III– INSULIN RESISTANCE

A) Definition:

Insulinresistance(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 thepancreassubsequently 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 whichnormalamountsof insulin areinadequateto produce a normalinsulin response fromfat, muscle andliver cells. In fat cells, it reducestheeffects ofinsulinandresultsin elevatedhydrolysisof storedtriglycerides in the absence of measures which either increaseinsulin 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, itreduces 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 anddiabetes 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 α subunits (135kd) and two β subunits (95kd) covalently linked through disulfide bonds to form α2 β2 heterotetramer figure(9) ***(Ueki and Khan, 2000).***

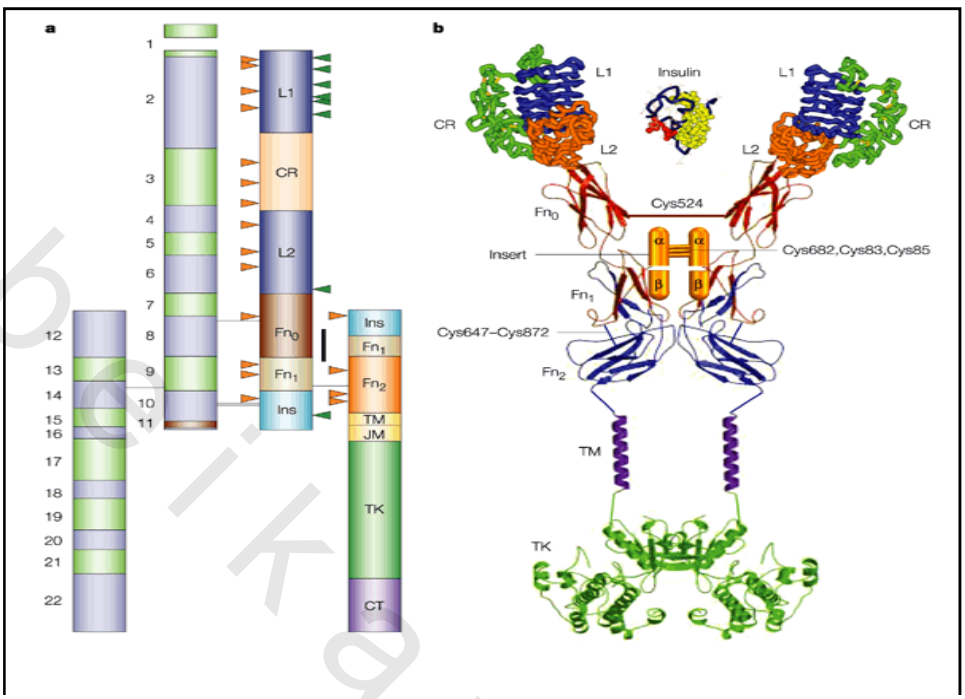

Figure (9): Structure of insulin receptor*(Meyts & Whittaker, 2002)*

The α subunits is entirely extracellular and contains the sites for insulin binding, whereas the β subunits has a small extracellular portion, a transmembrane domain and an intracellular insulin-regulated tyrosine kinase activity. Binding of insulin to the α 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 intracellularproteins (e.g. insulin receptor substrate-1(IRS-1), IRS-2, IRS-3, IRS-4) which mediate insulin signaling ***(Sacks, 2006).***

Both α and β 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-dervied vesicles where cleavage of the proreceptor occurs at a tetrabasic site located at the junction of the α and β subunits. This is followed by terminal glycosylation and possible fatty acid acylation ***(Ueki and Khan, 2000).***

C) Physiology of Insulin Action

Followingsecretionfrom the β-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 β subunits of the insulin receptor is a tyrosine kinase, which is activated when insulin binds to the α 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 (β) 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 β-cells are unable to produce sufficient insulin to maintain normal blood sugarlevels

(euglycemia) ***(Rasouli et al., 2005)***. The inability of the β-cells to produce sufficient insulin in a condition of hyperglycemia is what characterizes the transition from insulin resistance toType 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 TNFα) 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).***

Insulinitselfcan 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 (TNFα), and Interleukins-1 and-6***(Antuna-Puente et al., 2008).***

In numerous experimental models, these proinfammatory 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 cytokinegenes. 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 2diabetes 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 increasedinsulinsensitivityand 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 reportsare 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 resistanceis 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 themostreliabletestforprediabetes,butitisnotassensitiveasthe 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, thattype ofmeasurement 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).***

PeopleofAfrican, Mediterranean, orSoutheast Asiandescent, or peoplewithfamilymemberswith sickle cell anemia or a thalassemia, are particularly at risk of interference. People in these groups may have a less commontypeofhemoglobin,known as a hemoglobin variant, that can interfere with some A1C tests***(American Diabetes Association, 2014).***AnA1Cof5.7 to 6.4 percent indicatesprediabetes ***(American Diabetes Association, 2014)***.

4- Measuring Insulin Resistance

a-Hyperinsulinemic euglycemic clamp

Thegoldstandardforinvestigatingandquantifyinginsulinresistanceisthe"hyperinsulinemiceuglycemicclamp,"so-calledbecauseitmeasurestheamountofglucosenecessarytocompensateforanincreasedinsulinlevelwithoutcausinghypoglycemia.Thetestisrarelyperformedinclinicalcare,butisusedinmedicalresearch,forexample,toassesstheeffectsofdifferentmedications.TherateofglucoseinfusioniscommonlyreferredtoindiabetesliteratureastheGINFvalue*(**Sartietal.,2006).*

Theproceduretakesabout2hours.Throughaperipheralvein,insulinisinfusedat10-120mUperm^{2}perminute.Inordertocompensatefortheinsulininfusion,glucose20%isinfusedtomaintainbloodsugarlevelsbetween5and5.5mmol/l.Therateofglucoseinfusionisdeterminedbycheckingthebloodsugarlevelsevery5to10minutes*(**Moadabetal.,2010).*

Low-doseinsulininfusionsaremoreusefulforassessingtheresponseoftheliver,whereashigh-doseinsulininfusionsareusefulforassessingperipheral(i.e.,muscleandfat)insulinaction*(**Wierzbickietal.,2008)*.

Therateofglucoseinfusionduringthelast30minutesofthetestdeterminesinsulinsensitivity.Ifhighlevels(7.5mg/minorhigher)arerequired,thepatientisinsulin-sensitive.Verylowlevels(4.0mg/minorlower)indicatethatthebody

isresistanttoinsulinaction.Levelsbetween4.0and7.5mg/minareno tdefinitiveandsuggest"impairedglucosetolerance,"anearlysignofi nsulinresistance***(vanetal.,2011).***

Thisbasictechniquecanbesignificantlyenhancedbytheuseo fglucosetracers.Glucosecanbelabeledwitheitherstableorradioacti veatoms.Commonly-usedtracersare3-^{3}Hglucose(radioactive),6,6^{2}H-glucose(stable)and1-^{13}CGlucose(stable).Priortobeginningthehyperinsulinemicperiod, a3htracerinfusionenablesonetodeterminethebasalrateofglucosepr oduction(***Lutseyetal.,2008)***.

Duringtheclamp,theplasmatracerconcentrationsenablethe calculationofwhole-bodyinsulin-stimulatedglucosemetabolism,aswellastheproductionofglucoseb ythebody(i.e.,endogenousglucoseproduction)(***Uruskaetal.,2010)***.

b-ModifiedInsulinSuppressionTest

Anothermeasureofinsulinresistanceisthemodifiedinsulins uppressiontestdevelopedbyGeraldReavenatStanfordUniversity. Thetestcorrelateswellwiththeeuglycemicclampwithlessoperator-dependenterror.Thistesthasbeenusedtoadvancethelargebodyofre searchrelatingtothemetabolicsyndrome(***Dushayetal.,2005)***.

Patientsinitiallyreceive25µgofoctreotide(Sandostatin)in5 mlofnormalsalineover3to5minIVasaninitialbolus,andthenwillbei nfusedcontinuouslywithanintravenousinfusionofsomatostatin(0. 27µgm/m^{2}/min)tosuppressendogenousinsulinandglucosesecretio

n.Insulinand20%glucoseistheninfusedatratesof32and267mg/m^2/min,respectively***(Diamantetal.,2006)***.

Bloodglucoseischeckedatzero,30,60,90,and120minutes,andthenevery10minutesforthelasthalf-hourofthetest.Theselast4valuesareaveragedtodeterminethesteady-stateplasmaglucoselevel(SSPG).SubjectswithaSSPGgreaterthan150mg/dlareconsideredtobeinsulin-resistant***(Hugetal.,2005).***

c-Alternatives

Giventhecomplicatednatureofthe"clamp"technique(andthepotentialdangersofhypoglycemiainsomepatients),alternativeshavebeensoughttosimplifythemeasurementofinsulinresistance.ThefirstwastheHomeostaticModelAssessment(HOMA),andamorerecentmethodistheQuantitativeinsulinsensitivitycheckindex(QUICKI)(***Morenoetal.,2010)***.

Bothemployfastinginsulinandglucoselevelstocalculateinsulinresistance,andbothcorrelatereasonablywiththeresultsofclampingstudies.**Wallaceetal.**pointedoutthatQUICKIisthelogarithmofthevaluefromoneoftheHOMAequations***(Wallaceetal.,2004).***

HOMAwasfirstdevelopedin1985byMatthews*etal.****(Pazienzaetal.,2007)***.Themethodassessesβ-cellfunctionandIR(HOMA-IR)frombasalglucoseandinsulin,orC-peptide,concentrations.HOMAisamemberofafamilyofparadigmaticmodelswhicharephysiological-basedstructuralmodelswiththeoreticalsolutionsadjustedtopopulat

ion.Thus,datafromindividualscanbeusedtoyieldestimatesofβ-cellfunctionandinsulinsensitivityfromthesolutionofthemodelwithoutfurthercomputation(***Whiteetal.,2008)***.

HOMAisamodeloftherelationshipbetweenglucoseandinsulindynamicsthatpredictsfastingsteady-stateglucoseandinsulinconcentrationsforawiderangeofpossiblecombinationsofinsulinresistance(IR)andβ-cellfunction.Insulinlevelsdependonthepancreaticβ-celleffectonglucoseconcentrations,whileglucoseconcentrationsareregulatedthroughinsulin-mediatedglucoseproductionbytheliver.Thus,deficientβ-cellfunctionwillechoadiminishedresponseofβ-celltoglucose-stimulatedinsulinsecretion***(Araseetal.,2009).***

Similarly,IRisreflectedinthediminishedsuppressiveeffectofinsulinonhepaticglucoseproduction***(Moucarietal.,2008)***.Therefore,HOMAdescribesthisglucose–insulinhomeostasisbymeansofasetofsimple,mathematicallyderivednonlinearequations.TheapproximatingequationforIRhasbeensimplifiedandusesafastingplasmasampleinwhichglucose(fastingbloodglucose;FBG)andinsulin(fastinginsulin;FI)aremeasured,togetherwithaconstant***(Tanetal.,2008).***

TheproductofFBG×FIisanindexofIR.

HOMA-IR=(glucose×insulin)/22.5.InsulinconcentrationisreportedinμU/Landglucoseinmmol/L.

Theconstantof22.5isanormalizingfactor,

i.e.normalFIof5μU/mL×thenormalFBGof4.5mmol/Ltypicalofa'normal'healthyindividual=22.5***(Kawaguchietal.,2007).***

ManystudiesusetheHOMA-IRasthediagnosticcriteriaforinsulinresistance***(Tanetal.,2008).***I.R.wasconsideredtobepresentincaseswithHOMA≥3.0***(Wallaceetal.,2004).***

SUBJECTSANDMETHODS

Thestudywasconductedat bothClinicalPathologyandObstetrics andGynecologyDepartments– AinShamsUniversityhospitalsintheperiodbetweenOctober2013andApril2014.Informedconsentswereobtainedfromallparticipantsbeforeenrollmentinthestudy.

I-Subjects:

Thisstudywasconductedonninety(90)females.Theyweredividedintothefollowinggroups:-

GroupI:Pre-eclampsiaGroup(PE)(n=60):

Thisgroupincludedsixty(60)pregnantwomenwithsingletonpregnancybetween28and32weeksofgestation,attendingtheObstetrics out-patientclinicsufferingfrompre-eclampsiaduringthethirdtrimesterofpregnancy**.**Theiragesrangedfrom20to39years with a mean age of 25.00±4.5 .ThediagnosisofPEwasbasedonthepresenceofhypertensionandsignificantproteinuria***(Arikanetal.,2009).***Theywereclassifiedaccordingtoseverityofpre-eclampsiaintotwosubgroups:

a) SubgroupIa(mildpre-eclampticfemales;n=30):

ThissubgroupincludedthirtypregnantfemalessufferingfrommildPE,their ages ranged from 20 to 39 years, with a mean age of 25±4.00 years.Thediagnosiswasbasedonthefollowingcriteria:

Presenceofhypertension(bloodpressureof140/90mmHgor higher,onatleasttwooccasions,atleast6hoursapart,afterthe20thweekofgestation)andproteinuria(dipstickshowing+1inarandomurinesample)***(Arikanetal.,2009).***

b) SubgroupIb(severepre-eclampticfemales;n=30):

Thissubgroupincludedthirty(30)pregnantfemalessuffering fromseverePE,their ages ranged from 20 to 39 years with a mean age of 24.00±5.00 years.Thediagnosiswasbasedononeormoreofthefollowingcriteria :

Bloodpressureof160/110mmHgorhigherontwooccasionsatle astsixhoursapart,aurinedipstickof≥+2inarandomurinesample,oliguri aoflessthan400mLin24hoursurinesample,pulmonaryoedemaorcyan osis,visualorcerebraldisturbance,impairedliverfunctions,thrombo-cytopenia,epigastricorrightupperquadrantpainandHELLPsyndrome (haemolysis,elevatedliverenzymesandlowplatelets)***(Arikanetal.,2009).***

GroupII:HealthyControlGroup(n=30):

Thisgroupincludedthirty(30)age-matchedfemales.Thisgroupwassubdividedinto:

SubgroupIIa:fifteen(15)age-matched**,**theiragesrangedfrom20to39yearswith a mean age of 25.00±4.00,healthy,non–pregnantfemales.

SubgroupIIb:fifteen(15)age-matched,theiragesrangedfrom20to39yearswith a mean age of 25.00±3.00, pregnantfemaleswithnon-complicatedsingletonpregnancybetween28and32weeksofgestation.

Allindividualsincludedinthisstudyweresubjectedtothefollowingafteraninformedoralconsent:

1- Fullhistorytakingfocusingoninformationregardingthecurrentpregnancyincludingage,medicalandobstetrichistory,significantendocrinedisorderinthecurrentpregnancyorinthepastandothersymptomsdependentonthesystemicinvolvement,suchashypertension,renalorliverfunctiondisturbances.

2- Thoroughclinicalexaminationwithspecialemphasisonedema,bloodpressuremeasurementandMaternalbodymassindex(BMI);Index(Wt(kg)/height²(m²))

3- Determinationofgestationalageaccordingtothedateofthelastmenstrualperiodandconfirmedbyfirsttrimesterultrasound.

4-Laboratoryinvestigationsincluding:

a. Liverenzymes(AST,ALT).
b. Renalfunctiontests(BUN,creatinine).
c. Completebloodcount.
d. Urineproteinestimationbydipsticktestinamorningurinesample.
e. 50goralglucosechallengetest(GCT)forpregnantwomenonly.

f. HOMA-IR:calculatedbymeasuringfastinginsulin(mU/mL)andfastingbloodglucose(mmol/L).
g. AssayofmaternalserumresistinebyELISA.

ExclusionCriteria:

Patientswithgestationaldiabetesmellitus,chronichypertension(hypertensionbeforepregnancyandpersistentlyelevatedbloodpressurebeforethe20thweekofgestation).,diabetesmellitus,polyhydramnios,renaldiseases,multiplegestation.Agelessthan20yearsormorethan39years.

II-SAMPLING:

A-BloodSamples:

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

ThecollectedbloodwasdividedintoanEDTAtubeforCBCandaplaintesttubeforserumseparation.Aftercompleteclotformation,sampleswerecentrifugedat1500xgfor10minutes.Theseparatedserumwasdividedintotwoaliquots.Onewasdesignatedfortheassayof BUN,AST,ALT,50goralglucosechallengetestandserumcreatinineassessment.Theotheraliquotwasstoredat-20ºCforsubsequentassayofresistin.Theseparated8-12hoursfastingserumfortheassayoffastingglucoseandfastinginsulinlevelsfordetectionofinsulinresistance(IR).Hemolysedsampleswerediscarded,repeatedfreezingandthawingwasavoided.

B.UrineSamples:

Fordetectionofurinaryproteins,morningmidstreamurinesamplewascollectedfromeachsubjectinacleancontainer.Thespecimenwasexaminedimmediately*(NationalCommitteeforClinicalLaboratoryStandards,2007)*.

III-METHODS:

A. AnalyticalMethods:

1-Detectionofurineprotiens:

Urinaryproteinwastestedforbydipstickmethod(ComboStik,DOC.);whichisbasedonthechangeinthecolourofthedyebromophenolblue,inthepresenceofabindingproteinasalbumin.Bromophenolblue,bufferedtopH3withcitrate,ispresentpredominantlyintheprotonatedform(yellowform).Whenproteinisadded,theaffinityoftheanionicformoftheindicatordyeforproteincausesashiftoftheequilibriumbetweenanionicandprotonatedformoftheindicatortowardformationoftheblueanionicspecies.

Theintensityofthebluecolourisproportionaltotheconcentrationofproteininthespecimen.Thisassayissemi-quantitativeasresultsof+1correspondstoproteinconcentrationof30mg/dL,+2correspondstoproteinconcentrationof100mg/dL,+3correspondstoproteinconcentrationof300mg/dLand+4correspondstoproteinconcentrationof1000mg/dL*(Akdenizetal.,2011)*.

2-CompleteBloodCount(CBC):

CBCwasdoneusingMaxMCoulter*.

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

** Beckman Instruments Inc., Scientific Instruments Division, Fullerton, CA92634-3100,USA.

3-

RoutineLaboratoryTests:AST,ALT,BUNandCreatininewerecarriedoutonSynchronPROautoanalyzer**.

4-50gglucosechallengetest(GCT).

ItisthemostwidelyusedscreeningtestforgestationaldiabetesandisrecommendedbytheamericanDiabetesAssociation(ADA).Thisisperformedwithoutregardtotimeornatureofthelastmeal,at24-28weeks'gestation.Womenaregiven50gofglucosebymouthandplasmaglucoseismeasuredat1hour.Apositivetestisaplasmavenousglucoseconcentration≥7.8mmol/L(≥140mg/dL)***(AmericanDiabetesAssociation,2014).***

5-Fastinginsulin:

Fastingseruminsulinconcentrationsweremeasuredusingacommerciallyavailableenzyme-linkedimmuno-sorbentassay(ELISA)kitDRG®Insulin*

a. Principleoftheassay:

Themicrotiterwellsarecoatedwithamonoclonalantibodydirectedtowardsauniqueantigenicsiteontheinsulinmolecule.Analiquotofpatientsamplecontainingendogenousinsulinisincubatedinthecoatedwellwithenzymeconjugate,whichisananti-insulinantibodyconjugatedwithbiotin.Afterincubationtheunboundconjugateiswashedoff.Duringthesecondincubationstepstreptavidinperoxidaseenzymecomplexbindstothebiotin-anti-

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insulinantibody.TheamountofboundHRPcomplexisproportionalt otheconcentrationofinsulininthesample.Havingaddedthesubstrat esolution,theintensityofcolordevelopedisproportionaltotheconce ntrationofinsulininthepatientsample***(Flieretal.,1979).***

b-Assayprocedure:

i. 25µlofeachstandard,controlsandsampleswereaddedwithnew disposabletipsintoappropriatewells.
ii. 25µlofEnzymeConjugatewereaddedintoeachwell.
iii. Thoroughlymixingwasdonefor10seconds.
iv. Incubationfor30minutesatroomtemperaturewithoutcovering theplatewasdone.
v. Thecontentsofthewellsbrisklywereshakenout.
vi. ThewellswererinsedwithdilutedWashSolution(400µl perwell).Thewellswereblottedonabsorbentpapertore moveresidualdroplets.
vii. 50µlofEnzymeComplexwereaddedtoeachwell.
viii. Wellswerethenincubatedfor30minutesatroomtemperature.
ix. Brisklyshakeoutthecontentsofthewellswasdone.
x. ThewellswererinsedwithdilutedWashSolution(400µl perwell).Thewellswereblottedonabsorbentpapertore moveresidualdroplets.
xi. 50µlofSubstrateSolutionwereaddedtoeachwell.
xii. Wellswerethenincubatedfor15minutesatroomtemperature.

xiii. Stoppingtheenzymaticreactionwasdonebyadding50µlofStopSolutiontoeachwell.

xiv. TheODwasreadat455nmwithamicrotiterplatereaderwithin10minutesafteraddingtheStopSolution.

c- Calculationofresults:

i. Theduplicatereadingsforeachstandard,control,andsamplewereaveragedandtheaveragezerostandardopticaldensitywassubstracted.

ii. Theopticaldensityforthestandardswasplottedversustheconcentrationofthestandards,andthebestcurvewasdrawnusinglog/log paper.

iii. TheInsulinconcentrationofeachsamplewasdetermined,byfindingtheabsorbancevalueontheyaxisandextendingahorizontallinetothestandardcurve.Atthepointofintersection,averticallinetothexaxiswasextendedandthecorrespondingInsulinconcentrationdetermined.

d-Referencerange:

Reportedreferenceleveloffastinginsulininhealthyindividualsis(2-25µIU/mL)***(Flieretal.,1979).***

e-Sensitivity:

TheanalyticalsensitivityoftheDRGELISAwascalculatedb yadding2standarddeviationstothemeanof20replicateanalysesofth eZeroStandardandwasfoundtobe1.76µIU/mL***(Flieretal.,1979)***.

f- Linearity:

Thismethodislinearupto100µIU/mL***(Flieretal.,1979).***

6-Homeostasismodelassessment(HOMA)Score:

ItwasfirstdescribedunderthenameHOMAby**Matthews*etal.in 1985***.

ItusesthefollowingformulatodetermineInsulinresistance(IR):

HomeostasismodelassessmentHOMAScore=

{Fastingglucose(*mmol/L*)xfastinginsulin(*mU/L*)}/22.5.

Or

HomeostasismodelassessmentHOMAScore=

{Fastingglucose(*mg/dL*)xfastinginsulin(*mU/L*)}/405.

I.R.wasconsideredtobepresentincaseswithHOMA ≥3.0***(Wallaceetal.,2004).***

6-MeasurementofSerumResistin:

Resistinconcentrationsweremeasuredusingacommerciallyavailableenzyme-linkedimmune-sorbentassay(ELISA)kitsuppliedbyAssaypro,LLC*

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

a.Principle:

Theusedmethodisdesignedfordetectionofhumanresistininplasma,serumandcellculturesupernatants.Thisassayemploysaquantitativesandwichenzymeimmunoassaytechniquewhichmeasuresresistin.Amurinemonoclonalantibodyspecificforresistinhasbeenpre-coatedontoamicroplate.Resistinstandardsandsamplesissandwichedbytheimmobilizedantibodyandabiotinylatedpolyclonalantibodyspecificforresistin,whichisrecognizedbyastreptavidin-peroxidaseconjugate.Allunboundmaterialisthenwashedawayandaperoxidaseenzymesubstrateisadded.Thecolordevelopmentisstoppedandtheintensityofthecolorismeasured.

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 µL).

v. Streptavidin-Peroxidase conjugate (SP Conjugate): A 100-fold concentrate (90 µL).

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. EIADiluentConcentrate(10x):30mLoftheconcentratedEIA diluentwasdilutedwith270mLreagentgradewatertoafinalv olumeof300mL.

ii. StandardCurve:The64ngofhumanresistinstandardwasreco nstitutedwith4mLofEIAdiluenttogenerateastocksolutionof 16ng/mL.Thestandardwasallowedtositfor10minuteswithg entleagitationpriortomakingdilutions.Triplicatestandardp ointswerepreparedbyseriallydilutingtheresistinstandardsol ution(16ng/mL)twofoldwithequalvolumeofEIAdiluenttop roduce8,4,2,1,0.5and0.25ng/mLsolutions.EIAdiluentserv esasthezerostandard(0ng/mL).

iii. Biotinylatedresistinantibody(100x):Theantibodywasspinn eddownbrieflyandthe80µLoftheconcentratedbiotinylatedr esistinantibodywasdilutedwith7.920mLEIAdiluenttoafina lvolumeof8mL.

iv. SPconjugate(100x):TheSPconjugatewasspinneddownbrieflyandthe90µLoftheconcentratedSPconjugatewasdilutedwith8.910mLEIAdiluenttoafinalvolumeof9mL.

v. WashBufferConcentrate(20x):30mLoftheconcentratedwashbufferwasdilutedwith570mLreagentgradewatertoafinal volumeof600mL.

d.Assayprocedure:

Allreagents,workingstandardsandsampleswereallowedtoreachroomtemperatureandmixedthoroughlybygentleinversionbeforeuse.Theassaywasperformedatroomtemperature(20-30^{0}C).Theassayprocedurewasasfollows:

i. Serumsampleswerediluted byadding(100µLserum+500µLEIAdiluents).

ii. 50µLofeachstandarddilutionsanddilutedserumsampleswerdispensedwithnewdisposabletipsintoappropriatewells.Wellswerecoveredandincubatedfortwohoursatroomtemperature.Thetimerwasstartedafterthelastsampleaddition.

iii. Eachwellwasaspiratedandwashed5timeswiththedilutedwash bufferusinganautomaticmicroplatewasher.Theplatewasinvertedonabsorbentpapertoweltobedried.

iv. 50µLofbiotinylatedresistinantibodyweredispensedtoeachwell andincubatedfortwohoursatroomtemperature.

v. Eachwellwasaspiratedandwashed5timeswiththedilutedwash bufferusinganautomaticmicroplatewasher.Theplatewasinvertedonabsorbentpapertoweltobedried.

vi. 50µLofstreptavidin-peroxidaseconjugateweredispensedtoeachwellandincubatedfor 30minutesatroomtemperature.

vii. Eachwellwasaspiratedandwashed5timeswiththedilutedwashbufferusinganautomaticmicroplatewasher.Theplatewasinvertedonabsorbentpapertoweltobedried.

viii. 50µLofchromogensubstratewereaddedtoeachwellandincubatedforabout10minutesatroomtemperature.Theplatewasgentlytappedtoensurethoroughmixingandthebubblesinthewellswere breakedwithpipettetip.

ix. 50µLofstopsolutionwereaddedtoeachwell.

x. Theabsorbanceofthesolutioninthewellswasreadwithin10minutesusingamicroplatereadersetatawavelengthof450nm.

e.Calculationofresults:

Themeanabsorbancevalueforeachsetofreferencestandards andsampleswerecalculated.Astandardcurvewasconstructedbyplottingthemeanabsorbanceobtainedforeachreferencestandardagainstitsconcentrationinng/mLonlineargraphpaper,withabsorbanceonthevertical(y)axisandconcentrationonthehorizontal(x)axis.The bestfittingcurvewasdrawn.Usingthemeanabsorbancevalueforeachsample,thecorrespondingconcentrationofresistin(ng/mL)wasdeterminedfromthestandardcurve.

B-StatisticalMethods:

Statisticalanalysiswasdoneusingsoftwareversion SPFF.

1-DescriptiveStatistics:

a-Themean__ andstandarddeviation(SD)

Thesewereusedincaseofparametricdataandwerecalculated asfollows:

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

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

where:∑=Sumofobservedvalues

X=Agivenvalueofavariable

$\bar{X}$=Arithmeticmean

n=Totalnumberofvalues(observations)

b-Themedianandinterquartilerange:

Theywereusedincaseofskeweddata.Valueswerearrangedin anascendingorder.Eachofthesevalueswasgivenaranknumber,wit hthesmallestvaluegiventhesmallestranknumber.Theranknumber ofthemedianwascalculatedas(n+1)÷2,wherenisthenumberofobse rvations.Theoriginalvaluecorrespondingtotheranknumberwasthe nobtained.

Theinterquartilerangeisthatbetweenthe25thand75thpercen tiles(25thPand75thP,respectively).

Theranknumberofthe25thpercentile=0.25x(n+1),andthato fthe75thpercentile=0.75x(n+l),wherenisthenumberofobservatio ns.Theoriginalvaluescorrespondingtotheseranknumberswerethe nobtained(**Bourkeetal.,1985).**

2-SignificanceTests:

a-TheStudent'sttest:

The't'valuewascalculatedfromthet-table,theprobability(p)valueforthecalculated't'valuewasthendeduced,withthedegreesoffreedombeingequaltothesumofthetwosamplesizesminus2.pvalues>0.05indicateanon-significantdifference,p<0.05indicatesasignificantdifference,andp<0.01indicatesahighlysignificantdifference***(Fogiel,1986).***

$$t=\frac{\overline{X1}-\overline{X2}}{\sqrt{\frac{(s_1)^2}{n_1}+\frac{(s_2)^2}{n_2}}}$$

where,

$\overline{X1}$ =themeanofsample1.

$\overline{X2}$=themeanofsample2.

S_1=standarddeviationinsample1.

S_2=standarddeviationinsample2.

n_1=thenumberofsubjectsinsample1.

n_2=thenumberofsubjectsinsample2.

b.Wilcoxon'sranksumtest(Mann-WhitneyUtest):

Datafromeachtwocomparedgroupswerecombinedandarrangedfromthelowesttothehighest.Ranknumbersweregiventothesevalues,thesmallestranknumberbeinggiventothelowestvalue,andt

hegreatestranknumberbeinggiventothehighestvalue.Whentwovalueswereidentical,theywereeachgiventheaverageofthenexttworanks.

Thetwogroupswerethenseparatedandthesumofranksofeachgroupwascalculated.The"z"valuewasthencalculatedasfollows:

$$Z_T=\frac{(\mu_{T-T-1/2})}{\sigma_T}$$

$$\mu_T=\frac{n_1(n_{1+}n_2+1)}{2}$$

where,

n1isthesamplesizeofthenumericallysmallergroup

n2isthatofthelargergroup.

Tiscalculatedfromthenumericallysmallergroup,andiscalculatedfromthenormalranktotal(T1),whenthelowerrankspredominateinthisgroup.WhenhigherrankspredominateTisequalton1(n1+n2+l)-T1

$$\sigma_T=\sqrt{\frac{n_1\mu}{6}}$$

Fromstatisticaltables,theprobability(p)valuesforthecalculated"z"valueswerethendeduced,wherep>0.05representsnon-significantdifference,p<0.05representssignificantdifference,and p<0.01representsahighlysignificantdifference***(Fogiel,1986).***

3-CorrelationStudy:

Spearman'srankcorrelationcoefficient(rs)wasusedtoassess thedegreeofassociationbetweentwosetsofvariablesifoneorbothoft hemwasskewed.Thesignificanceofcorrelationwasthendetermine dfromcertaintableslookingforthecriticalrvalue(rc)fortheobserved n***(Bourkeetal.,1985).***

Inordertocalculaters,theobservationsofeachvariable(xandy)wereranked,thentheranknumbersofthexandyvariableswereassig nedseparately,fromlowesttohighest,givingtheaverageofthenextt worankstosimilarobservations.

Thenforeachpair,therankofvariableywassubtractedfromth erankofvariablexandtheresultwascalled"D".Each"D"wassquared andaddedtoobtain∑D².TheSpearman'srankcorrelationcoefficient wasthencalculatedasfollows:

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

where,nisthenumberofpairsofobservationsofanxandyvariable.

Thesignificanceofcorrelationwasthendeterminedfromcert aintableslookingforthecriticalvalue(rc)fortheobservedn.Signific antcorrelationwaspredictedifrs≥rcorrs≤-rc***(Bourkeetal.,1985).***

Pearson'scorrelationcoefficientwhenappliedtoasampleisco mmonlyrepresentedbytheletter*r*andmaybereferredtoasthe*sample correlationcoefficient*orthe*samplePearsoncorrelationcoefficient* .Wecanobtainaformulafor*r*bysubstitutingestimatesofthecovarian

cesandvariancesbasedonasampleintotheformulaabove.Thatform ulafor*r*is:

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

Anequivalentexpressiongivesthecorrelationcoefficientast hemeanoftheproductsofthestandardscores.Basedonasampleofpai reddata(X_i,Y_i),thesamplePearsoncorrelationcoefficientis:

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

where

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

arethestandardscore,samplemean,andsamplestandarddeviation,r espectively.

4-EvaluationofDiagnosticPerformance:

a.Diagnosticsensitivity:

Itmeasurestheincidenceoftruepositiveresultsinpatientsgro up.

Diagnosticsensitivity=TP/(TP+FN)×100

Where:

TP(truepositive)isthenumberofdiseasedpatientsaccurately classifiedbythetestand**FN**(falsenegative)isthenumberofdiseased patientsmisclassifiedbythetest.

b.Diagnosticspecificity:

Itmeasurestheincidenceoftruenegativeresultsinanon-diseasedgroup.

Diagnosticspecificity=TN/(TN+FP)×100

Where:

TN(truenegative)isthenumberofnon-diseasedsubjectsaccuratelyclassifiedbythetestand**FP**(falsepositiv e)isthenumberofnon-diseasedsubjectsmisclassifiedbythetest.

c.Diagnosticefficiency:

Itisthepercentoftruepositiveandnegativeresultsamongallresu lts.

Diagnosticefficiency=(TN+TP)/(TN+TP+FN+FP)×100

d.Positivepredictivevalue:

Itisthepercentoftruepositiveresultsamongallpositiveresults.

Positivepredictivevalue=TP/(TP+FP)×100

e.Negativepredictivevalue:

Itisthepercentoftruenegativeresultsamongallnegativeresults.

Negativepredictivevalue=TN/(TN+FN)×100

(EdwardandSchultz,1999).

Receiver-operatingcharacteristic(ROC)curveanalysis:

Theoveralldiagnosticperformanceofeachtestwasassessedbyreceiver-operatingcharacteristics(ROC)curveanalysis.TheROC-curvewasplottedasfollows:

-TheX-axisrepresentsthefalse-positiverate(1-specificity%).

-TheY-axisrepresentsthediagnosticsensitivity%.

- Thedotteddiagonallineextendingfromthelowerleftcornertotheupperrightcornerrepresentsatestwithnodiscrimination.
- Acurvethatextendsfromthelowerleftcornertotheupperleftcornerthentotheupperrightcornerisconsideredaperfecttest.
- Theareaunderthecurve*(AUC)*describestheoveralltestperformance.

-

Thebestcutoffpointisthepointnearesttotheupperleftcorner***(Zweigand Campbell,1993).***

RESULTS

Results 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 ranksum 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 no statistically significant difference regarding age and gestational age (GA) (Tables 3 &4 and Figure 1).

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

pregnantcontrols(groupIIa)regardingthedifferentstudiedparamet ersusingstudent´sttestforparametricdataandWilcoxon's ranksumtestfornon-parametricdatarevealedahighlystatisticallysignificantincreaseint hehealthypregnantcontrolsgroupthanthehealthynon-pregnantcontrolsgroupregardingFBG,fastinginsulin,HOMA-IRandBMIwhiletherewasahighlystatisticallysignificantdecreasei nthehealthypregnantcontrolsgroupthanthehealthynon-pregnantcontrolsgroupregardingBUNandHB.Italsorevealedastat isticallysignificantincreaseinthehealthypregnantcontrolsgroupth anthehealthynon-pregnantcontrolsgroupregardingSBPandresistin,whiletherewasa statisticallysignificantdecreaseinthehealthypregnantcontrolsgrou pthanthehealthynon-pregnantcontrolsgroupregardingALTandanon-significantdifferenceinage,DBP,creatinine,AST,andPLT(Tables 5 & 6andFigure 2).

Oncomparingthemildpre-eclampticgroups(groupIa)andseverepre-eclampticgroup(groupIb)usingstudent´sttestforparametricdataa ndWilcoxon's ranksumtestfornon-parametricdata,therewasahighlystatisticallysignificantincreasein theseverepre-eclampticgroupthanthemildpre-eclampticgroupregardingGA,SBP,DBP,AST,ALT,50gglucosete st,HBandresistinwitharecordedstatisticallysignificantincreaseins everepre-eclampticgroupthanthemildpre-

eclampticgroupregardingBUNandHOMA-IR.Incontrast,therewasastatisticallysignificantdecreaseinseverepre-eclampticgroupthanthemildpre-eclampticgroupregardingPLTandnon-significantdifferenceinage,BMI,CRE,FBGandfastinginsulin(Tables7 & 8andFigure 3).

Statisticalcomparisonbetweenpatientsgroup(groupI)andcontrolpregnantgroup(groupIIb)regardingthedifferentstudiedparametersusingstudent´sttestforparametricdataandWilcoxon's ranksumtestfornon-parametricdatarevealedahighlystatisticallysignificantincreaseinSBP,DBP,50gglucosetest,CRE,BUN,ALT,FBG,fastinginsulin,HOMA-IRandresistininpatientgroupwhiletherewasastatisticallysignificantdecreaseinHBinpatientsgroupthancontrolpregnantgroupandwitharecordedstatisticallysignificantregardingAST.Anon-significantstatisticallydifferencewasrecordedbetweenthetwogroupsregardingage,GA,PLTandBMI(Tables 9 & 10andfigure4).

Statisticalcomparisonbetweenpatientsgroup(groupI)andcontrolnonpregnantgroup(groupIIa)regardingthedifferentstudiedparametersusingstudent´sttestforparametricdataandWilcoxon's ranksumtestfornon-parametricdatarevealedahighlystatisticallysignificantincreaseinSBP,DBP,BMI,CRE,FBG,fastinginsulin,HOMA-IRandresistininpatientgroupwitharecordedhighlystatisticallysignificantdecreaseinPLTsandHB.Therewasastatisticallysignificantd

ecreaseinASTinpatientsgroup.Anon-significantdifferencewasrecordedbetweenthetwogroupsregardingage,ALTandBUN(Table11 & 12andfigure5).

Correlationstudybetweenresistinandtheotherstudiedparametersinpre-eclampticpatientscollectivelyusingSpearman's rankcorrelationcoefficienttest(r_s)isshownin(Tables 13 and 14).Itrevealedasignificantpositivecorrelationbetweenresistinand SBP,DBP,HOMA-IR,GA,50gglucosetest,fastinginsulin,FBG,AST,BUNandALTand anon-significantcorrelationbetweenresistinandage,GA,BMI,CRE,HBandPLT.

CorrelationstudybetweenHOMA-IRandtheotherstudiedparametersinpre-eclampticpatientscollectivelyusingSpearman's rankcorrelationcoefficienttest(r_s)isshownin(Tables15 and 16andfigure6).Itrevealedasignificantpositivecorrelationbetween HOMA-IRandresistin,DBP,GA,GA,50gglucosetest,fastinginsulin,FBGandALTandanon-significantcorrelationbetweenHOMA-IRandage,SBP,BMI,CRE,BUN,AST,HBandPLT.

Receiveroperatingcharacteristic(ROC)curveanalysiswasappliedtoassessthediagnosticutilityofresistinindiscriminatingpre-eclampticpatientsfromthecontrolgroup.Thebestdiagnosticcutoffl

evelforresistinwas10ng/mL.Thishadadiagnosticsensitivityof88.33%,specificity96.76%,negativepredictivevalue80.6%,positivepredictivevalue98.1%.Theareaunderthecurve(AUC)was96.7asshownin(Figure7).

Inaddition,whenROCcurveanalysiswasappliedtoassessthediagnosticperformanceofresistinindiscriminatingseverepre-eclampticpatientsfromthosewithmildpre-eclampsia,itwasfoundthatthebestcutoffvaluewas17ng/mL.Thishadadiagnosticsensitivityof96.76%,specificity70%,negativepredictivevalue95.5%,positivepredictivevalue76.3%.Theareaunderthecurve(AUC)was90.8asshownin(Figure8).

Receiveroperatingcharacteristic(ROC)curveanalysiswasappliedtoassessthediagnosticutilityofHOMA-IRindiscriminatingpre-eclampticpatientsfromthecontrolgroup.Thebestdiagnosticcutofflevelforresistinwas2.05ng/mLgivingadiagnosticsensitivityof98.33%,specificity86.67%,negativepredictivevalue96.3%,positivepredictivevalue93.7%.Theareaunderthecurve(AUC)was97.3asshownin(Figure9).

Inaddition,whenROCcurveanalysiswasappliedtoassessthediagnosticperformanceofHOMA-IRindiscriminatingseverepre-eclampticpatientsfromthosewithmildpre-eclampsia,itwasfoundthatthebestcutoffvaluewas3.69ng/mL.Thishadadiagnosticsensitivityof93.33%,specificity40%,negativepred

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

Table (1): DescriptiveStatisticsofage, GA, SBP, DBP and BMI intheDifferentStudiedGroups.

Parameter	GroupIa(n=30) $\overline{X}$ ±SD Medianand(IQ R)*	GroupIb(n=30) $\overline{X}$ ±SD Medianand(IQ R)*	GroupIIa(n=15) $\overline{X}$ ±SD Medianand(IQ R)*	GroupIIb(n=15) $\overline{X}$ ±SD Medianand(IQ R)*
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) $\overline{X}$ ± SD Median and (IQR) *	Group I b(n=30) $\overline{X}$ ± SD Median and (IQR) *	Group II a(n=15) $\overline{X}$ ± SD Median and (IQR) *	Group II b(n=15) $\overline{X}$ ± 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 $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata**.**

Table (3): StatisticalComparisonbetweenPatientsGroupandControlGroupRegardingage, GA, SBP, DBP and BMI,UsingStudent´stTestforParametricDataandWilcoxon'sRankSumTestfornon-ParametricData.

Parameters	Group I (n=60) $\overline{X}$ ± SD Median and (IQR) *	Group II (n=30) $\overline{X}$ ± 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 $\overline{X}$ ± 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 Dataand Wilcoxon's Rank Sum Test for non- Parametric Data.

Parameters	Group I (n=60) $\overline{X}$ ± SD Median and (IQR) *	Group II (n=30) $\overline{X}$ ± 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

- Dataareexpressedas $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata**.**
- **p>0.05:**Nonsignificantdifference.
- **p<0.05**:Significantdifference.
- **p<0.01**:Highlysignificantdifference.

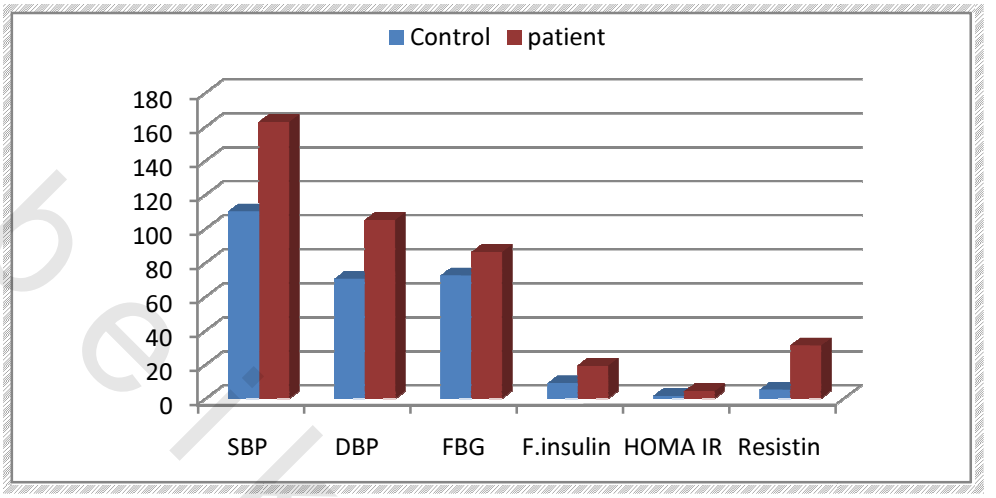


Figure (1): Comparison between control and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

Table (5): StatisticalComparisonbetweentheControlgroupsRegardingage, SBP, DBP and BMI,UsingStudent´stTestforParametricDataandWilcoxon'sRankSumTestfornon-ParametricData.

Parameters	GroupIIb(n=15) $\overline{X}$ ±SD Medianand(IQR)*	GroupIIa(n=15) $\overline{X}$ ±SD Medianand(IQR)*	p
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 $\overline{X}$ ± 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) $\overline{X}$ ± SD Median and (IQR) *	Group II a (n=15) $\overline{X}$ ± SD Median and (IQR) *	p
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 $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.05**:Significantdifference.
- **p<0.01**:Highlysignificantdifference.

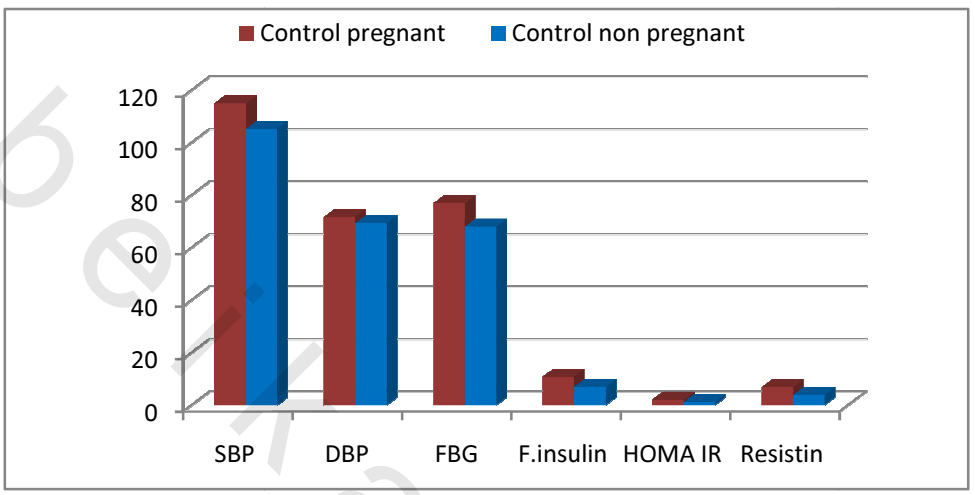


Figure (2): Comparison between control pregnant and control non pregnant groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

Table (7): StatisticalComparisonbetweenPre-eclampticFemalesClassifiedAccordingtoDiseaseSeverityRegardingage, GA, SBP, DBP and BMI,UsingStudent´stTestforParametricDataandWilcoxon'sRankSumTestfornon-ParametricData.

Parameters	GroupIa(n=30) $\overline{X}$ ±SD Medianand(IQR)*	GroupIb(n=30) $\overline{X}$ ±SD Medianand(IQR)*	p
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 $\overline{X}$ ± 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) $\overline{X}$ ± SD Median and (IQR) *	Group I b (n=30) $\overline{X}$ ± 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 $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01**:Highlysignificantdifference.

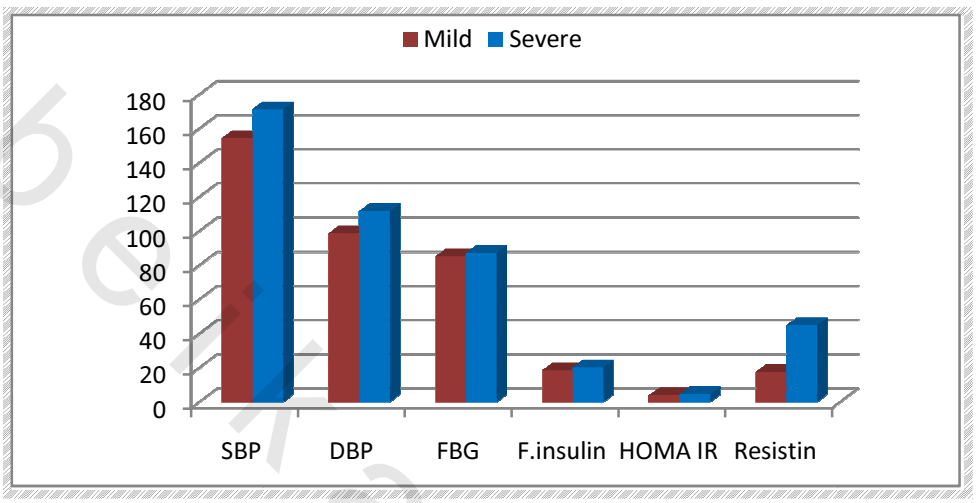


Figure (3): Comparison between mild pre-eclampsia and sever pre-eclampsia groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin

Table (9): StatisticalComparisonbetweenPatient'sGroupandControlpregnantGroupRegardingage, GA, SBP, DBP and BMI,UsingStudent´stTestforParametricDataandWilcoxon'sRankSumTestfornon-ParametricData

Parameters	Group I (n=60) $\overline{X}$ ± SD Median and (IQR) *	Group II b(n=15) $\overline{X}$ ± SD Median and (IQR) *	p
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 $\overline{X}$ ± 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) $\overline{X}$ ± SD Median and (IQR) *	Group II b (n=15) $\overline{X}$ ± SD Median and (IQR) *	p
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 $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01**:Highlysignificantdifference.

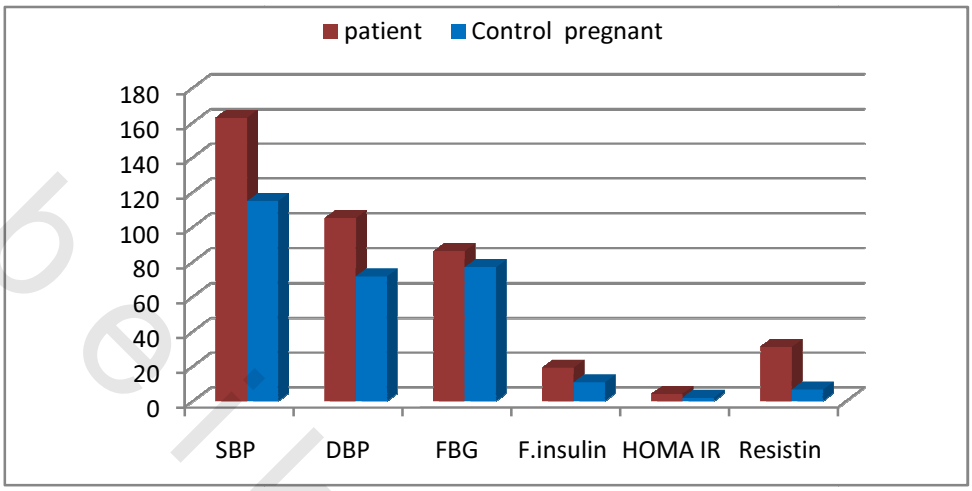


Figure (4): Comparison between control pregnant and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

Table (11): StatisticalComparisonbetweenPatientsGroupandControlnonpregnantGroupRegardingage, SBP, DBP

and BMI,UsingStudent´stTestforParametricDataandWilcoxon'sRankSumTestfornon-ParametricData.

Parameters	Group I (n=60) $\overline{X}$ ± SD Median and (IQR) *	Group II a (n=15) $\overline{X}$ ± SD Median and (IQR) *	p
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 $\overline{X}$ ± 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) $\overline{X}$ ± SD Median and (IQR) *	Group II a (n=15) $\overline{X}$ ± 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/µL)	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(µU/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 $\overline{X}$ ±SDforparametricdata.
- Medianand(Interquartilerange)*fornon-parametricdata.
- **p>0.05:**Nonsignificantdifference.
- **p<0.01**:Highlysignificantdifference.

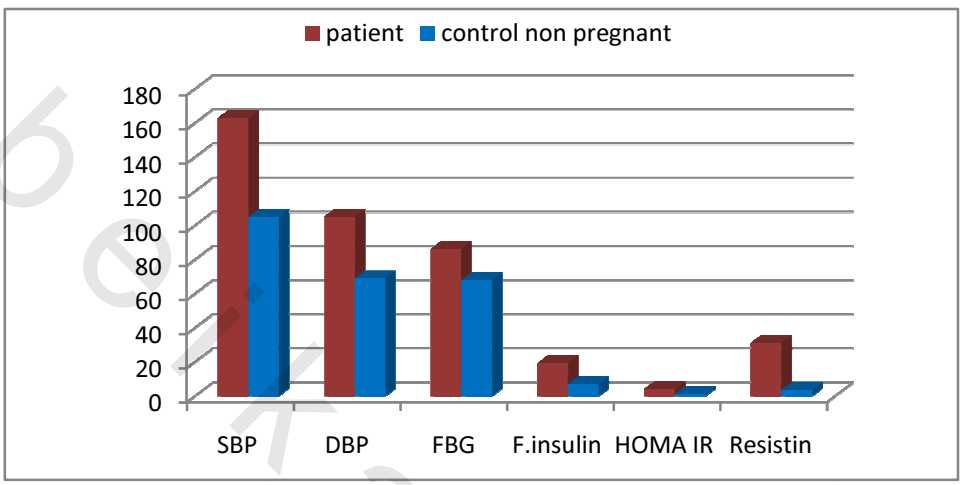


Figure (5): Comparison between control non pregnant and patient groups as regards mean values of SBP, DBP, FBG, fasting insulin, HOMA-IR and resistin.

Table (13): CorrelationStudybetweenresistin&HOMA IR, age, GA, SBP, DBP and BMI,inPre-eclampticPatientsUsingSpearman'sRankCorrelationCoefficientTest($r_{s)}$:

	Resistin	
	rs	**p-value**
HOMAIR	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
Fastinginsulin(µU/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(x103/µL)	-0.056	>0.05
HB(g)	0.183	>0.05

- **p>0.05**:Nonsignificantcorrelation.
- **p<0.01**:Highlysignificantcorrelation.

Table (15): CorrelationStudybetweenHOMA-IR& Resistin, age, GA, SBP, DBP and BMI,inPre-eclampticPatientsUsingPearsoncorrelationTest(r):

	HOMAIR	
	r	**p-value**
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):

	HOMA IR	
	r	**p-value**
50gglucosetest(mg/dL)	0.606	<0.001
FBG(mg\dl)	0.840	<0.001
Fastinginsulin(µU/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(x103/µL)	-0.089	>0.05
HB(g)	0.062	>0.05

- **p>0.05**:Nonsignificantcorrelation.
- **p<0.01**:Highlysignificantcorrelation.

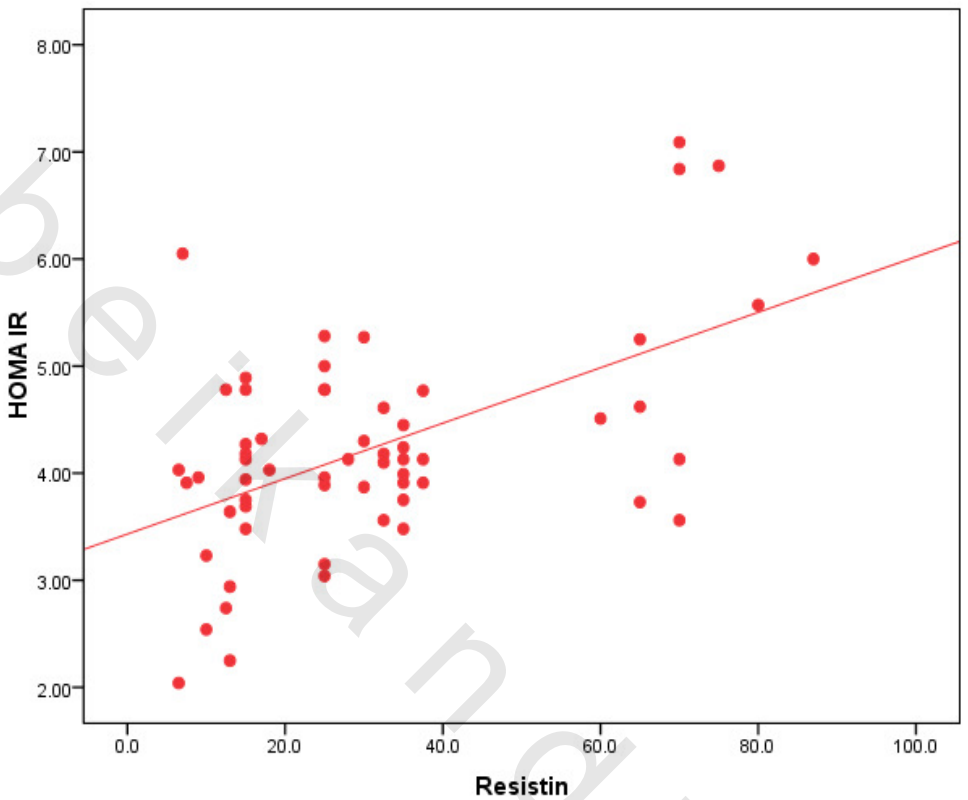
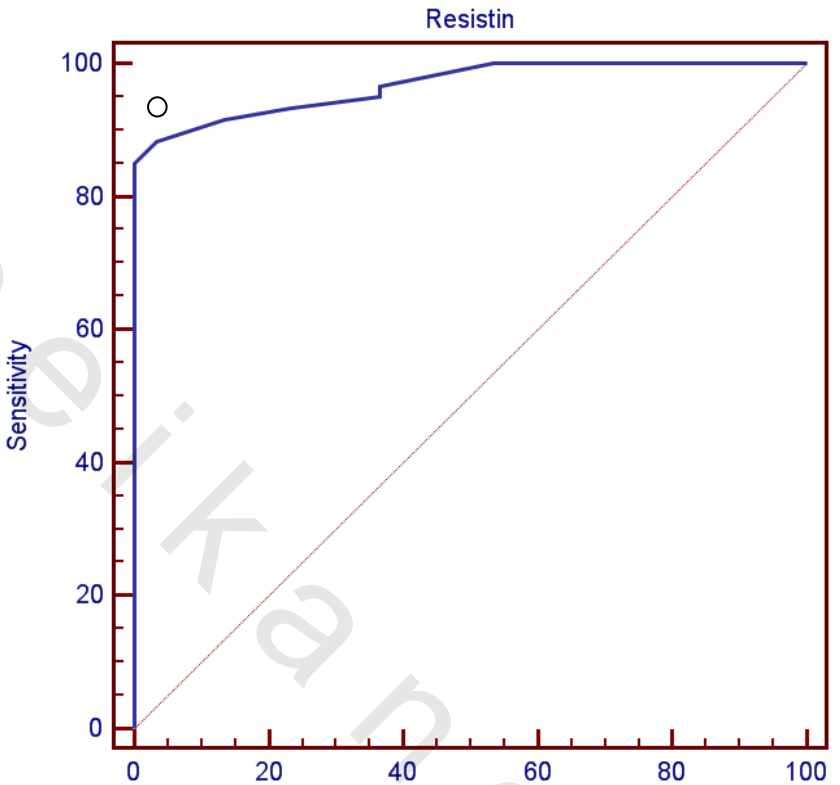
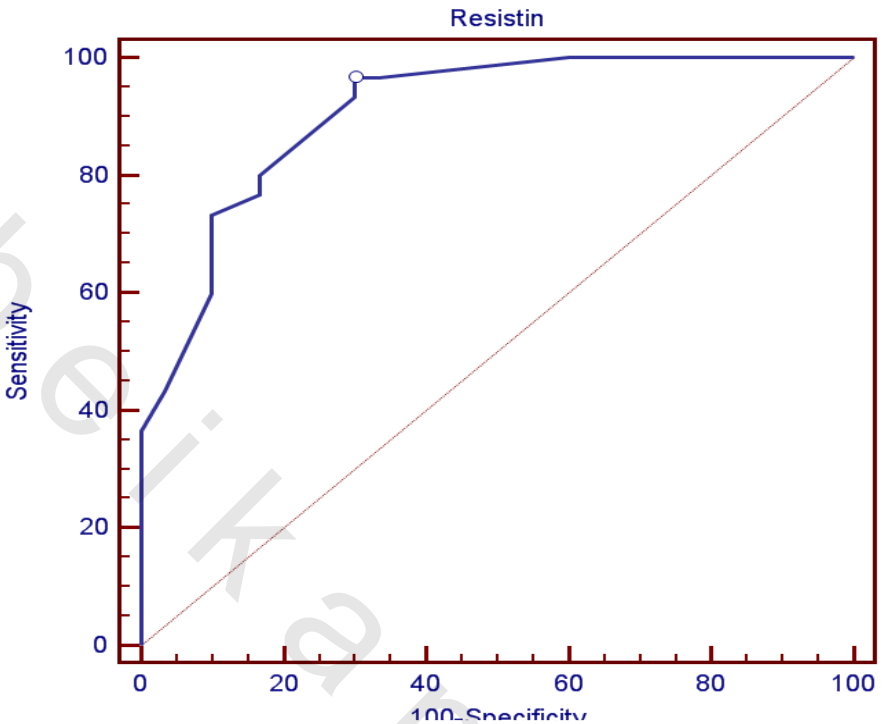


Figure (6): Correlation Study between HOMA-IR and resistin in Pr-eclamptic Patients.



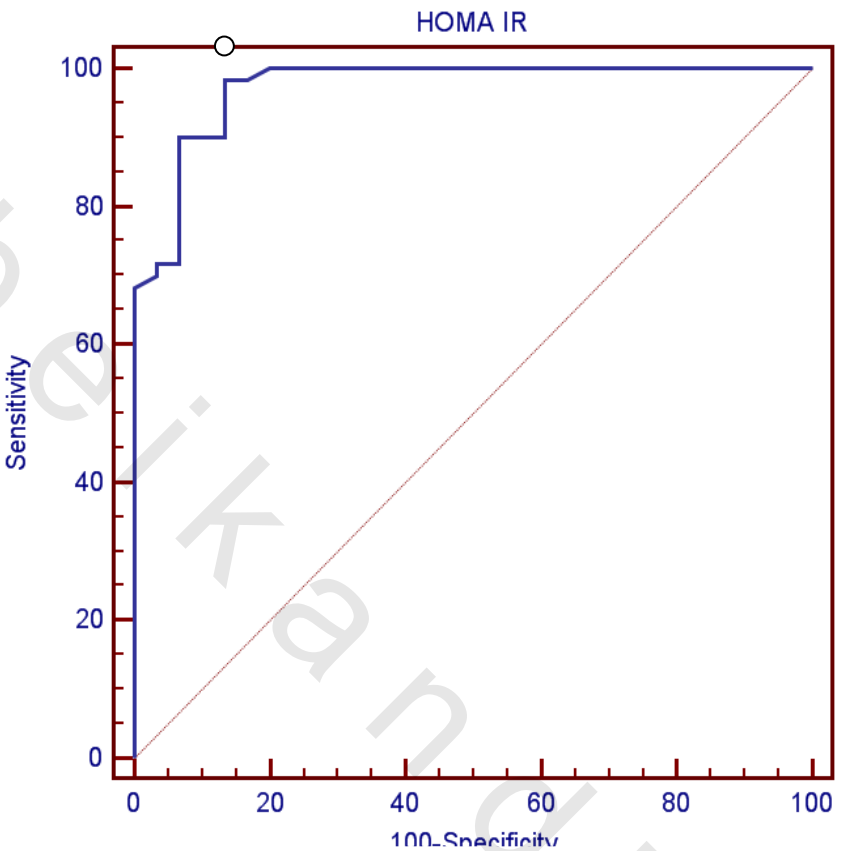
Cutoffpoint	AUC	Sensitivity	Specificity	+PV	-PV
>10	96.7	88.33	96.67	98.1	80.6

Figure (7): ROCCurveanalysisshowingthediagnosticperformanceofResistinindiscriminatingpre-eclampticfemalesfromhealthycontrols.



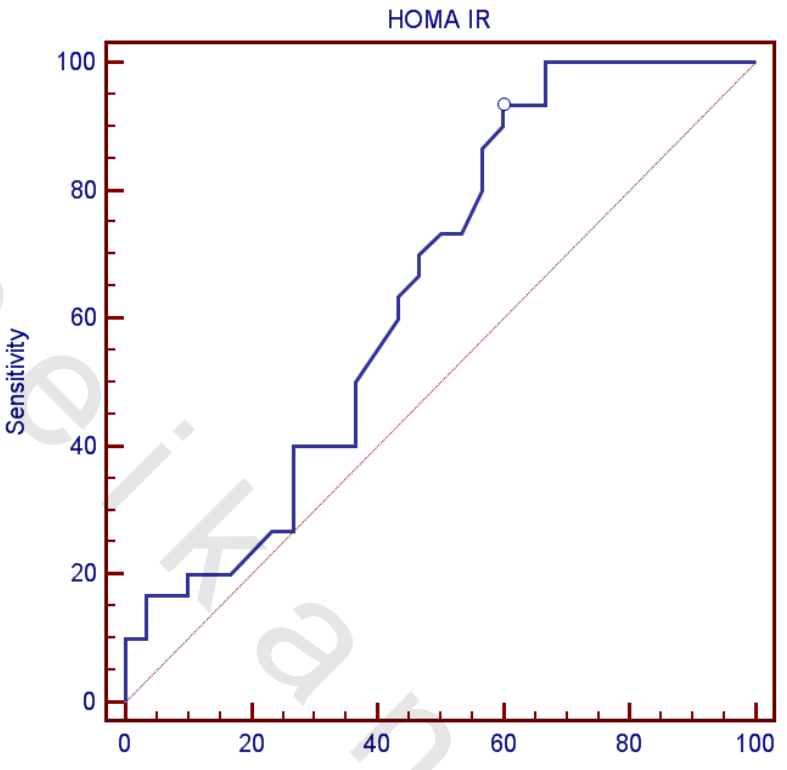
Cutoffpoint	AUC	Sensitivity	Specificity	+PV	-PV
>17	90.8	96.67	70.00	76.3	95.5

Figure (8): ROCCurveanalysisshowingthediagnosticperformanceofresistinin discriminatingmildfromseverecasesofpre-eclampsia.



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

Figure (9): ROCCurveanalysisshowingthediagnosticperformanceof HOMAI Rindiscriminatingpre-eclampticfemalesfromhealthycontrols.



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

Figure (10): ROCCurveanalysisshowingthediagnosticperformanceof HOMA-IRindiscriminatingmildfromseverecasesofpre-eclampsia.

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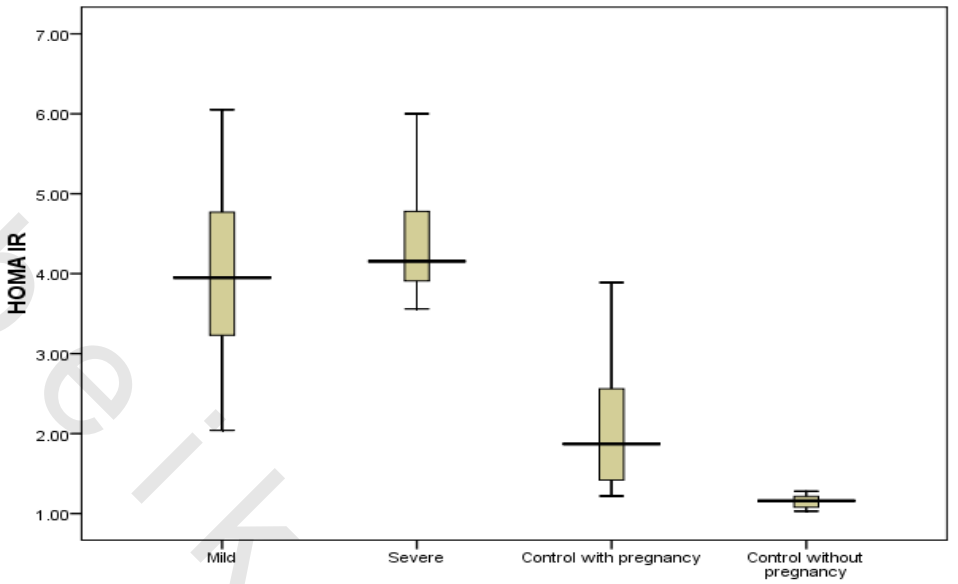

Figure (11): Comparisonbetweenallstudiedgroupsasregardvaluesof HOMA-IR.

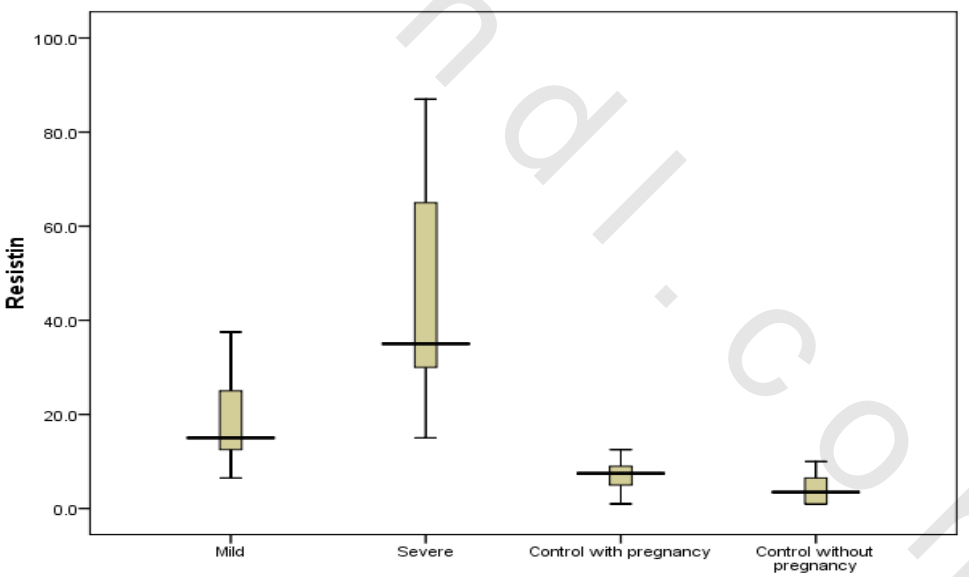

Figure (12): Comparisonbetweenallstudiedgroupsasregardvaluesof Resistin.