antuna-Puente et al., 2008*).

In numerous experimental models, these proinflammatory cytokines profoundly disrupt normal insulin action in fat and muscle cells, and may be a major factor in causing the whole-body insulin resistance observed in patients with visceral adiposity (*Muniyappa et al., 2008*).

A great deal of attention into the production of proinflammatory cytokines has focused on the IKK-beta/NF-kappa-B pathway, a protein network that enhances transcription of cytokine genes. Second, visceral adiposity is related to an accumulation of fat in the liver, a condition known as non alcoholic fatty liver disease (NAFLD). The result of NAFLD is an excessive release of free fatty acids into the blood stream (due to increased lipolysis), and an increase in hepatic glucose production, both of which have the effect of exacerbating peripheral insulin resistance and increasing the likelihood of Type 2 diabetes mellitus (*Sjöholm et al., 2005*).

Insulin resistance is also often associated with a hypercoagulable state (impaired fibrinolysis) and increased inflammatory cytokine levels. It is also occasionally found in patients who use insulin. In this case, the production of antibodies against insulin leads to lower-than-expected glucose level reductions (glycemia) after a specific dose of

insulin. With the development of human insulin and analogues in the 1980s and the decline in the use of animal insulins (e.g., pork, beef), this type of insulin resistance has become uncommon (*De Taeye et al., 2005*).

Magnesium (Mg) is present in living cells and its plasma concentration is remarkably constant in healthy subjects. Plasma and intracellular Mg concentrations are tightly regulated by several factors. Among them, insulin seems to be one of the most important (*Kahn et al., 2005*).

In vitro and in vivo studies have demonstrated that insulin may modulate the shift of Mg from extracellular to intracellular space. Intracellular Mg concentration has also been shown to be effective in modulating insulin action (mainly oxidative glucose metabolism), offset calcium-related excitation-contraction coupling, and decrease smooth cell responsiveness to depolarizing stimuli (*Reaven et al., 2006*).

Poor intracellular Mg concentrations, as found in type 2 diabetes mellitus and in hypertensive patients, may result in a defective tyrosine-kinase activity at the insulin receptor level and exaggerated intracellular calcium concentration. Both events are responsible for the impairment in insulin action and a worsening of insulin resistance in noninsulin-dependent diabetic and hypertensive patients (*Daskalopoulou et al., 2006*).

By contrast, in type 2 diabetes mellitus patients daily Mg administration, restoring a more appropriate intracellular Mg

concentration, contributes to improve insulin-mediated glucose uptake. The benefits deriving- from daily Mg supplementation in type 2 diabetes mellitus patients are further supported by epidemiological studies showing that high daily Mg intake are predictive of a lower incidence of type 2 diabetes mellitus (*Cheng et al., 2006*).

## **E) Causes of Insulin Resistance**

There are several levels of insulin resistance causation including diet, cellular, molecular, genetic, and disease.

### **1- Diet**

Grounds exist for linking insulin resistance to a high-carbohydrate diet. The high amounts of ordinary sucrose (i.e., table sugar) in the typical developed-world diet is also suspected of having some causative effect on the development of insulin resistance (*Florez et al., 2006*).

Insulin resistance has certainly risen in step with the increase in sugar consumption and the substantial commercial usage of high fructose corn syrup (HFCS) since its introduction to the food trades; the effect may also be due to other parallel diet changes however (*Semple et al., 2008*).

Other studies have also linked to the increased amounts of fructose (e.g., in HFCS, currently the least expensive nutritive sweetener available in industrial quantities); in humans, fructose causes changes in blood lipid profiles, among

other things, mostly due to its effects on liver function (*Fuke et al., 2010*).

An American study has shown that glucosamine (often prescribed for joint problems) may cause insulin resistance (*Meilleur et al., 2010*).

## **2- Cellular**

At the cellular level, excessive circulating insulin appears to be a contributor to insulin resistance via down-regulation of insulin receptors. This occurs due to prolonged and repeated elevations of circulating insulin. Since the usual instances of type 2 insulin resistance are distinct from pathological over production of insulin, this does not seem to be the typical cause of the insulin resistance leading to type 2 diabetes mellitus, the largest clinical issue connected with insulin resistance (*Klok et al., 2007*).

The presence of insulin resistance typically precedes the diagnosis of types 2 diabetes mellitus, however, and as elevated blood glucose levels are the primary stimulus for insulin secretion and production, habitually excessive carbohydrate intake is a likely contributor. Additionally, some type 2 cases require so much external insulin that this down-regulation contributes to total insulin resistance(*Grant et al., 2005*).

Inflammation also seems to be implicated in causing insulin resistance. Vitamin D deficiency is also associated with insulin resistance (*Tilg et al., 2008*).

---

### **3- Molecular**

Insulin resistance has been proposed at a molecular level to be a reaction to excess nutrition by superoxide dismutase in cell mitochondria that acts as an antioxidant defense mechanism (*Kim et al., 2008*).

This link seems to exist under diverse causes of insulin resistance. It is also based on the finding that insulin resistance can be rapidly reversed by exposing cells to mitochondrial uncouplers, electron transport chain inhibitors, or mitochondrial superoxide dismutase mimetics (*Klok et al., 2007*).

### **4- Genetic**

Individual variability is a cause with an inherited component, as sharply increased rates of insulin resistance and type 2 diabetes are found in those with close relatives who have developed type 2 diabetes (*Fauci et al., 2008*). A major influence on the genetic susceptibility to diabetes type 1 is exerted by certain alleles of class 1 MCH genes. Several alleles of the DR3 and DR4 loci are associated with susceptibility to diabetes type 1, especially DR3\DR4 heterozygotes. Some alleles of DR2 confer relative resistance to diabetes. Numerous genes or loci conferring susceptibility to diabetes type 2 have been identified at several sites in the human genome (*Klok et al., 2007*).

## 5- Disease

Sub-clinical Cushing's syndrome and hypogonadism (low testosterone levels) seem to be the major insulin resistance causes (*Swinburn et al., 2005*).

Recent research and experimentation has uncovered a non-obesity related connection to insulin resistance and type 2 diabetes. It has long been observed that patients who have had some kinds of bariatric surgery have increased insulin sensitivity and even remission of type 2 diabetes (*Sjöström et al., 2006*).

It was discovered that diabetic / insulin resistant non obese rats whose proximal small intestine and duodenum has been surgically removed also experienced increased insulin sensitivity and remission of Type 2 diabetes (*Kiortsis et al., 2005*). This suggested similar surgery in humans, and early reports are that the same effect is seen in humans, at least the small number who have participated in the experimental surgical program (*Jayagopal et al., 2005*). The speculation is that some substance is produced in that portion of the small intestine which signals body cells to become insulin resistant. If the producing tissue is removed, the signal ceases and body cells revert to normal insulin sensitivity. No such substance has been found as yet, so its existence remains speculation (*Shih et al., 2010*).



## F) Insulin Resistance Associated Conditions

It was found that insulin resistance is usually associated with:

- 1- Abnormally sedentary lifestyle, whether the result of the effects of aging on the body or lack of physical exercise (both of which can also produce obesity).
- 2- Haemochromatosis.
- 3- Gastroparesis.
- 4- Tobacco smoking.
- 5- Coffee (A Canadian study has found that consumption of caffeine makes insulin more resistant to alterations in blood sugar in patients with and without diabetes).
- 6- Polycystic ovarian syndrome (PCOS).
- 7- Hypercortisolism (e.g., steroid use or Cushing's disease).
- 8- Drugs (e.g., rifampicin, isoniazid, olanzapine, risperidone, progestogens, corticosteroids, glucocorticoids, many antiretrovirals, possibly alcohol and methadone).
- 9- Genetic causes:
  - Insulin receptor mutations (Donohue Syndrome)
  - LMNA mutations (Familial Partial Lipodystrophy) (*Shih et al., 2010*).

Insulin resistance may also be caused by the damage of liver cells having undergone a defect of insulin receptors in hepatocytes (*Tan et al., 2008*).



## G) Insulin Resistance Symptoms:

1. Fatigue.
2. Brain fogginess and inability to focus. Sometimes the fatigue is physical, but often it is mental.
3. High blood sugar.
4. Intestinal bloating as mostintestinal gasis produced from carbohydrates in the diet. Insulin resistance sufferers who eat carbohydrates sometimes suffer from gas.
5. Sleepiness asmany people with insulin resistance get sleepy immediately after eating a meal containing more than 20% or 30% carbohydrates.
6. Weight gain, fat storage, difficulty losing weight. For most people, too much weight is too much fat. The fat in insulin resistance is generally stored in and around abdominal organs in both males and females. It is currently suspected that hormonal effects from such fat are a precipitating cause of insulin resistance.
7. Increased blood triglyceride levels.
8. Increased blood pressure. Many people with hypertension are either diabetic or pre-diabetic and have elevated insulin levels due to insulin resistance. One of insulin's effects is on arterial walls throughout the body.
9. Depression; because of the derangedmetabolismresulting from insulin resistance, psychological effects are not uncommon. Depression is said to be the prevalent psychological symptom. *(Fauci et al.,2008)*

## H) Insulin Resistance Diagnosis

### 1-Fasting Insulin Levels

A fasting serum insulin level of greater than the upper limit of normal for the assay used (approximately 60 pmol/L) is considered evidence of insulin resistance(*Pasquali et al., 2005*).

### 2-Glucose tolerance testing (GTT)

During a glucose tolerance test, which may be used to diagnose diabetes mellitus, a fasted patient takes a 75 gram oral dose of glucose. Blood glucose levels are then measured over the following 2 hours(*Essah et al., 2006*).

Interpretation is based on WHO guidelines. After 2 hours a Glycemia less than 7.8 mmol/L (140 mg/dl) is considered normal, a glycaemia of between 7.8 to 11.0 mmol/dl (140 to 197 mg/dl) is considered as Impaired Glucose Tolerance (IGT) and a glycaemia of greater than or equal to 11.1 mmol/dl (200 mg/dl) is considered DiabetesMellitus (*Savino et al., 2010*).

An OGTT can be normal or mildly abnormal in simple insulin resistance. Often, there are raised glucose levels in the early measurements, reflecting the loss of a postprandial peak in insulin production. Extension of the testing (for several more hours) may reveal a hypoglycemic "dip," which is a result of an overshoot in insulin production after the failure of the physiologic postprandial insulin response(*Hirschler et al., 2009*).

### 3- Glycated hemoglobin

**A1C test.** Sometimes called hemoglobin A1c, HbA1c, or glycohemoglobin test, this test reflects average blood glucose levels over the past 3 months. This test is the most reliable test for prediabetes, but it is not as sensitive as the other tests. In some individuals, it may miss prediabetes that could be caught by glucose tests (*Fauci et al., 2008*).

Although some health care providers can quickly measure A1C in their office, that type of measurement called a point-of-care test is not considered reliable for diagnosis. For diagnosis of prediabetes, the A1C test should be analyzed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) (*Pasquali et al., 2005*).

People of African, Mediterranean, or Southeast Asian descent, or people with family members with sickle cell anemia or a thalassemia, are particularly at risk of interference. People in these groups may have a less common type of hemoglobin, known as a hemoglobin variant, that can interfere with some A1C tests (*American Diabetes Association, 2014*). An A1C of 5.7 to 6.4 percent indicates prediabetes (*American Diabetes Association, 2014*).

## 4- Measuring Insulin Resistance

### a-Hyperinsulinemic euglycemic clamp

The gold standard for investigating and quantifying insulin resistance is the "hyperinsulinemic euglycemic clamp," so-called because it measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. The test is rarely performed in clinical care, but is used in medical research, for example, to assess the effects of different medications. The rate of glucose infusion is commonly referred to in diabetes literature as the GINF value (*Sarti et al., 2006*).

The procedure takes about 2 hours. Through a peripheral vein, insulin is infused at 10-120 mU per m<sup>2</sup> per minute. In order to compensate for the insulin infusion, glucose 20% is infused to maintain blood sugar levels between 5 and 5.5 mmol/l. The rate of glucose infusion is determined by checking the blood sugar level every 5 to 10 minutes (*Moadab et al., 2010*).

Low-dose insulin infusions are more useful for assessing the response of the liver, whereas high-dose insulin infusions are useful for assessing peripheral (i.e., muscle and fat) insulin action (*Wierzbicki et al., 2008*).

The rate of glucose infusion during the last 30 minutes of the test determines insulin sensitivity. If high levels (7.5 mg/min or higher) are required, the patient is insulin-sensitive. Very low levels (4.0 mg/min or lower) indicate that the body

is resistant to insulin action. Levels between 4.0 and 7.5 mg/min are not definitive and suggest "impaired glucose tolerance," an early sign of insulin resistance (*van et al., 2011*).

This basic technique can be significantly enhanced by the use of glucose tracers. Glucose can be labeled with either stable or radioactive atoms. Commonly-used tracers are  $^3\text{H}$  glucose (radioactive),  $6,6^2\text{H}$ -glucose (stable) and  $^{13}\text{C}$  glucose (stable). Prior to beginning the hyperinsulinemic period, a 3h tracer infusion enables one to determine the basal rate of glucose production (*Lutsey et al., 2008*).

During the clamp, the plasma tracer concentrations enable the calculation of whole-body insulin-stimulated glucose metabolism, as well as the production of glucose by the body (i.e., endogenous glucose production) (*Uruska et al., 2010*).

### **b-Modified Insulin Suppression Test**

Another measure of insulin resistance is the modified insulin suppression test developed by Gerald Reaven at Stanford University. The test correlates well with the euglycemic clamp with less operator-dependent error. This test has been used to advance the large body of research relating to the metabolic syndrome (*Dushay et al., 2005*).

Patients initially receive 25  $\mu\text{g}$  of octreotide (Sandostatin) in 5 ml of normal saline over 3 to 5 min IV as an initial bolus, and then will be infused continuously with an intravenous infusion of somatostatin (0.27  $\mu\text{g}/\text{m}^2/\text{min}$ ) to suppress endogenous insulin and glucose secretion

---

n. Insulin and 20% glucose is then infused at rates of 32 and 267 mg/m<sup>2</sup>/min, respectively (*Diamantetal., 2006*).

Blood glucose is checked at zero, 30, 60, 90, and 120 minutes, and then every 10 minutes for the last half-hour of the test. These last 4 values are averaged to determine the steady-state plasma glucose level (SSPG). Subjects with a SSPG greater than 150 mg/dl are considered to be insulin-resistant (*Hugetal., 2005*).

### **c-Alternatives**

Given the complicated nature of the "clamp" technique (and the potential dangers of hypoglycemia in some patients), alternatives have been sought to simplify the measurement of insulin resistance. The first was the Homeostatic Model Assessment (HOMA), and a more recent method is the Quantitative Insulin Sensitivity Check Index (QUICKI) (*Morenoetal., 2010*).

Both employ fasting insulin and glucose levels to calculate insulin resistance, and both correlate reasonably with the results of clamping studies. **Wallaceetal.** pointed out that QUICKI is the logarithm of the value from one of the HOMA equations (*Wallaceetal., 2004*).

HOMA was first developed in 1985 by *Matthewsetal.* (*Pazienzaetal., 2007*). The method assesses  $\beta$ -cell function and IR (HOMA-IR) from basal glucose and insulin, or C-peptide, concentrations. HOMA is a member of a family of paradigmatic models which are physiological-based structural models with theoretical solutions adjusted to populat

ion. Thus, data from individuals can be used to yield estimates of  $\beta$ -cell function and insulin sensitivity from the solution of the model without further computation (*White et al., 2008*).

HOMA is a model of the relationship between glucose and insulin dynamics that predicts fasting steady-state glucose and insulin concentrations for a wider range of possible combinations of insulin resistance (IR) and  $\beta$ -cell function. Insulin levels depend on the pancreatic  $\beta$ -cell effect on glucose concentrations, while glucose concentrations are regulated through insulin-mediated glucose production by the liver. Thus, deficient  $\beta$ -cell function will elicit a diminished response of  $\beta$ -cell to glucose-stimulated insulin secretion (*Arase et al., 2009*).

Similarly, IR is reflected in the diminished suppressive effect of insulin on hepatic glucose production (*Moucariet al., 2008*). Therefore, HOMA describes this glucose-insulin homeostasis by means of a set of simple, mathematically derived nonlinear equations. The approximating equation for IR has been simplified and uses a fasting plasma sample in which glucose (fasting blood glucose; FBG) and insulin (fasting insulin; FI) are measured, together with a constant (*Tan et al., 2008*).

The product of  $\text{FBG} \times \text{FI}$  is an index of IR.

HOMA-

$\text{IR} = (\text{glucose} \times \text{insulin}) / 22.5$ . Insulin concentration is reported in  $\mu\text{U/L}$  and glucose in  $\text{mmol/L}$ .



The constant of 22.5 is a normalizing factor,

i.e.  $\text{normal FI of } 5 \mu\text{U/mL} \times \text{the normal FBG of } 4.5 \text{ mmol/L}$  typical of a normal healthy individual = 22.5 (*Kawaguchi et al., 2007*).

Many studies use the HOMA-IR as the diagnostic criteria for insulin resistance (*Tan et al., 2008*). I.R. was considered to be present in cases with  $\text{HOMA} \geq 3.0$  (*Wallace et al., 2004*).

## SUBJECTS AND METHODS

The study was conducted at both Clinical Pathology and Obstetrics and Gynecology Departments— Ain Shams University hospitals in the period between October 2013 and April 2014. Informed consents were obtained from all participants before enrollment in the study.

### I-Subjects:

This study was conducted on ninety (90) females. They were divided into the following groups:-

#### **Group I: Pre-eclampsia Group (PE) (n=60):**

This group included sixty (60) pregnant women with singleton pregnancy between 28 and 32 weeks of gestation, attending the Obstetrics out-patient clinics suffering from pre-eclampsia during the third trimester of pregnancy. Their ages ranged from 20 to 39 years with a mean age of  $25.00 \pm 4.5$ . The diagnosis of PE was based on the presence of hypertension and significant proteinuria (*Arikanetal., 2009*). They were classified according to the severity of pre-eclampsia into two subgroups:

#### **a) Subgroup Ia (mild pre-eclamptic females; n=30):**

This subgroup included thirty pregnant females suffering from mild PE, their ages ranged from 20 to 39 years, with a mean age of  $25 \pm 4.00$  years. The diagnosis was based on the following criteria:

Presence of hypertension (blood pressure of 140/90 mmHg or higher, on at least two occasions, at least 6 hours apart, after the 20th week of gestation) and proteinuria (dipstick showing +1 in a random urine sample) (*Arikanetal., 2009*).

**b) Subgroup Ib (severe pre-eclamptic females; n=30):**

This subgroup included thirty (30) pregnant females suffering from severe PE, their ages ranged from 20 to 39 years with a mean age of  $24.00 \pm 5.00$  years. The diagnosis was based on one or more of the following criteria :

Blood pressure of 160/110 mmHg or higher on two occasions at least six hours apart, a urine dipstick of  $\geq +2$  in a random urine sample, oliguria of less than 400 mL in 24 hours urine sample, pulmonary oedema or cyanosis, visual or cerebral disturbance, impaired liver functions, thrombocytopenia, epigastric or right upper quadrant pain and HELLP syndrome (haemolysis, elevated liver enzymes and low platelets) (*Arikan et al., 2009*).

**Group II: Healthy Control Group (n=30):**

This group included thirty (30) age-matched females. This group was subdivided into:

**Subgroup IIa:** fifteen (15) age-matched, their ages ranged from 20 to 39 years with a mean age of  $25.00 \pm 4.00$ , healthy, non-pregnant females.

**Subgroup IIb:** fifteen (15) age-matched, their ages ranged from 20 to 39 years with a mean age of  $25.00 \pm 3.00$ , pregnant females with non-complicated singleton pregnancy between 28 and 32 weeks of gestation.

All individuals included in this study were subjected to the following after an informed oral consent:

1-

Full history taking focusing on information regarding the current pregnancy including age, medical and obstetric history, significant endocrine disorder in the current pregnancy or in the past and other symptoms dependent on the systemic involvement, such as hypertension, renal or liver function disturbances.

2-

Thorough clinical examination with special emphasis on edema, blood pressure measurement and Maternal body mass index (BMI);  $\text{Index}(\text{Wt}(\text{kg})/\text{height}^2(\text{m}^2))$

3-

Determination of gestational age according to the date of the last menstrual period and confirmed by first trimester ultrasound.

4-Laboratory investigations including:

- a. Liver enzymes (AST, ALT).
- b. Renal function tests (BUN, creatinine).
- c. Complete blood count.
- d. Urine protein estimation by dipstick test in a morning urine sample.
- e. 50g oral glucose challenge test (GCT) for pregnant women only.

f. HOMA-

IR:calculatedbymeasuringfastinginsulin(mU/mL)andfasting bloodglucose(mmol/L).

g. AssayofmaternalserumresistinedbyELISA.

### **ExclusionCriteria:**

Patientswithgestationaldiabetesmellitus,chronichypertension(hypertensionbeforepregnancyandpersistentlyelevatedblood pressurebeforethe20thweekofgestation),diabetesmellitus,polyhydramnios,renaldiseases,multiplegestation.Agelessthan20yearsor morethan39years.

## **II-SAMPLING:**

### **A-BloodSamples:**

Fivemilliliters(5mL)ofvenousbloodwerewithdrawnfromallstudiedsubjectsundercompleteasepticprecautions,andthesamedonefor othersampleafterovernightfasting(8-12hs).

ThecollectedbloodwasdividedintoanEDTAtubeforCBCandplaintesttubeforserumseparation.Aftercompleteclotformation, sampleswerecentrifugedat1500xgfor10minutes.Theseparatedserumwasdividedintotwoaliquots.Onewasdesignatedfortheassayof BUN,AST,ALT,50goralglucosechallengetestandserumcreatinine assessment.Theotheraliquotwasstoredat-20°Cforsubsequentassayofresistin.Theseparated8-12hoursfastingserumfortheassayoffastingglucoseandfastinginsulin levelsfordetectionofinsulinresistance(IR).Hemolysedsamples werediscarded,repeatedfreezingandthawingwasavoided.

## **B. Urine Samples:**

For detection of urinary proteins, morning midstream urine sample was collected from each subject in a clean container. The specimen was examined immediately (*National Committee for Clinical Laboratory Standards, 2007*).

## **III-METHODS:**

### **A. Analytical Methods:**

#### **1-Detection of urine proteins:**

Urinary protein was tested for by dipstick method (ComboStik, DOC.); which is based on the change in the colour of the dye bromophenol blue, in the presence of a binding protein as albumin. Bromophenol blue, buffered to pH 3 with citrate, is present predominantly in the protonated form (yellow form). When protein is added, the affinity of the anionic form of the indicator dye for protein causes a shift of the equilibrium between an anionic and protonated form of the indicator toward formation of the blue anionic species.

The intensity of the blue colour is proportional to the concentration of protein in the specimen. This assay is semi-quantitative as results of +1 correspond to protein concentration of 30 mg/dL, +2 correspond to protein concentration of 100 mg/dL, +3 correspond to protein concentration of 300 mg/dL and +4 correspond to protein concentration of 1000 mg/dL (*Akdeniz et al., 2011*).

#### **2-Complete Blood Count (CBC):**

CBC was done using MaxM Coulter\*.

---

\* Beckman Coulter, Inc., 22 Raio Juste-Olivier, 1260 Nyon-Switzerland.

\*\* Beckman Instruments Inc., Scientific Instruments Division, Fullerton, CA 92634-3100, USA.

### **3-**

**Routine Laboratory Tests:** AST, ALT, BUN and Creatinine were carried out on Synchron PRO autoanalyzer<sup>\*\*</sup>.

### **4-50g glucose challenge test (GCT).**

It is the most widely used screening test for gestational diabetes and is recommended by the American Diabetes Association (ADA). This is performed without regard to time or nature of the last meal, at 24-28 weeks' gestation. Women are given 50g of glucose by mouth and plasma glucose is measured at 1 hour. A positive test is a plasma venous glucose concentration  $\geq 7.8$  mmol/L ( $\geq 140$  mg/dL) (*American Diabetes Association, 2014*).

### **5-Fasting insulin:**

Fasting serum insulin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit DRG® Insulin<sup>\*</sup>

#### **a. Principle of the assay:**

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule. A aliquot of patient sample containing endogenous insulin is incubated in the coated well with enzyme conjugate, which is an anti-insulin antibody conjugated with biotin. After incubation the unbound conjugate is washed off. During the second incubation step streptavidin peroxidase enzyme complex binds to the biotin-anti-

---

<sup>\*</sup>DRG International, Inc. USA, fax: (908) 233 0758 E-mail: corp@drg-international.com.

insulin antibody. The amount of bound HRP complex is proportional to the concentration of insulin in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of insulin in the patient's sample (*Flier et al., 1979*).

**b-Assay procedure:**

- i. 25  $\mu$ l of each standard, controls and samples were added with new disposable tips into appropriate wells.
- ii. 25  $\mu$ l of Enzyme Conjugate were added into each well.
- iii. Thoroughly mixing was done for 10 seconds.
- iv. Incubation for 30 minutes at room temperature without covering the plate was done.
- v. The contents of the wells briskly were shaken out.
- vi. The wells were rinsed 3 times with diluted Wash Solution (400  $\mu$ l per well). The wells were sharply stroked on absorbent paper to remove residual droplets.
- vii. 50  $\mu$ l of Enzyme Complex were added to each well.
- viii. Wells were then incubated for 30 minutes at room temperature.
- ix. Briskly shake out the contents of the wells was done.
- x. The wells were rinsed 3 times with diluted Wash Solution (400  $\mu$ l per well). The wells were sharply stroked on absorbent paper to remove residual droplets.
- xi. 50  $\mu$ l of Substrate Solution were added to each well.
- xii. Wells were then incubated for 15 minutes at room temperature.

- xiii. Stopping the enzymatic reaction was done by adding 50  $\mu$ l of Stop Solution to each well.
- xiv. The OD was read at 455 nm with a microtiter plate reader within 10 minutes after adding the Stop Solution.

**c- Calculation of results:**

- i. The duplicate readings for each standard, control, and sample were averaged and the average zero standard optical density was subtracted.
- ii. The optical density for the standards was plotted versus the concentration of the standards, and the best curve was drawn using log/log paper.
- iii. The Insulin concentration of each sample was determined, by finding the absorbance value on the y-axis and extending a horizontal line to the standard curve. At the point of intersection, a vertical line to the x-axis was extended and the corresponding Insulin concentration determined.

**d-Reference range:**

Reported reference level of fasting insulin in healthy individuals is (2-25  $\mu$ IU/mL) (*Flier et al., 1979*).

**e-Sensitivity:**

The analytical sensitivity of the DRGELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard and was found to be 1.76  $\mu$ IU/mL (*Flier et al., 1979*).

### **f- Linearity:**

This method is linear up to 100  $\mu$ IU/mL (*Flier et al., 1979*).

### **6-Homeostasis model assessment (HOMA) Score:**

It was first described under the name HOMA by *Matthews et al. in 1985*.

It uses the following formula to determine Insulin resistance (IR):

$$\text{Homeostasis model assessment HOMA Score} = \frac{\{\text{Fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)}\}}{22.5}$$

Or

$$\text{Homeostasis model assessment HOMA Score} = \frac{\{\text{Fasting glucose (mg/dL)} \times \text{fasting insulin (mU/L)}\}}{405}$$

I.R. was considered to be present in cases with HOMA  $\geq 3.0$  (*Wallace et al., 2004*).

### **6-Measurement of Serum Resistin:**

Resistin concentrations were measured using a commercial available enzyme-linked immunosorbent assay (ELISA) kit supplied by Assaypro, LLC\*

---

\* Assaypro LLC 30 Triad South Drive St. Charles, MO 63304 T (636) 447-9175 F (636) 447-9475 www.assaypro.com.

**a. Principle:**

The used method is designed for detection of human resistin in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures resistin. A murine monoclonal antibody specific for resistin has been pre-coated onto a microplate. Resistin standards and samples are sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for resistin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

**b. Reagents:**

- i. Resistin microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against resistin.
- ii. Sealing tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- iii. Resistin standard (2 vials): Human resistin in a buffered protein base (64 ng, lyophilized).
- iv. Biotinylated resistin antibody (100x): A 100-fold biotinylated polyclonal antibody against human resistin (80  $\mu$ L).
- v. Streptavidin-Peroxidase conjugate (SP Conjugate): A 100-fold concentrate (90  $\mu$ L).

- vi. EIA diluent concentrate (10x): A 10-fold buffered protein base (30mL).
- vii. Wash buffer concentrate (20x): A 20-fold concentrated buffered surfactant (30 mL).
- viii. Chromogen substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 mL).
- ix. Stop solution: A 0.5 N hydroxychloric acid to stop the chromogen substrate reaction (12 mL).

**c.Reagentspreparation:**

- i. EIA Diluent Concentrate (10x): 30mL of the concentrated EIA diluent was diluted with 270mL reagent grade water to a final volume of 300mL.
- ii. Standard Curve: The 64ng of human resistin standard was reconstituted with 4mL of EIA diluent to generate a stock solution of 16ng/mL. The standard was allowed to sit for 10 minutes with gentle agitation prior to making dilutions. Triplicate standard points were prepared by serially diluting the resistin standard solution (16ng/mL) twofold with the equal volume of EIA diluent to produce 8, 4, 2, 1, 0.5 and 0.25ng/mL solutions. EIA diluent serves as the zero standard (0ng/mL).
- iii. Biotinylated resistin antibody (100x): The antibody was spinned down briefly and the 80 $\mu$ L of the concentrated biotinylated resistin antibody was diluted with 7.920mL EIA diluent to a final volume of 8mL.

- iv. SP conjugate (100x): The SP conjugate was spinned down briefly and the 90  $\mu$ L of the concentrated SP conjugate was diluted with 8.910 mL EI Adiluent to a final volume of 9 mL.
- v. Wash Buffer Concentrate (20x): 30 mL of the concentrated wash buffer was diluted with 570 mL reagent grade water to a final volume of 600 mL.

#### **d. Assay procedure:**

All reagents, working standards and samples were allowed to reach room temperature and mixed thoroughly by gentle inversion before use. The assay was performed at room temperature (20-30<sup>0</sup> C). The assay procedure was as follows:

- i. Serum samples were diluted by adding (100  $\mu$ L serum + 500  $\mu$ L EI Adiluent).
- ii. 50  $\mu$ L of each standard dilutions and diluted serum samples were dispensed with new disposable tips into appropriate wells. Wells were covered and incubated for two hours at room temperature. The timer was started after the last sample addition.
- iii. Each well was aspirated and washed 5 times with the diluted wash buffer using an automatic microplate washer. The plate was inverted on absorbent paper towel to be dried.
- iv. 50  $\mu$ L of biotinylated resin antibody were dispensed to each well and incubated for two hours at room temperature.
- v. Each well was aspirated and washed 5 times with the diluted wash buffer using an automatic microplate washer. The plate was inverted on absorbent paper towel to be dried.

- vi. 50 $\mu$ L of streptavidin-peroxidase conjugate were dispensed to each well and incubated for 30 minutes at room temperature.
- vii. Each well was aspirated and washed 5 times with the diluted wash buffer using an automatic microplate washer. The plate was inverted on absorbent paper towel to be dried.
- viii. 50 $\mu$ L of chromogen substrate were added to each well and incubated for about 10 minutes at room temperature. The plate was gently tapped to ensure thorough mixing and the bubbles in the wells were broken with pipette tip.
- ix. 50 $\mu$ L of stop solution were added to each well.
- x. The absorbance of the solution in the wells was read within 10 minutes using a microplate reader set at a wavelength of 450 nm.

#### **e. Calculation of results:**

The mean absorbance value for each set of reference standards and samples were calculated. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. The best fitting curve was drawn. Using the mean absorbance value for each sample, the corresponding concentration of resistin (ng/mL) was determined from the standard curve.

### **B-Statistical Methods:**

Statistical analysis was done using software version SPSS.

#### **1-Descriptive Statistics:**

### **a- The mean and standard deviation (SD)**

These were used in case of parametric data and were calculated as follows:

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

$$SD = \sqrt{\frac{\sum (X - \bar{X})^2}{n - 1}}$$

where:  $\sum$  = Sum of observed values

$X$  = A given value of a variable

$\bar{X}$  = Arithmetic mean

$n$  = Total number of values (observations)

### **b- The median and interquartile range:**

They were used in case of skewed data. Values were rearranged in an ascending order. Each of these values was given a rank number, with the smallest value given the smallest rank number. The rank number of the median was calculated as  $(n+1) \div 2$ , where  $n$  is the number of observations. The original value corresponding to the rank number was the one obtained.

The interquartile range is that between the 25th and 75th percentiles (25th P and 75th P, respectively).

The rank number of the 25th percentile =  $0.25 \times (n+1)$ , and that of the 75th percentile =  $0.75 \times (n+1)$ , where  $n$  is the number of observations. The original values corresponding to these rank numbers were the ones obtained (**Bourke et al., 1985**).

## 2-Significance Tests:

### a-The Student's t test:

The 't' value was calculated from the table, the probability (p) value for the calculated 't' value was then deduced, with the degrees of freedom being equal to the sum of the two sample sizes minus 2. p values > 0.05 indicate a non-significant difference, p < 0.05 indicates a significant difference, and p < 0.01 indicates a highly significant difference (*Fogiel, 1986*).

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(s_1)^2}{n_1} + \frac{(s_2)^2}{n_2}}}$$

where,

$\bar{X}_1$  = the mean of sample 1.

$\bar{X}_2$  = the mean of sample 2.

$S_1$  = standard deviation in sample 1.

$S_2$  = standard deviation in sample 2.

$n_1$  = the number of subjects in sample 1.

$n_2$  = the number of subjects in sample 2.

### b. Wilcoxon's rank sum test (Mann-Whitney U test):

Data from each two compared groups were combined and arranged from the lowest to the highest. Rank numbers were given to these values, the smallest rank number being given to the lowest value, and t

the greatest rank number being given to the highest value. When two values were identical, they were each given the average of the next two ranks.

The two groups were then separated and the sum of ranks of each group was calculated. The "z" value was then calculated as follows:

$$Z_T = \frac{(\mu_T - T - 1/2)}{\sigma_T}$$
$$\mu_T = \frac{n_1(n_1 + n_2 + 1)}{2}$$

where,

**n1** is the sample size of the numerically smaller group

**n2** is that of the larger group.

**T** is calculated from the numerically smaller group, and is calculated from the normal rank total ( $T_1$ ), when the lower ranks predominate in this group. When higher ranks predominate  $T$  is equal to  $1(n_1 + n_2 + 1) - T_1$

$$\sigma_T = \sqrt{\frac{n_2 \mu}{6}}$$

From statistical tables, the probability (p) values for the calculated "z" values were then deduced, where  $p > 0.05$  represents non-significant difference,  $p < 0.05$  represents significant difference, and  $p < 0.01$  represents a highly significant difference (*Fogiel, 1986*).

### **3-Correlation Study:**

Spearman's rank correlation coefficient ( $r_s$ ) was used to assess the degree of association between two sets of variables if one or both of them was skewed. The significance of correlation was then determined from certain tables looking for the critical value ( $r_c$ ) for the observed  $n$  (*Bourke et al., 1985*).

In order to calculate  $r_s$ , the observations of each variable ( $x$  and  $y$ ) were ranked, then the rank numbers of the  $x$  and  $y$  variables were assigned separately, from lowest to highest, giving the average of the next two ranks to similar observations.

Then for each pair, the rank of variable  $y$  was subtracted from the rank of variable  $x$  and the result was called "D". Each "D" was squared and added to obtain  $\sum D^2$ . The Spearman's rank correlation coefficient was then calculated as follows:

$$r_s = 1 - \frac{6 \times \sum D^2}{n(n^2 - 1)}$$

where,  $n$  is the number of pairs of observations of an  $x$  and  $y$  variable.

The significance of correlation was then determined from certain tables looking for the critical value ( $r_c$ ) for the observed  $n$ . Significant correlation was predicted if  $r_s \geq r_c$  or  $r_s \leq -r_c$  (*Bourke et al., 1985*).

Pearson's correlation coefficient when applied to a sample is commonly represented by the letter  $r$  and may be referred to as the *sample correlation coefficient* or the *sample Pearson correlation coefficient*. We can obtain a formula for  $r$  by substituting estimates of the covarian

ces and variances based on a sample into the formula above. That formula for is:

$$r = \frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^n (X_i - \bar{X})^2} \sqrt{\sum_{i=1}^n (Y_i - \bar{Y})^2}}$$

An equivalent expression gives the correlation coefficient as the mean of the products of the standard scores. Based on a sample of paired data  $(X_i, Y_i)$ , the sample Pearson correlation coefficient is:

$$r = \frac{1}{n-1} \sum_{i=1}^n \left( \frac{X_i - \bar{X}}{s_X} \right) \left( \frac{Y_i - \bar{Y}}{s_Y} \right)$$

where

$$\frac{X_i - \bar{X}}{s_X}, \bar{X} = \frac{1}{n} \sum_{i=1}^n X_i, \text{ and } s_X = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})^2}$$

are the standard score, sample mean, and sample standard deviation, respectively.

#### **4-Evaluation of Diagnostic Performance:**

##### **a. Diagnostic sensitivity:**

It measures the incidence of true positive results in patients group.

$$\text{Diagnostic sensitivity} = \frac{TP}{(TP+FN)} \times 100$$

Where:

**TP**(true positive) is the number of diseased patients accurately classified by the test and **FN**(false negative) is the number of diseased patients misclassified by the test.

**b. Diagnostic specificity:**

It measures the incidence of true negative results in a non-diseased group.

$$\text{Diagnostic specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100$$

Where:

**TN**(true negative) is the number of non-diseased subjects accurately classified by the test and **FP**(false positive) is the number of non-diseased subjects misclassified by the test.

**c. Diagnostic efficiency:**

It is the percent of true positive and negative results among all results.

$$\text{Diagnostic efficiency} = \frac{(TN + TP)}{(TN + TP + FN + FP)} \times 100$$

**d. Positive predictive value:**

It is the percent of true positive results among all positive results

$$\text{Positive predictive value} = \frac{TP}{(TP + FP)} \times 100$$

**e. Negative predictive value:**

It is the percent of true negative results among all negative results.

$$\text{Negative predictive value} = \frac{TN}{(TN + FN)} \times 100$$

*(Edward and Schultz, 1999).*

**Receiver-operating characteristic (ROC) curve analysis:**

The overall diagnostic performance of each test was assessed by receiver-operating characteristics (ROC) curve analysis. The ROC-curve was plotted as follows:

- The X-axis represents the false-positive rate (1-specificity %).

- The Y-axis represents the diagnostic sensitivity %.

- The dotted diagonal line extending from the lower left corner to the upper right corner represents a test with no discrimination.

- A curve that extends from the lower left corner to the upper left corner then to the upper right corner is considered a perfect test.

- The area under the curve (AUC) describes the overall test performance.

The best cutoff point is the point nearest to the upper left corner (*Zweig and Campbell, 1993*).

## RESULTS

**R**esults of the present study are expressed in Tables (1-16) and Figures (1-12).

Descriptive statistics of the various studied parameters in all studied groups are shown in Tables 1 and 2.

Statistical comparison between pre-eclamptic patients collectively (Group I), and the healthy control group (Group II) regarding the different studied parameters using student's *t* test for parametric data and Wilcoxon's rank sum test for non-parametric data revealed a highly statistically significant increase in the patients group than the control group regarding SBP, DBP, BMI, CRE, AST, ALT, 50g glucose test, FBG, fasting insulin, HOMA-IR and resistin. On the contrary, there was a highly statistically significant decrease in the patients group than the control group regarding HB. It also revealed a statistically significant increase in the patients group than the control group regarding BUN while there was a statistically significant decrease in the patients group than the control group regarding PLT and also a non-statistically significant difference regarding gestational age (GA) (Tables 3 & 4 and Figure 1).

Statistical comparison between healthy pregnant controls (group IIb) and healthy non-

pregnant controls (group IIa) regarding the different studied parameters using student's t test for parametric data and Wilcoxon's rank sum test for non-parametric data revealed a highly statistically significant increase in the healthy pregnant controls group than the healthy non-pregnant controls group regarding FBG, fasting insulin, HOMA-IR and BMI while there was a highly statistically significant decrease in the healthy pregnant controls group than the healthy non-pregnant controls group regarding BUN and HB. It also revealed a statistically significant increase in the healthy pregnant controls group than the healthy non-pregnant controls group regarding SBP and resistin, while there was a statistically significant decrease in the healthy pregnant controls group than the healthy non-pregnant controls group regarding ALT and a non-significant difference in age, DBP, creatinine, AST, and PLT (Tables 5 & 6 and Figure 2).

On comparing the mild pre-eclamptic groups (group Ia) and severe pre-eclamptic group (group Ib) using student's t test for parametric data and Wilcoxon's rank sum test for non-parametric data, there was a highly statistically significant increase in the severe pre-eclamptic group than the mild pre-eclamptic group regarding GA, SBP, DBP, AST, ALT, 50g glucose test, HB and resistin with a recorded statistically significant increase in severe pre-eclamptic group than the mild pre-

eclamptic group regarding BUN and HOMA-IR. In contrast, there was a statistically significant decrease in severe pre-eclamptic group than the mild pre-eclamptic group regarding PLT and non-significant difference in age, BMI, CRE, FBG and fasting insulin (Tables 7 & 8 and Figure 3).

Statistical comparison between patients group (group I) and control pregnant group (group IIb) regarding the different studied parameters using student's t test for parametric data and Wilcoxon's rank sum test for non-parametric data revealed a highly statistically significant increase in SBP, DBP, 50g glucose test, CRE, BUN, ALT, FBG, fasting insulin, HOMA-IR and resistin in patient group while there was a statistically significant decrease in HB in patients group than control pregnant group and with recorded statistically significant regarding AST. A non-significant statistical difference was recorded between the two groups regarding age, GA, PLT and BMI (Tables 9 & 10 and figure 4).

Statistical comparison between patients group (group I) and control nonpregnant group (group IIa) regarding the different studied parameters using student's t test for parametric data and Wilcoxon's rank sum test for non-parametric data revealed a highly statistically significant increase in SBP, DBP, BMI, CRE, FBG, fasting insulin, HOMA-IR and resistin in patient group with recorded highly statistically significant decrease in PLT and HB. There was a statistically significant

---

decrease in AST in patients group. A non-significant difference was recorded between the two groups regarding age, ALT and BUN (Table 11 & 12 and figure 5).

Correlation study between resistin and the other studied parameters in pre-eclamptic patients collectively using Spearman's rank correlation coefficient test ( $r_s$ ) is shown in (Tables 13 and 14). It revealed a significant positive correlation between resistin and SBP, DBP, HOMA-IR, GA, 50g glucose test, fasting insulin, FBG, AST, BUN and ALT and a non-significant correlation between resistin and age, GA, BMI, CRE, HbA and PLT.

Correlation study between HOMA-IR and the other studied parameters in pre-eclamptic patients collectively using Spearman's rank correlation coefficient test ( $r_s$ ) is shown in (Tables 15 and 16 and figure 6). It revealed a significant positive correlation between HOMA-IR and resistin, DBP, GA, 50g glucose test, fasting insulin, FBG and ALT and a non-significant correlation between HOMA-IR and age, SBP, BMI, CRE, BUN, AST, HbA and PLT.

Receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic utility of resistin in discriminating pre-eclamptic patients from the control group. The best diagnostic cut-off

---

level for resistin was 10 ng/mL. This had a diagnostic sensitivity of 88.33%, specificity 96.76%, negative predictive value 80.6%, positive predictive value 98.1%. The area under the curve (AUC) was 96.7 as shown in (Figure 7).

In addition, when ROC curve analysis was applied to assess the diagnostic performance of resistin in discriminating severe pre-eclamptic patients from those with mild pre-eclampsia, it was found that the best cutoff value was 17 ng/mL. This had a diagnostic sensitivity of 96.76%, specificity 70%, negative predictive value 95.5%, positive predictive value 76.3%. The area under the curve (AUC) was 90.8 as shown in (Figure 8).

Receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic utility of HOMA-IR in discriminating pre-eclamptic patients from the control group. The best diagnostic cutoff level for resistin was 2.05 ng/mL giving a diagnostic sensitivity of 98.33%, specificity 86.67%, negative predictive value 96.3%, positive predictive value 93.7%. The area under the curve (AUC) was 97.3 as shown in (Figure 9).

In addition, when ROC curve analysis was applied to assess the diagnostic performance of HOMA-IR in discriminating severe pre-eclamptic patients from those with mild pre-eclampsia, it was found that the best cutoff value was 3.69 ng/mL. This had a diagnostic sensitivity of 93.33%, specificity 40%, negative pred

ictivevalue85.7%,positivepredictivevalue60.9%.Theareaunderthecurve(AUC)was64.9asshownin(Figure10).

**Table (1):** Descriptive Statistics of age, GA, SBP, DBP and BMI in the Different Studied Groups.

Parameter	Group Ia(n=30)	Group Ib(n=30)	Group IIa(n=15)	Group IIb(n=15)
	$\bar{X} \pm SD$ Median and (IQR)*			
Age(Years)	25.00±4.00	24.00±5.00	25.00±4.00	25.00±3.00
GA(weeks)	27.00±2.50	30.00±1.00	-	27.00±2.50
SBP(mmHg)	154.00±4.00	171.00±6.00	105.00±10.00	115.00±10.00
DBP(mmHg)	98.00±2.50	112.00±3.00	69.00±8.00	72.00±8.00
BMI	27.80±3.10	28.60±2.30	23.53±3.71	27.50±2.82

**Table (2):** Descriptive Statistics of the Various Studied Parameters in the Different Studied Groups.

Parameter	Group I a (n=30)	Group I b(n=30)	Group II a(n=15)	Group II b(n=15)
	$\bar{X} \pm SD$ Median and (IQR)*			
50gglucosetest(mg/dL)	127.00±7.00	132.00±5.50	-	109.00±5.33
CRE(mg/dL)	0.86±0.19	0.83±0.42	0.66±0.15	0.61±0.23
BUN(mg/dL)	10.00±4.00	15.00±8.50	12.00±3.00	6.87±1.19
AST(IU/L)	17.00±5.00	34.50±9.00	18.50±3.50	18.50±4.00
ALT(IU/L)	24.00±8.00	39.00±14.00	27.00±10.00	18.50±6.00
PLTS(x103/μL)	208.00±42.00	183.00±54.00	239.00±63.00	195.13±47.0
HB(g)	9.60±1.70	10.80±1.40	12.50±0.43	11.70±0.90
FBG(mg/dl)	85.00±7.00	87.00±6.00	68.00±4.94	77.00±5.00
Fastinginsulin(μU/mL)	18.40±3.20	20.33±4.78	6.99±0.64	10.87±4.11
HOMA-IR	3.93±0.95	4.50±0.98	1.17±0.13	2.10±0.92
Resistin(ng/ml)	15(11.8–25)*	35(29.5–66.25)*	3.5(1–6.5)*	7.5(3.5–9)*

- **IQR\*** = Interquartilerange.
- Dataareexpressedas  $\bar{X} \pm SD$ forparametricdata.
- Medianand(Interquartilerange)\*fornon-parametricdata.

**Table (3):** Statistical Comparison between Patients Group and Control Group Regarding age, GA, SBP, DBP and BMI, Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non-Parametric Data.

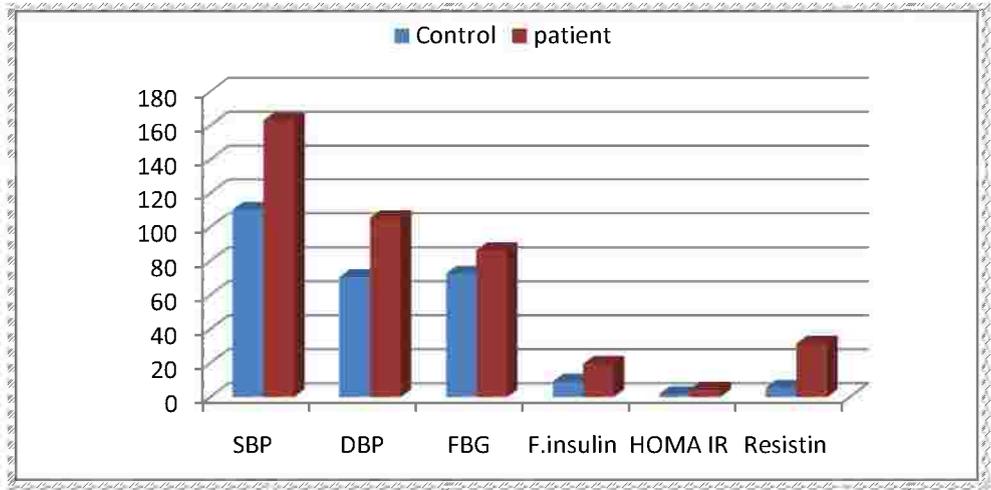
Parameters	Group I (n=60) $\bar{X} \pm SD$ Median and (IQR) *	Group II (n=30) $\bar{X} \pm SD$ Median and (IQR) *	P
Age(Years)	25.00±4.50	25.00±3.50	>0.05
GA(weeks)	28.00±2.50	27.00±2.50	>0.05
SBP(mmHg)	162.00±10.00	110.00±11.00	<0.01
DBP(mmHg)	105.00±7.00	70.00±8.00	<0.01
BMI	28.00±3.00	25.52±3.81	<0.01

- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range) \* for non- parametric data.
- **p>0.05:** Non significant difference.
- **p<0.05:** Significant difference.
- **p<0.01:** Highly significant difference.

**Table (4):** Statistical Comparison between Patients Group and Control Group Regarding the Different Studied Parameters Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non- Parametric Data.

Parameters	Group I (n=60) $\bar{X} \pm SD$ Median and (IQR) *	Group II (n=30) $\bar{X} \pm SD$ Median and (IQR) *	p
50gglucosetest(mg/dL)	129.00±7.00	109.00±5.00	<0.01
CRE(mg/dL)	0.85±0.22	0.64±0.19	<0.01
BUN(mg/dL)	12.00±7.00	9.00±4.00	<0.05
AST(IU/L)	26.00±11.00	18.00±4.00	<0.01
ALT(IU/L)	31.00±14.00	23.00±10.00	<0.01
PLTS(x103/μL)	195.00±50.00	253.00±197.00	<0.05
HB(g)	10.24±1.70	12.11±0.78	<0.01
FBG(mg\dl)	86.00±7.00	28.00±7.00	<0.01
Fastinginsulin(μU/mL)	19.40±4.14	8.93±3.50	<0.01
HOMA-IR	4.24±1.01	1.64±0.80	<0.01
Resistin*(ng\ml)	31.22±21.17	5.37±3.49	<0.01

- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range)\* for non-parametric data.
- **p>0.05:** Nonsignificant difference.
- **p<0.05:** Significant difference.
- **p<0.01:** Highly significant difference.



**Figure (1):** Comparison between control and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

**Table (5):**  
 Statistical Comparison between the Control groups Regard  
 dingage, SBP, DBP and  
 BMI, Using Student 'st Test for Parametric Data and Wilco  
 xon's Rank Sum Test for non-Parametric Data.

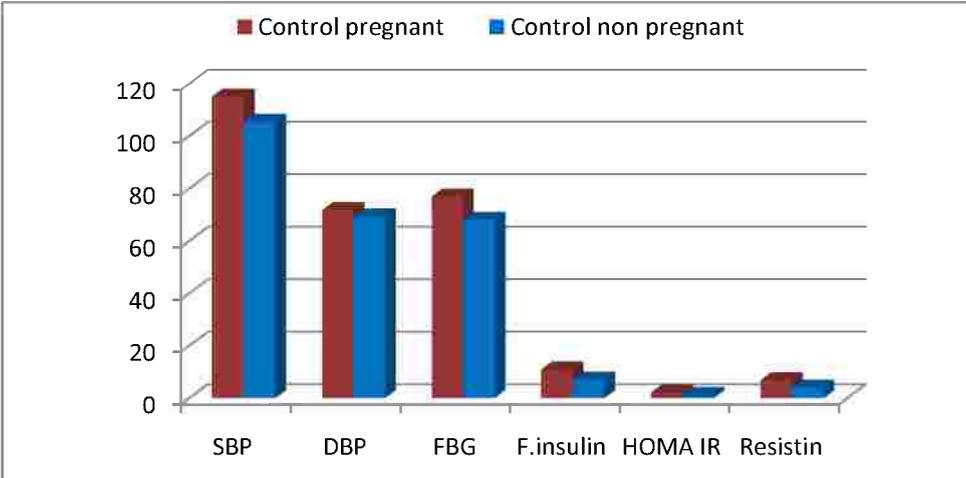
Parameters	Group IIb (n=15)	Group IIa (n=15)	p
	$\bar{X} \pm SD$ Median and (IQR)*	$\bar{X} \pm SD$ Median and (IQR)*	
Age (Years)	25.00±3.00	25.00±4.00	>0.05
SBP (mmHg)	115.00±10.00	105.00±10.00	<0.05
DBP (mmHg)	72.00±8.00	69.00±8.00	>0.05
BMI	27.50±2.82	23.53±3.71	<0.01

- **z\*** = Wilcoxon's Rank Sum Test for non- parametric data.
- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range) \* for non- parametric data.
- **p>0.05:** Non significant difference.
- **p<0.05:** Significant difference.
- **p<0.01:** Highly significant difference.

**Table (6):** Statistical Comparison between the Control groups Regarding the Different Studied Parameters Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non- Parametric Data.

Parameters	Group II b (n=15)	Group II a (n=15)	P
	$\bar{X} \pm SD$ Median and (IQR) *	$\bar{X} \pm SD$ Median and (IQR) *	
CRE(mg/dL)	0.61±0.23	0.66±0.15	>0.05
BUN(mg/dL)	6.87±1.19	12.00±3.00	<0.01
AST(IU/L)	18.50±4.00	18.50±3.50	>0.05
ALT(IU/L)	18.50±6.00	27.00±10.00	<0.05
PLTS(x103/μL)	195.13±47.0	239.00±63.00	>0.05
HB(g)	11.70±0.90	12.50±0.43	<0.01
FBG(mg\dl)	77.00±5.00	68.00±4.94	<0.01
Fastinginsulin(μU/mL)	10.87±4.11	6.99±0.64	<0.01
HOMA-IR	2.10±0.92	1.17±0.13	<0.01
Resistin(ng\ml)	7.5(3.5–9)*	3.5(1–6.5)*	<0.05

- **z\*** =Wilcoxon'sRankSumTestfornon-parametricdata.
- Dataareexpressedas  $\bar{X} \pm SD$ forparametricdata.
- Medianand(Interquartilerange)\*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.05:**Significantdifference.
- **p<0.01:**Highlysignificantdifference.



**Figure (2):** Comparison between control pregnant and control non pregnant groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

**Table (7):** Statistical Comparison between Pre-eclamptic Females Classified According to Disease Severity Regarding age, GA, SBP, DBP and BMI, Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non-Parametric Data.

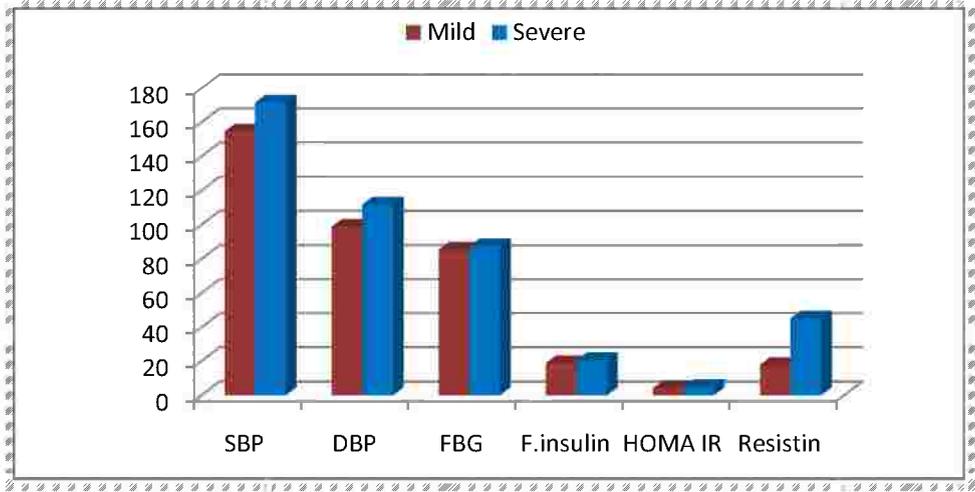
Parameters	Group Ia (n=30)	Group Ib (n=30)	P
	$\bar{X} \pm SD$ Median and (IQR)*	$\bar{X} \pm SD$ Median and (IQR)*	
Age (Years)	25.00 ± 4.00	24.00 ± 5.00	>0.05
GA (weeks)	27.00 ± 2.50	30.00 ± 1.00	<0.01
SBP (mmHg)	154.00 ± 4.00	171.00 ± 6.00	<0.01
DBP (mmHg)	98.00 ± 2.50	112.00 ± 3.00	<0.01
BMI	27.80 ± 3.10	28.60 ± 2.30	>0.05

- $z^*$  = Wilcoxon's Rank Sum Test for non-parametric data.
- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range) \* for non-parametric data.
- **p > 0.05:** Non significant difference.
- **p < 0.01:** Highly significant difference.

**Table (8):** Statistical Comparison between Pre-eclamptic Females Classified According to Disease Severity Regarding the Different Studied Parameters Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non- Parametric Data.

Parameters	Group I a (n=30) $\bar{X} \pm SD$ Median and (IQR) *	Group I b (n=30) $\bar{X} \pm SD$ Median and (IQR) *	P
50gglucosetest(mg/dL)	127.00±7.00	132.00±5.50	<0.01
CRE(mg/dL)	0.86±0.19	0.83±0.42	>0.05
BUN(mg/dL)	10.00±4.00	15.00±8.50	<0.05
AST(IU/L)	17.00±5.00	34.50±9.00	<0.01
ALT(IU/L)	24.00±8.00	39.00±14.00	<0.01
PLTS(x103/μL)	208.00±42.00	183.00±54.00	>0.05
HB(g)	9.60±1.70	10.80±1.40	<0.01
FBG(mg\dl)	85.00±7.00	87.00±6.00	>0.05
Fastinginsulin(μU/mL)	18.40±3.20	20.33±4.78	>0.05
HOMA-IR	3.93±0.95	4.50±0.98	<0.05
Resistin(ng/ml)	15(11.8–25)*	35(29.5–66.25)*	<0.01

- $z^*$  =Wilcoxon'sRankSumTestfornon-parametricdata.
- Dataareexpressedas  $\bar{X} \pm SD$ forparametricdata.
- Medianand(Interquartilerange)\*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01:**Highlysignificantdifference.



**Figure (3):** Comparison between mild pre-eclampsia and sever pre-eclampsia groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin

**Table (9):**  
 Statistical Comparison between Patient's Group and Control pregnant Group Regarding age, GA, SBP, DBP and BMI, Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non-Parametric Data

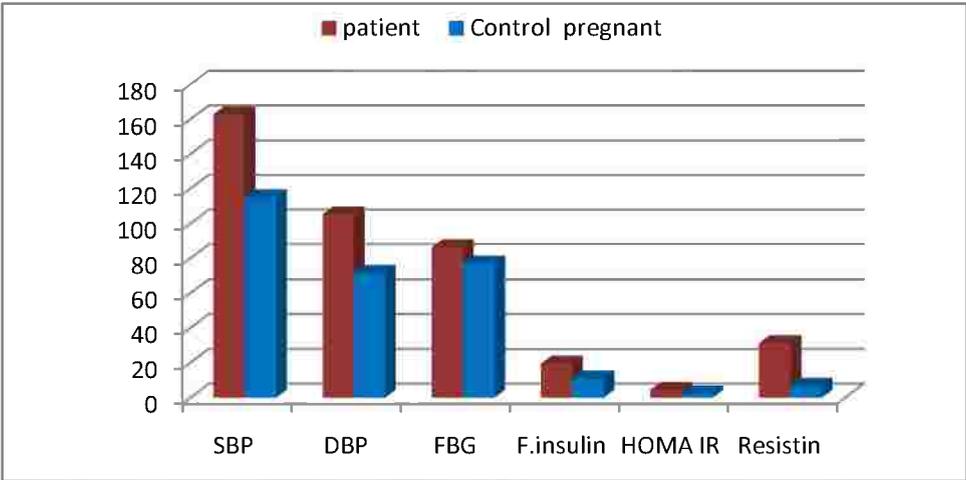
Parameters	Group I (n=60)	Group II (n=15)	p
	$\bar{X} \pm SD$ Median and (IQR) *	$\bar{X} \pm SD$ Median and (IQR) *	
Age(Years)	25.00±4.50	25.50±3.00	>0.05
GA(weeks)	28.00±2.50	27.00±2.50	>0.05
SBP(mmHg)	162.00±10.00	115.00±10.00	<0.01
DBP(mmHg)	105.00±7.00	72.00±8.00	<0.01
BMI	28.00±3.00	27.50±2.82	>0.05

- $z^*$  = Wilcoxon's Rank Sum Test for non- parametric data.
- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range) \* for non- parametric data.
- **p>0.05:** Non significant difference.
- **p<0.01:** Highly significant difference.

**Table (10):** Statistical Comparison between Patient's Group and Control pregnant Group Regarding the Different Studied Parameters Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non- Parametric Data

Parameters	Group I (n=60)	Group II b (n=15)	p
	$\bar{X} \pm SD$ Median and (IQR) *	$\bar{X} \pm SD$ Median and (IQR) *	
50gglucosetest(mg/dL)	129.00±7.00	109.00±5.33	<0.01
CRE(mg/dL)	0.85±0.22	0.61±0.23	<0.01
BUN(mg/dL)	12.00±7.00	6.87±1.19	<0.01
AST(IU/L)	26.00±11.00	18.50±4.00	<0.05
ALT(IU/L)	31.00±14.00	18.50±6.00	<0.01
PLTS(x103/μL)	195.00±50.00	195.13±47.0	>0.05
HB(g)	10.24±1.70	11.70±0.90	<0.05
FBG(mg\dl)	86.00±7.00	77.00±5.00	<0.01
Fastinginsulin(μU/mL)	19.40±4.14	10.87±4.11	<0.01
HOMA-IR	4.24±1.01	2.10±0.92	<0.01
Resistin(ng\ml)	31.22±21.17	7.5(3.5-9)*	<0.01

- $z^*$  =Wilcoxon'sRankSumTestfornon-parametricdata.
- Dataareexpressedas  $\bar{X} \pm SD$ forparametricdata.
- Medianand(Interquartilerange)\*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01:**Highlysignificantdifference.



**Figure (4):** Comparison between control pregnant and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

**Table (11):** Statistical Comparison between Patients Group and Control nonpregnant Group Regarding age, SBP, DBP

and  
 BMI, Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non-Parametric Data.

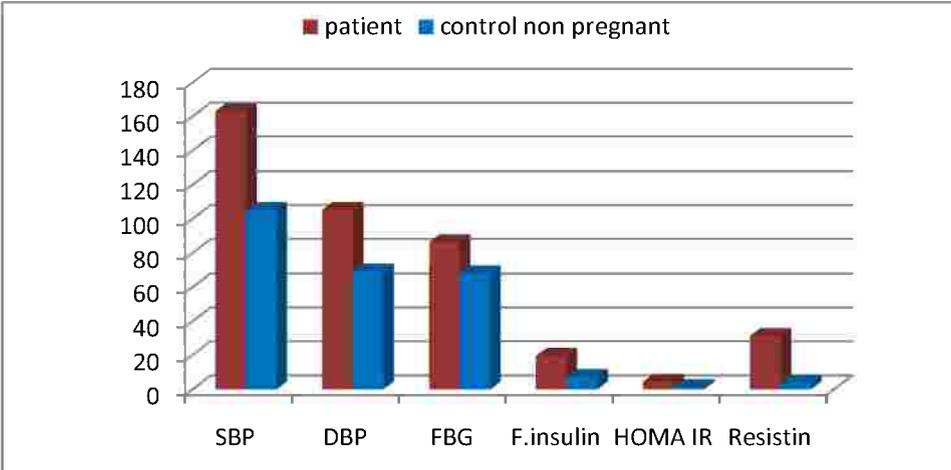
Parameters	Group I (n=60)	Group II a (n=15)	p
	$\bar{X} \pm SD$ Median and (IQR) *	$\bar{X} \pm SD$ Median and (IQR) *	
Age(Years)	25.00±4.50	25.00±4.00	>0.05
SBP(mmHg)	162.00±10.00	105.00±10.00	<0.01
DBP(mmHg)	105.00±7.00	69.00±8.00	<0.01
BMI	28.00±3.00	23.53±3.71	<0.01

- **z\*** = Wilcoxon's Rank Sum Test for non- parametric data.
- Data are expressed as  $\bar{X} \pm SD$  for parametric data.
- Median and (Interquartile range) \* for non- parametric data.
- **p>0.05**: Non significant difference.
- **p<0.01**: Highly significant difference.

**Table (12):** Statistical Comparison between Patients Group and Control non pregnant Group Regarding the Different Studied Parameters Using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for non-Parametric Data.

Parameters	Group I (n=60) $\bar{X} \pm SD$ Median and (IQR) *	Group II a (n=15) $\bar{X} \pm SD$ Median and (IQR) *	p
CRE(mg/dL)	0.85±0.22	0.66±0.15	<0.01
BUN(mg/dL)	12.00±7.00	12.00±3.00	>0.05
AST(IU/L)	26.00±11.00	18.50±3.50	<0.05
ALT(IU/L)	31.00±14.00	27.00±10.00	>0.05
PLTS(x103/ $\mu$ L)	195.00±50.00	239.00±63.00	<0.01
HB(g)	10.24±1.70	12.50±0.43	<0.01
FBG(mg\dl)	86.00±7.00	68.00±4.94	<0.01
Fastinginsulin( $\mu$ U/mL)	19.40±4.14	6.99±0.64	<0.01
HOMA-IR	4.24±1.01	1.17±0.13	<0.01
Resistin(ng\ml)	31.22±21.17	3.5(1–6.5)*	<0.01

- **z** \* =Wilcoxon'sRankSumTestfornon-parametricdata.
- Dataareexpressedas  $\bar{X} \pm SD$ forparametricdata.
- Medianand(Interquartilerange)\*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01:**Highlysignificantdifference.



**Figure (5):** Comparison between control non pregnant and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

**Table (13):** Correlation Study between resistin & HOMA IR, age, GA, SBP, DBP and BMI, in Pre-eclamptic Patients Using Spearman's Rank Correlation Coefficient Test ( $r_s$ ):

	Resistin	
	rs	p-value
HOMA IR	0.544	<0.001
Age (Years)	-0.090	>0.05
GA (weeks)	0.474	<0.001
SBP (mmHg)	0.575	<0.001
DBP (mmHg)	0.626	<0.001
BMI	0.202	>0.05

**Table (14):** Correlation Study between resistin and the Other Studied Parameters in Pre-eclamptic Patients Using Spearman's Rank Correlation Coefficient Test ( $r_s$ ):

	Resistin	
	rs	p-value
50gglucosetest (mg/dL)	0.494	<0.001
FBG (mg/dl)	0.331	<0.05
Fasting insulin ( $\mu$ U/mL)	0.469	<0.001
CRE (mg/dL)	-0.096	>0.05
BUN (mg/dL)	0.292	<0.05
AST (IU/L)	0.558	<0.001
ALT (IU/L)	0.505	<0.001
PLTS ( $\times 10^3/\mu$ L)	-0.056	>0.05
HB (g)	0.183	>0.05

- **p > 0.05:** Nonsignificant correlation.
- **p < 0.01:** Highly significant correlation.

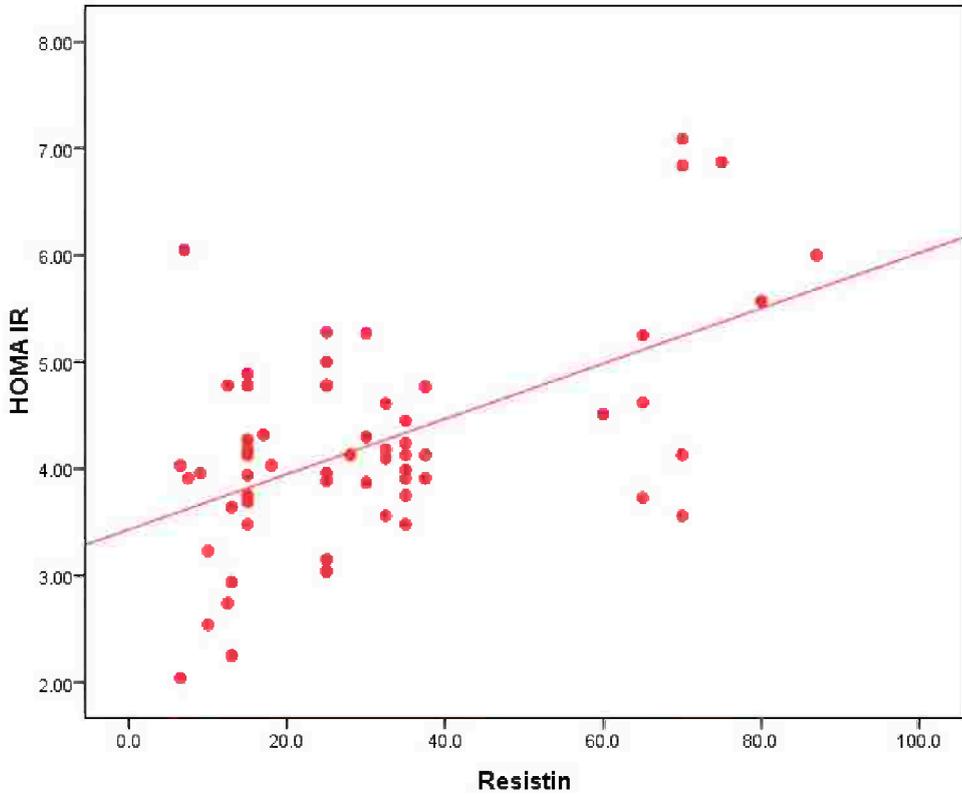
**Table (15):** Correlation Study between HOMA-IR & Resistin, age, GA, SBP, DBP and BMI, in Pre-eclamptic Patients Using Pearson correlation Test (r):

	<b>HOMA IR</b>	
	<b>r</b>	<b>p-value</b>
Resistin(ng/ml)	0.544	<0.001
Age(Years)	-0.085	>0.05
GA(weeks)	0.260	<0.05
SBP(mmHg)	0.234	>0.05
DBP(mmHg)	0.334	<0.001
BMI	0.153	>0.05

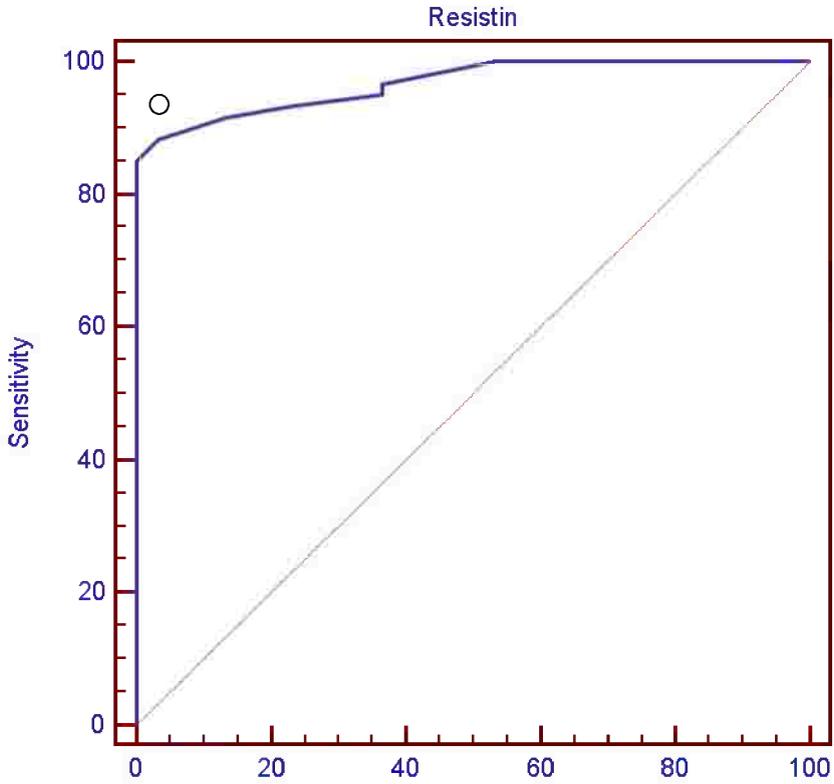
**Table (16):** Correlation Study between HOMA-IR & the Other Studied Parameters in Pre-eclamptic Patients Using Pearson correlation Test (r):

	<b>HOMA IR</b>	
	<b>r</b>	<b>p-value</b>
50gglucosetest(mg/dL)	0.606	<0.001
FBG(mg/dl)	0.840	<0.001
Fastinginsulin( $\mu$ U/mL)	0.812	<0.001
CRE(mg/dL)	-0.023	>0.05
BUN(mg/dL)	0.076	>0.05
AST(IU/L)	0.234	>0.05
ALT(IU/L)	0.311	<0.05
PLTS( $\times 10^3/\mu$ L)	-0.089	>0.05
HB(g)	0.062	>0.05

- **p>0.05:** Nonsignificant correlation.
- **p<0.01:** Highly significant correlation.

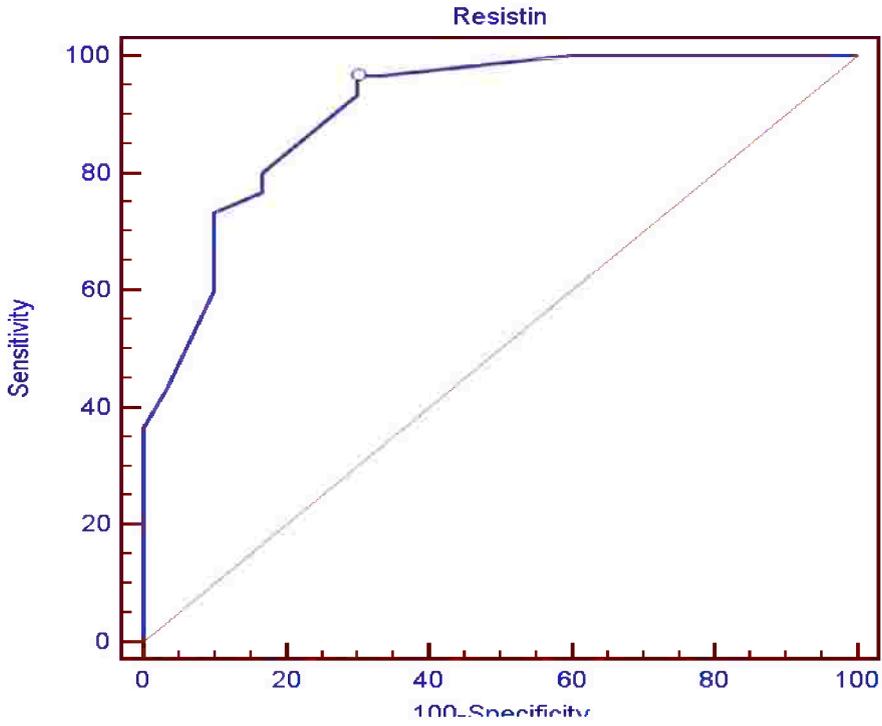


**Figure (6):**Correlation Study between HOMA-IR and resistin in Pr-eclamptic Patients.



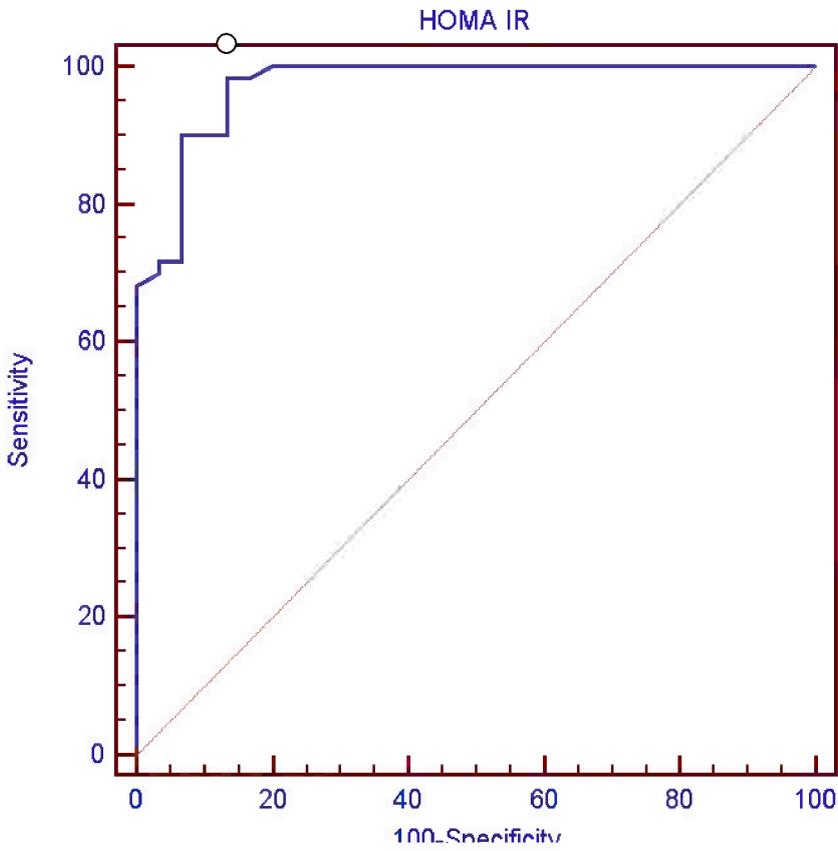
Cutoffpoint	AUC	Sensitivity	Specificity	+PV	-PV
>10	96.7	88.33	96.67	98.1	80.6

**Figure (7):** ROC Curve analysis showing the diagnostic performance of Resistin in discriminating pre-eclamptic females from healthy controls.



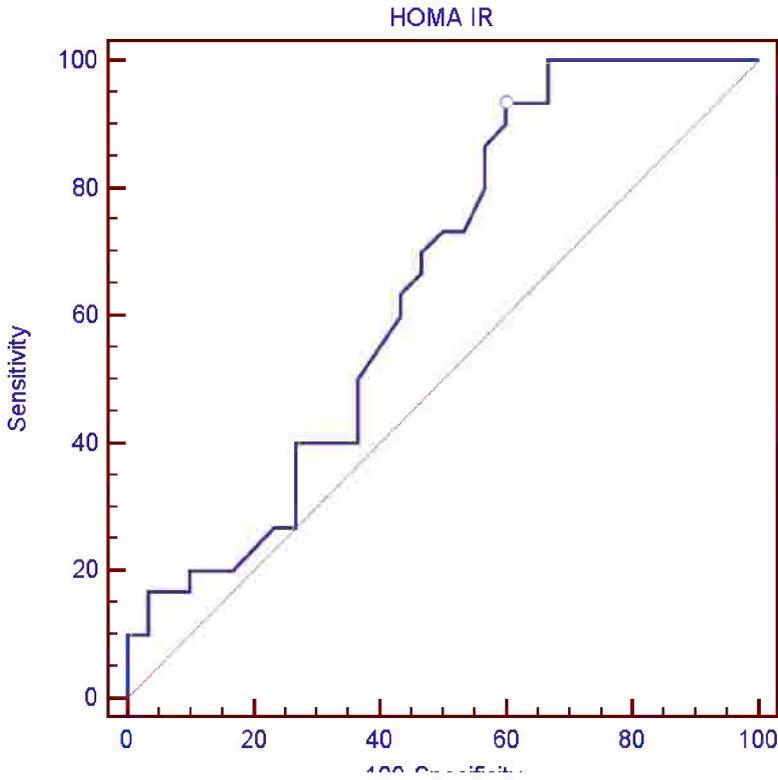
Cutoffpoint	AUC	Sensitivity	Specificity	+PV	-PV
>17	90.8	96.67	70.00	76.3	95.5

**Figure (8):** ROC Curve analysis showing the diagnostic performance of resistin in discriminating mild from severe cases of pre-eclampsia.



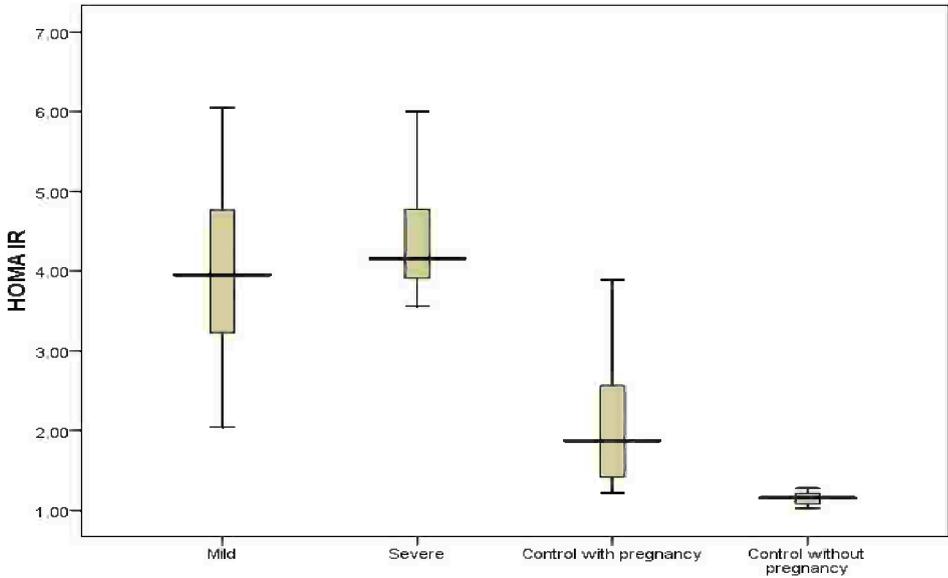
Cutoffpoint	AUC	Sensitivity	Specificity	+PV	-PV
>2.05	97.3	98.33	86.67	93.7	96.3

**Figure (9):** ROC Curve analysis showing the diagnostic performance of HOMA IR in discriminating pre-eclamptic females from healthy controls.

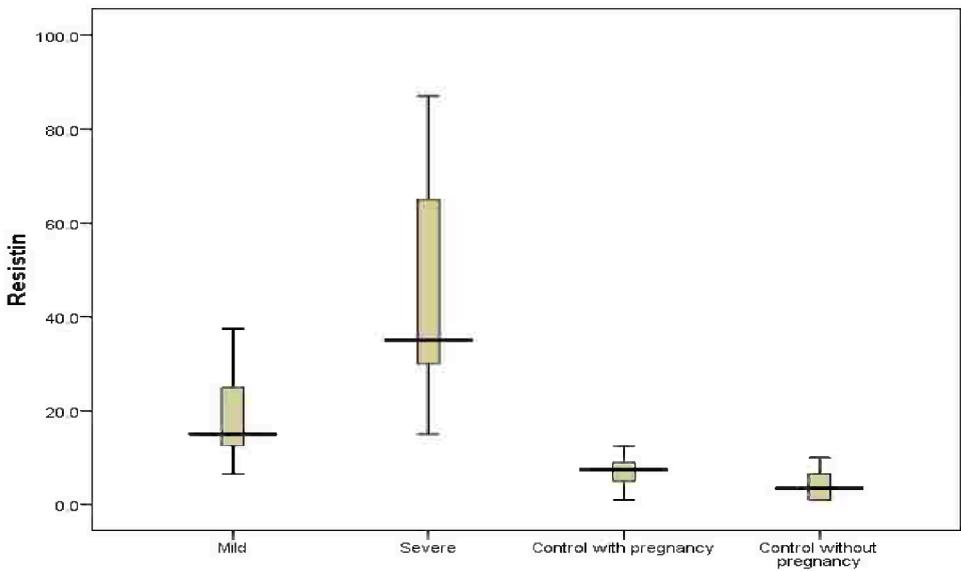


Cutoffpoint	AUC	Sensitivity	Specificity	+PV	PV
>3.69	64.9	93.33	40.00	60.9	85.7

**Figure (10):** ROC Curve analysis showing the diagnostic performance of HOMA-IR in discriminating mild from severe cases of pre-eclampsia.



**Figure (11):** Comparison between all studied groups as regard values of HOMA-IR.



**Figure (12):** Comparison between all studied groups as regard values of Resistin.