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Protein Kinase A, Insulin resistance markers and Their Correlation with Specific SNPs of IGF2BP2 Among Patient with Type 2 Diabetes Mellitus in Holy Najaf province

A Thesis
Submitted to the Council of the College of the Medicine/ University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical Biochemistry
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بسْمِ اللَّه الرَّحْمَٰنِ الرَّحِيمِ

﴿قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ﴾

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Acknowledgments

We certify that this thesis entitled " **Protein Kinase A, Insulin resistance markers and Their Correlation with Specific SNPs of IGF2BP2 Among Patient with Type 2 Diabetes Mellitus in Holy Najaf province** "

". was carried out under our supervision at the college of medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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Dedication

I dedication this work to:

My parents for Encouragement and support

My familiy

for their kindness with me

and to all my friends that help me

Thualfaqar

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Thanks and praise to Allah Almighty and his Messenger, may Allah blessings be upon him and his family and all his companions.

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Summary

The term diabetes describes a group of metabolic disorders characterized and identified by the presence of hyperglycemia.

Patient with type 2 diabetes mellitus (T2DM) can be found in almost every population in the world. This study was designed as a case-control study and was constructed to study the effect of diabetes mellitus type 2 on protein kinase A, insulin, hemoglobin A1C (HbA1c), fasting blood glucose (FBG) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels and to study the correlation of protein kinase A with, insulin, HbA1c, and HOMA levels in patient with type 2 diabetes to achieve this aim, 60 patients with type 2 diabetes, and with 60 apparently healthy participate as control group. The patients ages were range between (35-60) years, mean ± standard deviation (SD) was (47.2±13.1) years, and mean ± standard deviation (SD) for BMI was (25.31±2.59) Kg/m². The age of control group was ranged between (35-60) years with mean ± standard deviation (SD) was (46.7±12.9) years, and mean ± standerd deviation (SD) for BMI was (24.7±1.11) Kg/m². There was matching in age and BMI between patients and control (p-value >0.05).

The sera sample of both groups were used to measure the level of protein kinase A, insulin by using ELISA method, insulin resistance parameters (HOMA-IR) were calculated using HOMA2-Calculator . While, FBG and HbA1c had measured by AFIAS instrument.

Results of the present study revealed that there was a significant differences in the levels of protein kinase A between patients with type 2 diabetes mellitus and their control group ($p < 0.05$), there was a significant differences in the levels of insulin between patients and control groups

($p < 0.001$), also there was a significant difference in the levels of HbA1c between patient with type 2 diabetes mellitus and their control group ($p < 0.001$), there was a significant correlation between protein kinase A and insulin, HbA1c, and HOMA respectively.

The heterozygous genotype (GT) of IGF2BP2 gene (rs4402960 G/T) SNP in patient with type 2 diabetes mellitus group compared with control group was found to be non- significant difference also the homozygous genotype (TT) of IGF2BP2 gene (rs4402960 G/T) SNP in diabetic group compared with control group was found to be non -significantly difference.

The minor allele frequencies (T) of IGF2BP2gene **(**(rs4402960 G/T) SNP in diabetic and control group were found to be 67.5% and 32.5% respectively. It was non significantly difference ($P > 0.05$) in diabetic when compared with control group.

Polymorphism of IGF2BP2 gene (rs11705701 G/A) was not associate with patient with type 2 diabetes mellitus ,(this study detected only wild type GG genotype in all our studied participants, and none of them presented the mutant GA or AA genotypes).

In conclusion, from the results of this study, it is concluded that diabetes mellitus has a role in a significant decrement in protein kinase A, but there was an increase in HOMA-IR,HbA1c,FBG and insulin.

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List of Abbreviation

Abbreviation	Details
ABCD	Appropriate Blood Pressure Control in Diabetes
AC	Adenylyl cyclase
ADA‟s	American Diabetes Association's
AIP	Atherogenic index plasma
Afias	Automated fluorescent immunoassay system
BMI	Body Mass Index
CVD	Cardio vascular disese
cAMP	Cyclic adenosine 3′-5′ monophosphate
CSNP	Coding single nucleotide polymorphism
DM	Diabetes mellitus

dNTPs	Deoxynucleoside triphosphates
EPIC	European Prospective Investigation into Cancer and Nutrition
ELISA	Enzyme-linked immunosorbent assay
TBE	Tetra boric acid EDTA
FRET	Fluorescence resonance energy transfer
FPG	Random plasma glucose
GDM	Gestational diabetes mellitus
GLP-1	Glucagon-like peptide 1
GPCRs	G protein coupled receptors
GWAS	Genome-wide association studies
G6P	Glucose-6-phosphate
HGP	Hepatic glucose production
HbA1C	Glycosylated haemoglobin
HRP	Horseradish peroxidase
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IDF	International Diabetes Federation
IGF2BP2	Insulin like growth factor 2 mRNA binding protein2
IAPP	Islets amino acid poly peptide
LA	Lactic acidosis
MODY	Maturity-onset diabetes of the young
MHC	Major histocompatibility complex
PPG	Post-prandial plasma glucose
PKA	Protein kinase A
PCR	Polymerase chain reaction
PDE	Phosphodiesterase

RCTs	Randomized controlled trials
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SGLT2	Sodium-glucose transport protein 2
SNP	Single nucleotide polymorphism
sdLDL	Small dense low-density lipoprotein
SD	Standard deviation
STAT3	Signal transducer and activator of transcription 3
TBE	Tetra boric acid EDTA
T1DM	Type 1 diabetes
T2DM	Mellitus Type 2 diabetes mellitus
WHO	World Health Organization

Chapter One
Introduction and Literature Review

1. Introduction and Literature Review

1.1. Diabetes Mellitus

1.1.1. Definition

Diabetes Mellitus is a chronic condition that happens when the pancreas fails to produce enough insulin or when the body's insulin production is inefficient. the effects can be well-balanced with adequate health management and regular medical check-ups.frequent urination, increased thirst, and increased hunger are all feature of elevated blood glucose **(1)**.

patient with diabetes, if left untreated can lead to a slew of consequences include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and eye impairment are all serious long-term complications **(2)**.

Ketoacidosis or a non-ketotic hyperosmolar condition are the most serious clinical complication which can lead to dehydration, unconsciousness, and in the absence of adequate treatment and death **(3)**.

Type 1 DM occurs when an autoimmune illness affects pancreatic cells so that insulin production is reduced or impaired, whereas type 2 diabetes mellitus is caused by decrease insulin secretion and insulin resistance which impairs the individual's capacity to utilize insulin**(4)**.

T1DM can strike at any age, however it most commonly strikes in adolescents with a peak onset around puberty. T1DM is equally frequent in both sexes during childhood, although males are more likely to develop the condition in early adulthood **(5)**. Patient with type 2 diabetes mellitus is one of the most prevalent metabolic diseases in the world and it is caused by a combination of mainly two factors inadequate insulin production by pancreatic -cells and the failure of insulin-sensitive tissues to respond to insulin **(6)**.

1.1.2 Classification of Diabetes Mellitus

With this in mind, the expert group decided that defining a classification system that prioritizes clinical care and assists health providers in deciding whether or not to start insulin treatment, particularly at the time of diagnosis was the best option. the only classification system that could help with this right now uses clinical criteria to identify diabetes subtypes **(7)**.

The following is the WHO classification of diabetes mellitus:

1.Diabetes mellitus type 1 includes diabetes primarily caused by the destruction of pancreatic beta cells and is likely to develop ketoacidosis. This form includes auto-immune beta-cell destruction cases unknown for their aetiology **(8)**.

2.Diabetes mellitus type 2 (due to a progressive insulin secretory defect on the background of insulin resistance).

3.Gestational diabetes mellitus is a type of glucose intolerance that develops or is discovered during pregnancy.(non-overt diabetes diagnosed in the second or third trimester of pregnancy) **(9)**.

4.Diabetes is caused by other factors, such as monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young (MODY), exocrine pancreas diseases (such as cystic fibrosis), and drug- or chemical-induced patient with diabetes (such as after HIV/AIDS treatment or organ transplantation) **(10)**.

Table 1-1: show the classification and observations on types of DM **(7)**.

Feature	Type1	Type2	Gestational
Age of onest	Usually during childhood or puberty	Frequently after the age of 35	2 or 3 trimester of pregnancy
Prevalence	10%of diagnosis cases	90% of diagnosis cases	2-5% of pregnant woman
Genetic predisposition	Modrate	Very strong	
Nutrition	Undernourished	Mostly obese	
Biochemical defect	Auto immune destruction of B-cell	Insulin resistance and inability of B-cell to produce enough amount of insulin	B-cell are not able to compensate for the increase insulin resistance
Plasma insulin	Low to absent	High in the early stage	

1.1.3 Diabetes Diagnostic Criteria

1-Fasting plasms glucose (FPG) ≥7.0 mmol/L or above 126 mg/dl.

2-Glycated haemoglobin (Hba1C) ≥6.5% (in adults) .

3-Two-hour plasma glucose in a 75 g oral glucose tolerance test ≥11.1 mmol/L or above 200 mg/dl.

4-Random plasma glucose ≥11.1 mmol/L or above 200 mg/dl **(11)**.

1.2 Type 2 Diabetes

Patient with Type 2 diabetes often known as adult-onset diabetes can strike anyone at any age including children. Type 2 diabetes on the other hand, is more common in middle-aged and older persons. obese and sedentary people are also more likely to develop type 2 diabetes **(6)**.

Insulin resistance which arises when fat, muscle, and liver cells do not use insulin to transport glucose into the body's cells for use as energy is the most common cause of patient with type 2 diabetes. as a result, more insulin is required to help glucose enter cells. Initially, the pancreas responds by producing more insulin to meet the increased demand. when blood glucose levels rise such as after meals, the pancreas produces insufficient insulin over time **(12)**.

The hyper-insulinemia caused by insulin resistance in the early phase of type 2 diabetes mellitus causes a concomitant increase in amylin production which is then deposite as amyloid in the islets. amyloid is toxic to β-cells and surrounding them and may render them somewhat refractory to receiving the glucose signal. thus amyloid may contribute to the β-cells loss seen in advanced cases of type 2 diabetes mellitus **(13)**.

The risk of developing type 2 diabetes increases with **(14)**:

1-Family history of diabetes (particularly , parents).

2-Membership of some ethnic groups .

3-Age ≥ 45 years .

4-Hypertension (≥ 140/90 mmHg in adults) .

5-HDL cholesterol level <1.0 mmol/L (<0.38 g/L).

6-Reduced physical activity .

7-History of gestational diabetes mellitus (GDM).

1.2.1 Etiology

The pathogenesis of patient with T2DM primarily initiates with the inadequacy of pancreatic islet β-cells to respond to chronic fuel surfeit and hence causing glycemic load, insulin resistance, and obesity **(15)**. the major driving factors of the global patient with T2DM epidemic include overweight and obesity, sedentary lifestyle and increased consumption of unhealthy diets containing processed meat, refined grains and sugar- sweetened beverages **(16)**.

The epidemic of patient with T2DM necessitates the development of novel therapeutic and preventative strategies to attenuate the expansion of this debilitating disease **(17)**.

The mechanism of genetic initiation of DM is still unknown, but a single nucleotide polymorphism (SNP) is one of the most common cause of T2DM. the diabetic genome sequence different from normal one by single loci of genome around numerous genes and the single nucleotide variation make a phenotypically different trait that increase susceptibility of occurrence of the disease **(18)**.

1.2.2. Epidemiology

Diabetes mellitus is a major health problem and a common non communicable lifelong disease widely spread throughout the world . it is considered as one of the health challenges in 21st century. the global diabetes prevalence in 20–79 year olds in 2021 was estimated to be 10.5% (536.6 million people) as show Figure (1-1) **(19)**. according to international diabetes federation (IDF) in 2018 there were 1.4 million cases of diabetes in Iraq, the prevalence of DM in Iraq was 8.5% to 13.9% **(20)**.

In western countries, patient with Type 2 diabetes mellitus has essentially become the most epidemic disease because the western

typical lifestyle of sedentary behavior and huge calories diet.

However, the rate of diabetes is increased even in nonwestern country **(21)**.

Patient with Type 2 diabetes mellitus is the highest prevalence in comparison to another diabetes mellitus types. it accounts for about 90% of the overall prevalence of patient with diabetes.

The incidence of the disorder had been rapidly raised in the last years **(22).**

A local survey of almost 5400 people in Basrah. Southern Iraq, found a 19.7% age-adjusted prevalence of diabetes in people aged 19-94. there are few epidemiological research and randomized controlled trials (RCTs) on diabetes in Iraq making it difficult to completely comprehend the incidence of patient with diabetes in Iraq and the most effective treatments for Iraqis **(20)**.

Figure 1-1: Diabetes worldwide estimation for number of people and region in 2021 **(19)**.

1.2.3 Ethnicity

Patient with Type 2 Diabetes Mellitus prevalence and incidence vary widely by ethnicity and geographic area across the world with

Japanese, Hispanics, and Native Americans having the highest risk.

Asians had greater rates of incidence than white Americans and white Britons with black individuals having the highest risk while no clear explanation has been found relevant variables such as modern lifestyle factors (which increase obesity), socioeconomic and direct genetic predisposition and gene-environment interactions have been proposed **(23)**.

1.2.4. Pathophysiology of type 2 Diabetes

Patient with T2DM are thought to be born with a genetic susceptibility to insulin resistance **(24)**. Insulin suppresses hepatic glucose production in people with normal insulin sensitivity in the case of hepatic insulin resistance. However, gluconeogenesis persists even when the fasting insulin level is high resulting in hyperglycemia **(25)**.

In the fed state, hepatic glucose production suppression in response to insulin is also impaired. Post-meal glucose uptake occurs as a result of peripheral tissue insulin resistance and postprandial hyperglycemia develops **(26)**.

The obesity and physical inactivity are insulin-resistant situations that reveal pancreatic β-cell dysfunction when they fail to increase insulin production to compensate for insulin resistance consequences **(27)**. Glucose tolerance/ euglycemia is maintained as long as the β-cells can increase their insulin secretion to compensate for the influence of insulin resistance **(28)**. however, as β-cells lose their ability to compensate for insulin resistance, post-prandial plasma glucose and then fasting plasma glucose levels begin to rise leading to overt diabetes **(29)**.

The following are the main physiological defects that go into causing type 2 patient with diabetes **(30)** as shown in figure (1-2).

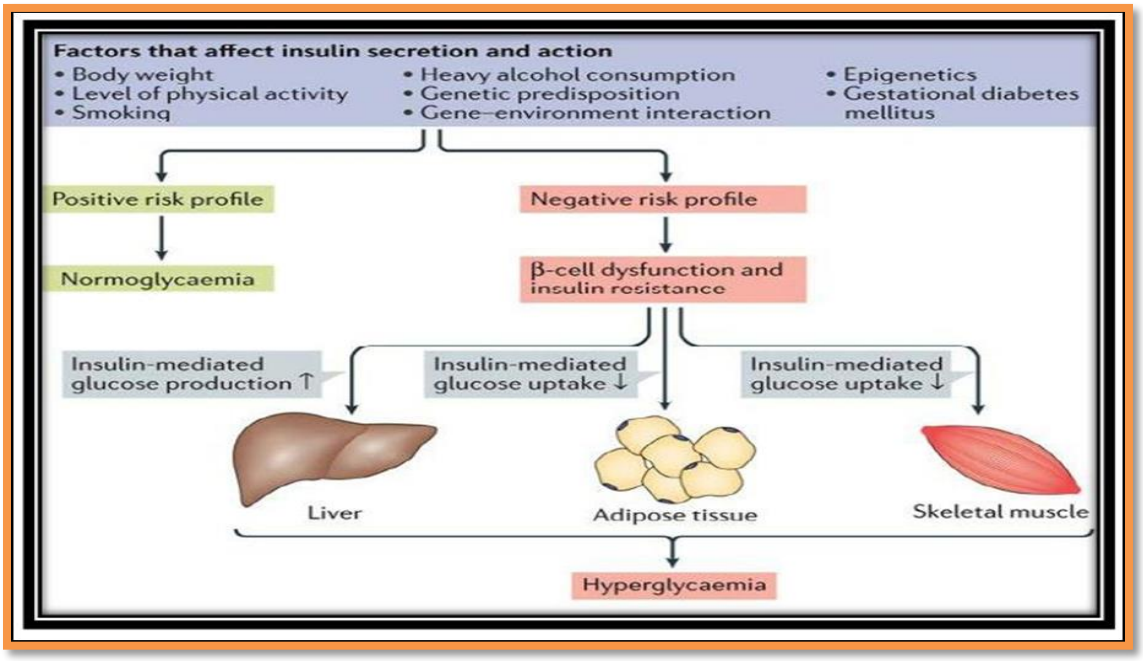

Figure 1-2: Hyperglycemia pathophysiology in type two diabetes mellitus **(30)**

1.2.4.1 β-Cell Dysfunction

The β -Cell dysfunction plays a key role in the progression of T2DM across the hyperglycemia range from prediabetes to diabetes with continuous islet regeneration and death. these cells are in a perpetual state of dynamic transformation. Multiple anomalies can throw this delicate equilibrium off. insulin production becomes insufficient to prevent rising blood glucose levels as β -cell failure advances **(31)**. Although the plasma insulin response to insulin resistance is frequently raised over the natural history of patient with T2DM, this does not mean that the β-cell is healthy.the beginning of β-cell failure is much earlier and the contribution to hyperglycemia is much greater as shown in figures (1-3) **(32)**.

Age: there is a progressive age-related decline in β-cell function.

Genes: β-Cell failure clusters in families.

Insulin resistance: Insulin resistance places an increased demand on the β cells to hyper secret insulin and thus contributes to the progressive β-cell failure in patient with T2DM.

Lipotoxicity: Lipid deposition in the β cell and chronic elevation of the plasma FFA concentration impair insulin secretion.

Glucotoxicity: Chronically elevated plasma glucose levels impair β-cell function, and this has been referred to as glucotoxicity.

Islets amylin poly peptide (IAPP): Excessive secretion of IAPP with subsequent amyloid deposition within the pancreas has also been shown to contribute to progressive β-cell failure in patient with T2DM.

TNF-α: Chronic exposure of β cells to TNF-α finally induces islet dysfunction and β cell apoptosis.

Incretins: There is severe resistance to the stimulatory effect of both GLP-1 and GIP. the resistance to GLP-1 can be observed in individuals with IGT and worsens progressively with progression to patient with T2DM **(33)**.

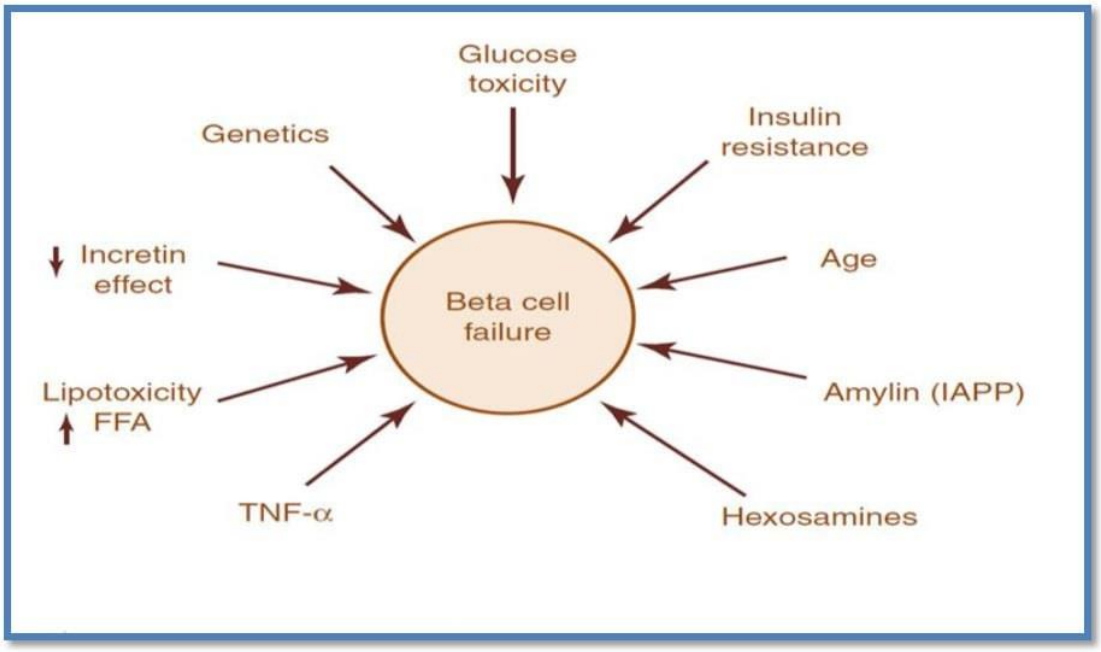

Figure 1-3: Pathogenic factors implicated in progressive β-cell failure **(33)**

1.2.4.2. Alpha Cells (Increased Glucagon Secretion)

There is a decrease in β-cell mass in patient with diabetes when compared to healthy people. There is no reduction in α-cell mass in

T2DM patients.It's also been suggested that in T2DM patients. β-cells dedifferentiate and become other cell types such as glucagon-secreting αcells **(34)**.

In support of these claims, even when insulin levels fall over time in T2DM, basal glucagon levels tend to remain elevated **(35)**.

1.2.4.3. Kidneys (Increased Glucose Reabsorption)

In patient with diabetes, the kidney's adaptive response to preserve glucose which allows the body to satisfy its energy demands particularly the brain and other neurological disorders which have an essential need for glucose becomes maladaptive. the kidney holds the glucose rather than releasing it into the urine to treat hyperglycemia. the SGLT2 transporter in the convoluted portion of the proximal tubule reabsorbs about 90% of the filtered glucose, whereas the SGLT1 transporter in the straight segment of the descending proximal tubule reabsorbs the remaining 10% **(36)**. In healthy people, no glucose occurs in the urine until the blood glucose level exceeds 180 mg/dL, however, in T2DM, this threshold is much higher **(37)**.

1.2.4.4. Brain(Neurotransmitter Dysfunction and Central Appetite Dysregulation)

In the pathophysiology of patient with T2DM, the nervous system is also important to regulate insulin and glucagon release, as well as hepatic glucose production.the sympathetic and parasympathetic nervous systems influence glucose metabolism directly through neural input and indirectly through circulation **(38)**. Insulin secretion was hindered when the vagus nerve was severed showing the importance of the vagus nerve in islet regulation **(39)**.

Insulin is an effective hunger suppressant. even though insulin resistance causes compensatory hyperinsulinemia in obese persons with

or without diabetes.food intake seems to be higher indicating that the appetite centers are also insulin resistant **(40)**.

1.2.5. Risk Factors and Mechanism of Diabetes Type 2

1.**Age:** Patient with diabetes is more common as people get older. patient with Type 2 diabetes is uncommon in most populations before the age of 30 but it rises rapidly and steadily as people get older, age is a substantial risk factor in prospective observational studies **(41)**.

2.**Gender:**The European Prospective Investigation into Cancer and Nutrition found that men have a higher risk of diabetes than women across all European countries **(42)**.

3.**Genetic component:**There is a significant genetic component to the condition, Higher concordance rates are indicating that patient with T2DM has a major genetic component. Furthermore, 40% of first-degree relatives of T2DM patients are at risk of developing diabetes compared to only 6% in the general population **(43)**.

4.**Lifestyle variables**: Sedentary lifestyles, physical inactivity, smoking, and alcohol use are all important contributors to the development of patient with T2DM **(44)**.

5.**Vitamins and type 2 diabetes**: There is growing evidence that vitamin D may have a role in the regulation of patient with T2DM, as there is a seasonal change in the glycemic state of patient with T2DM,and hypovitaminosis D which is more common in the winter, is likely to be linked to patient with T2DM aggravation **(45)**. Phylloquinone (vitamin K1) and menaquinones are two naturally occurring forms of vitamin K. Menaquinone-4 (vitamin K2) is the active form of vitamin K in bone tissue and is involved in bone quality maintenance. Furthermore.

A recent study found that vitamin K1 aids glucose homeostasis as higher vitamin K1 intake is linked to improved insulin sensitivity and

glycemic control Because vitamin K deficiency can lead to impaired glycemic management and bone quality **(46)**.

6-Obesity

Obesity is a state that characterized by a relatively excess fat stored in the adipose tissue , it is most important risk factor for T2DM according to extensive epidemiological research and it may influence the development of insulin resistance and disease progression **(47)**.

Obesity is a significant cause of mortality, in 2014, they described an approximated 1.9 billion overweight and nearly 600 million obese adults, which refers to 13% of the adult population in each world **(48)**.

As obesity levels rise, this has made this problem more prevalent.

The World Health Organization (WHO) estimates that there are 1.3 billion overweight adults globally. of these 300 million are clinically obese. the global increase in incidence of type-2 diabetes.

The currently worldwide estimate of overweight individuals (BMI>25Kg/m2) will increase from 1.3 billion to 2.0 billion by 2030 **(49)**.

1.2.6. Signs and Symptoms of Type 2 Diabetes

The most common feature of patient with diabetes type 2 is asymptomatic but the following signs and symptoms could appear include (thirst, polyuria, polyphagia, frequent hunger, feeling very fatigued, losing weight without trying, sores that heal slowly, dry, itchy skin, pins and needles in feet, losing feeling in feet, impaired eye sight) are all signs and symptoms of patient with diabetes. The only way to find out if a person has diabetes is to get a blood test order by a doctor **(50)**.

1.2.7 Complications of Type 2 Diabetes

1.2.7.1 Acute Complications of Type 2 Diabetes

Patient with type 2 diabetes can present with various acute crises that impart a substantial short-term risk of morbidity and mortality. these include(ketoacidosis, hypoglycamia, lactic acidosis and non-ketotic coma hyper-osmolarity) .

The lactic acidosis with more lactic acid up to 5.0 mEq / L with acidosis can be present. hypoxia, such as shock, hypoxemia, carbon monoxide exposure, sepsis, and certain medications, such as metformin, Its a common influencing factors for lactic acidosis, particularly in patient with renal failure also consider a widely known in treating patient with diabetes milltus type2 as a vital component **(51)**.

1.2.7.2 Chronic Complications of Type 2 Diabetes

There are two types of chronic complications:

A- one or more of the following are caused by the damage of small blood vessels that bring about microvascular **(52)**:

1- Diabetic nephropathy.

2- Diabetic neuropathy.

3- Diabetic retinopathy.

B- one or more of the following are caused by macrovascular disease**(53)**:

1- Cardiovascular disease to which accelerated atherosclerosis, is a contributor to coronary artery disease leading to angina or myocardial infarction (heart attack)

2- Diabetic myonecrosis (muscle wasting).

3- Stroke (mainly the ischemic type) **(53)**.

1.2.8. Management of Type 2 Diabetes

Patient with diabetes should not be treated solely based on symptoms. Glycemic targets are established based on evidence of what glucose levels are associated with the development of problems. insulin therapy is required to treat hyperglycemia in people with type 1 diabetes. in type 2 diabetes, if hyperglycemia is mild, patients may be given a one-month trial of diet, exercise and weight management to reduce hyperglycemia. if this regimen fails to provide acceptable blood glucose control, the doctor will need to prescribe oral anti-hyperglycaemic medications. the major goals of diabetes mellitus therapy are to remove hyperglycemia symptoms, achieve optimum control, limit or eliminate microvascular and macrovascular consequences of diabetes, treat related illnesses, and allow the patient to live as normally as possible **(54)**.

1.2.9. Pharmacological Therapy

When lifestyle modification fails, therapeutic methods should be used that consist of the following options include (sulfonylureas, meglitinides, metformin (a biguanide), thiazolidinediones (TZDs), alpha-glucosidase inhibitors,dipeptidyl peptidase IV (DPP-4) inhibitors, bile acid sequestrants, dopamine agonists, sodium-glucose transport protein 2 (SGLT2) inhibitors and oral glucagon-like peptide 1 (GLP-1) receptor agonists. In addition, glucagon-like peptide 1 (GLP-1) receptor agonists and amylin can be administered by injection) **(55)**.

1.3 Protein Kinase A (PKA)(2.7.11.11):

PKA is a serine/threonine kinase that regulates a wide range of cellular processes including metabolism, gene expression, and cell proliferation.Cyclic adenosine 3′-5′ monophosphate (cAMP) is a ubiquitous second messenger that acts as a signal transducer for a variety of external stimuli. It is associated in disease and regulates a

multitude of biological processes **(56)**. Downstream effector proteins such as cAMP-dependent protein kinase A, are activated by cyclic AMP. PKA is the most important cAMP downstream effector.

It's a broad specificity serine/threonine kinase that regulates a variety of physiological functions including (metabolism, cell proliferation, cell division, and cardiac myocyte contraction). it is a heterotetramer made up of two catalytic subunits (Cα, Cβ, or Cγ) that are maintained inactive in the holoenzyme by two regulatory (RI or RII) subunits that are structured as homodimers **(57)**.

The C subunits are released and hence activated when cAMP binds to the R subunits and they then phosphorylate local substrates **(58)**.

Recent fluorescence resonance energy transfer imaging-based research backed up this theory, suggesting that physiological cAMP levels induce only little separation of the C subunits from the holoenzyme, restricting PKA action to substrates in direct proximity. As a result, both the PKA holoenzyme and/or the dissociated C subunits appear to be active **(59)**. Cyclic AMP was first recognized as an intracellular mediator of glucagon and epinephrine's glycogenolytic impact in the liver **(60)**. Edwin Krebs and edmond fischer were given the nobel prize in physiology or medicine in 1992 for their discovery of the phosphorylation cascade PKA regulatory (R) subunits and exchange proteins directly activated by cAMP (Epac) are the most important cAMP receptors in mammals. Epac's role in metabolic control has lately been researched **(61)**. With the advent of genetically modified and pharmacological evidence, the physiological role of PKA in glucose metabolism is becoming well understood. cyclic AMP is made in cells from adenosine triphosphate (ATP), which is processed by adenylyl cyclase which is triggered by stimulatory Gα protein after Gs

protein-coupled receptors are active as show figure (1-3). the conversion of cAMP to AMP by phosphodiesterase leads to the end of cAMP signaling **(62)**. the ligand-dependent activation of G protein coupled receptors initiates cAMP/PKA signaling which is followed by Gsα activation, adenylyl cyclase activation and cAMP production by attaching to PKA regulatory subunits, cAMP activates PKA .

PKA catalytic subunits (C) are then released from the PKA tetramer to phosphorylate targets in the cytoplasm, plasma membrane (Ca_V, K_{ATP}), and nucleus (CREB). the binding of R subunits to various Akinase anchoring proteins is required for PKA subcellular localization (A-KAPs).

The breakdown of cAMP to AMP by phosphodiesterase is required for the cessation of cAMP signaling as shown in figure (1-4) **(63).**

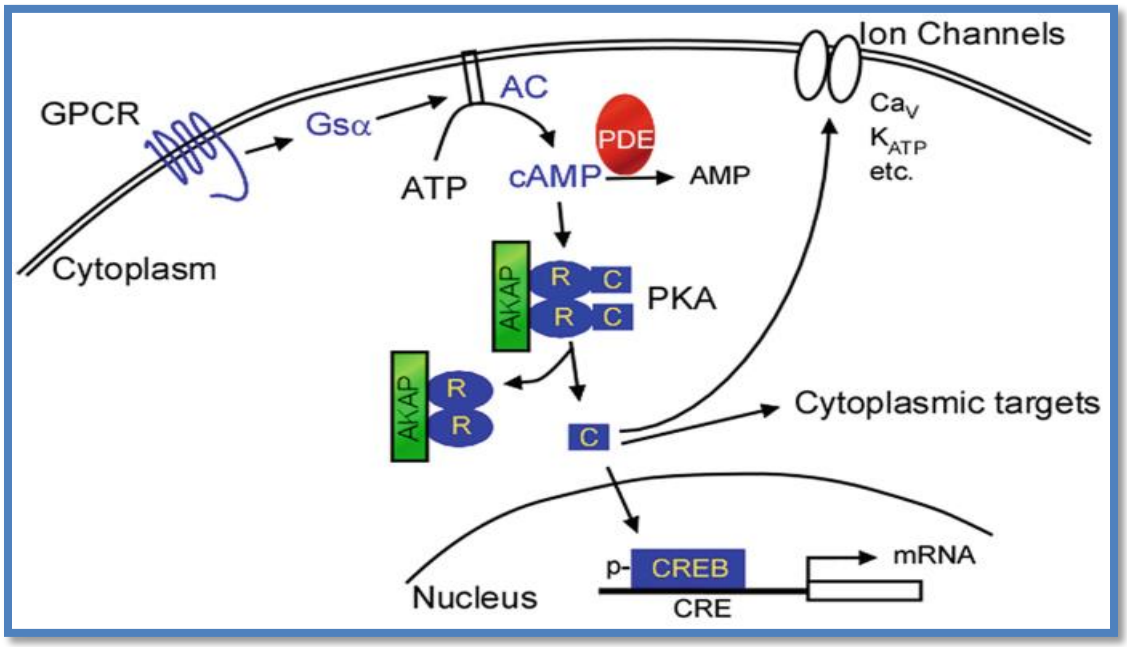

Figure 1-4: Cyclic AMP/PKA signaling pathway in human cells **(63)**

1.3.1- Cyclic adenosine 3′-5′ monophosphate (cAMP)/ Protein kinase A (PKA) in pancreatic islets β:

An early impairment in glucose-stimulated insulin release from pancreatic β-cells is a characteristic of a T2DM, following a meal, glucose levels in the circulation increase leading to increased uptake of glucose into β cells via the glucose transporter GLUT2.

Increased intracellular glucose then leads to production of ATP and inactivation of the ATP-sensitive potassium channel (K-ATP) and depolarization of the cell , cell depolarization activates voltage-gated calcium channel (Ca-v) on the plasma membrane and calcium influx into β cells. accumulation of Ca^{2+} in the cell then promotes insulin secretion via exocytosis **(64)**. Subsequently, insulin circulates and acts on a variety of tissues mainly fat, muscle, and liver, to increase glucose uptake and inhibit glucose production. this process is essential for postprandial glycemic control. although glucose is the primary regulator for insulin secretion, many hormones and neurotransmitters such as glucagon, glucagon-like peptide 1 (GLP1), epinephrine, and norepinephrine can either enhance or suppress insulin secretion through GPCRs on β cells **(65)**. cAMP/PKA pathway acts downstream of the GPCRs and regulates the activities of key molecules involved in insulin secretion, including GLUT2, K-ATP, and Ca-v . it has been shown that GLUT2 is phosphorylated at multiple sites in the carboxyl-terminal domain by PKA following either forskolin or GLP1 stimulation of β cells , PKA-dependent phosphorylation suppresses the catalytic activity of GLUT2 and reduces glucose uptake into β cells. the modification of cAMP levels has been proposed as one strategy for targeting the cAMP/PKA pathway **(66)**.

1.4 Glycated Hemoglobin (HbA1c)

Glycated hemoglobin is made by adding glucose to the amino groups of hemoglobin without using enzymes. the concentration of HbA1c is affected by both the amount of glucose in the blood and the erythrocyte's life span, HbA1c shows the integrated glucose concentration over the previous (8–12) weeks since erythrocytes are in the circulation for about 120 days as a result, it is free of the huge variations in blood glucose concentrations that occur on a daily basis **(67)**. The tetramer hemoglobin molecule is made up of two alpha and two beta globin chains and carries oxygen throughout the body. in the presence of high blood glucose levels, hemoglobin gets non-enzymatically glycated at a variety of sites across the structure of the molecule **(68)**. In patient with diabetes, the HbA1c level is primarily used to monitor their condition in order to be considered optimal diabetes treatment. several organizations have endorsed HbA1c goals of less than 6.5 to 7% as the gold standard **(69)**.

1.5 Insulin

Insulin is a polypeptide hormone composed of 51 amino acids and has a molecular weight of (5808) Dalton, secreted by pancreatic β-cells of Langerhans. the pancreas consists of clusters of endocrine tissue called the islets of Langerhans which are embedded in exocrine tissue. the islets are composed of four major phenotypically specific hormone-producing cells α, β, δ and PP-cells that produce glucagon, Insulin, somatostatin and pancreatic polypeptide, respectively.

The islets are innervated by autonomic nervous system and highly vascularized with a perfusion rate similar to that of the brain and can therefore respond promptly to changes of nutrient content **(70)**.

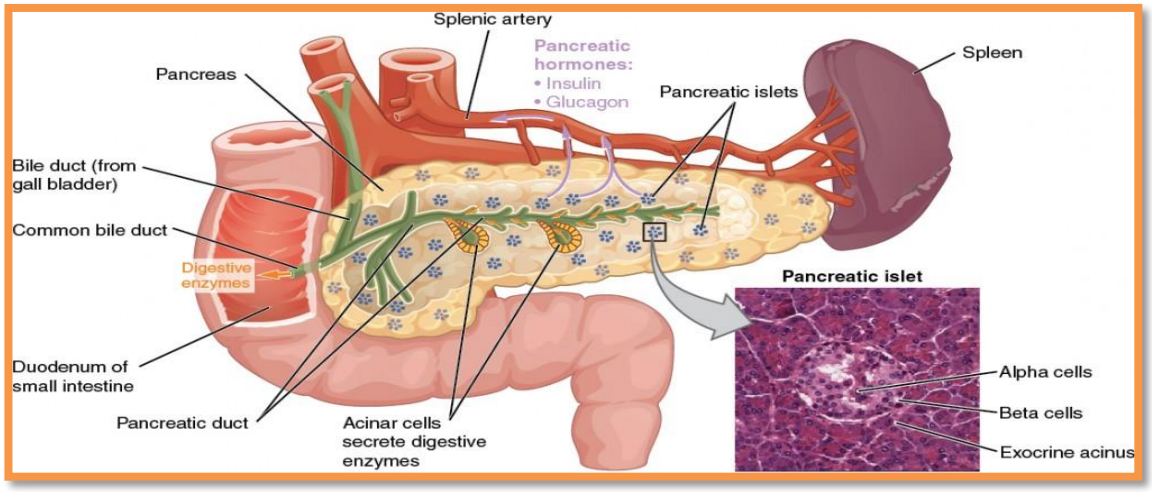

Figure 1-5: The pancreatic endocrine and exocrine function **(71)**

Insulin was the first peripheral hormone to be identified as cental regulator of carbohydrate and fat metabolism in the body. insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle **(70)**.

1.5.1 Structure and Chemical Properties of Insulin.

Insulin was found to be a polypeptide in 1928 with its amino acid sequence identified in 1952. it is a dipeptide containing A and B chains respectively, linked by disulphide bridges and containing 51 amino acids with a molecular weight of 5802. the A chain comprises 21 amino acids and the B chain 30 amino acids. the A chain has an N-terminal helix linked to an anti-parallel C-terminal helix; the B chain has a central helical segment. the two chains are joined by 2 disulphide bonds which join the N- and C-terminal helices of the A chain to the central helix of the B chain. in pro-insulin, a connecting peptide links the N-terminus of the A chain to the C-terminus of the B chain **(72)**.

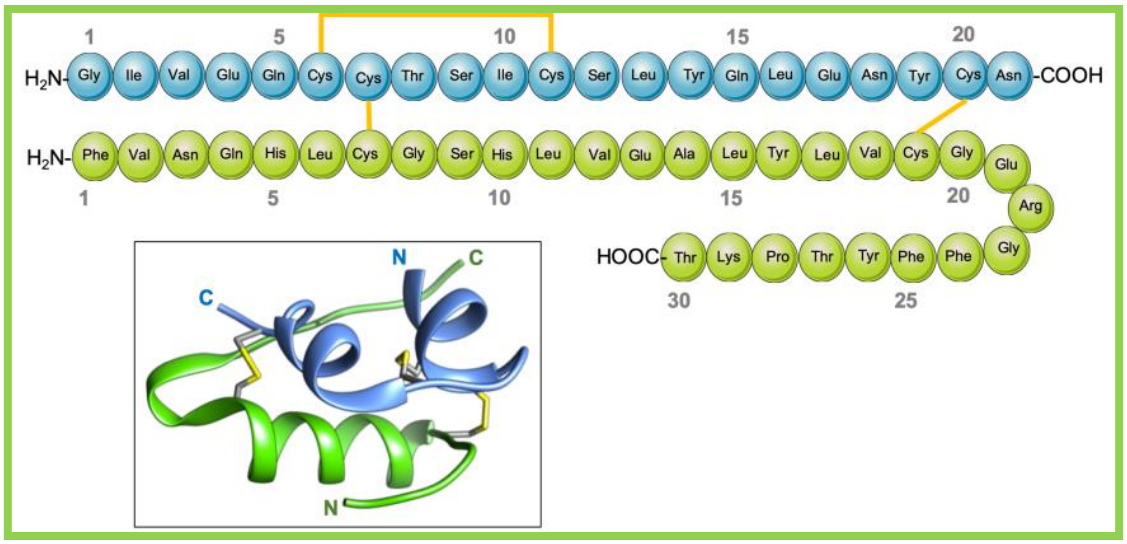

Figure 1-6: structure of human insulin **(73)**

1.5.2 Synthesis and Release of Insulin

Insulin is coded on the short arm of chromosome 11 and synthesized in the β cells of the pancreatic islets of langerhans as its precursor proinsulin, proinsulin is synthesised in the ribosomes of the rough endoplasmic reticulum (RER) from mRNA as pre-proinsulin **(74)**.

Insulin secretion from the islet cells into the portal veins is characteristically pulsatile reflecting the summation of coordinate secretory bursts from millions of islet cells **(75)**.

Factors influencing insulin biosynthesis and release may be influenced by alterations in synthesis at the level of gene transcription, translation, and post-translational modification in the Golgi as well as by factors influencing insulin release from secretory granules. Longer-term modification may occur via influences on β cell mass and differentiation **(76)**.

1.5.3 Mechanisms of Insulin Secretion

Increased levels of glucose induce the "first phase" of glucose mediated insulin secretion. the first phase consists of a brief spike lasting ~10 min followed by the second phase, which reaches a plateau at

2–3 h. It is widely thought that diminution of first-phase insulin release is the earliest detectable defect of β-cell function in individuals destined to develop type 2 diabetes and that this defect largely represents β-cell exhaustion after years of compensation for antecedent insulin resistance by the release of insulin from secretory granules in the β-cell.

Glucose entry into the β-cell is sensed by glucokinase, which phosphorylates glucose to glucose-6-phosphate generating ATP **(77)**. Closure of K^+ -ATP-dependent channels results in membrane depolarization and activation of voltage-dependent calcium channels leading to an increase in intracellular calcium concentration; this triggers pulsatile insulin secretion, regulation and mechanisms of insulin Secretion at the cellular level synthesis and secretion of insulin are regulated by both nutrient and non-nutrient secretagogues.in the context of environmental stimuli and the interplay of other hormones **(78)**.

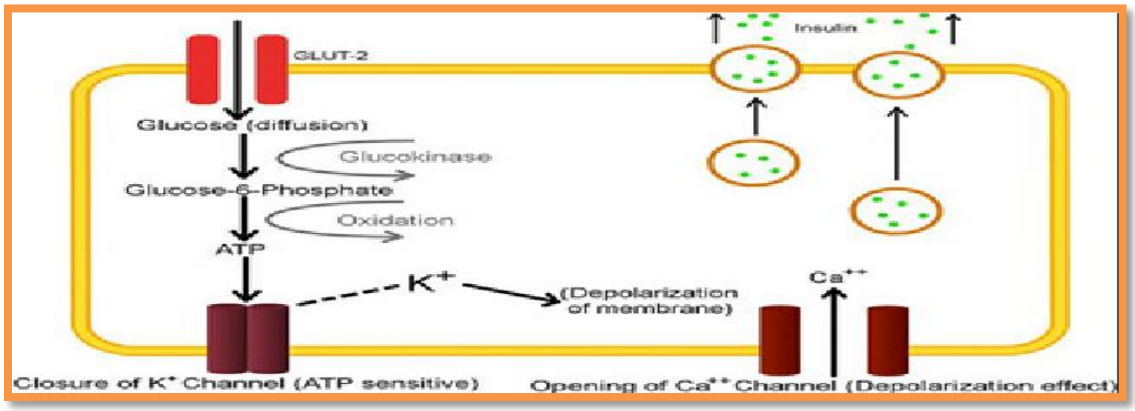

Figure 1-7: Regulation of insulin **(79)**

1.5.4 Insulin resistance

Insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. it results in inability of insulin to provide normal glucose and lipid homeostasis .

Hence, higher than normal concentrations of insulin are needed in

order to maintain normoglycaemia **(80)**. Insulin resistance describes a state of dys-regulation of glucose-insulin homeostasis in which the ability of insulin to stimulate glucose uptake in peripheral tissues such as skeletal muscle and adipose tissues is reduced. in addition to β-cell dysfunction, the major pathophysiological event contributing to the development of patient with T2DM is the resistance of target tissues to insulin which is usually related to abnormal insulin secretion **(81)**. insulin resistance is often found in people with visceral adiposite,hypertension, hyperglycemia and dyslipidemia involving elevated triglycerides, low-density lipoprotein (LDL) particles, and decreased (HDL) levels. islet-cell function defects are common in T2DM patients and the severity of the defects varies depending on the stage of disease progression **(82)**.

1.5.5. Etiology of Insulin Resistance

The etiology of insulin resistance includes genetic and environmental factors. the genetic factors seems to be polygenic in nature and several genes have been suggested as potential candidates.

The environmental factors include reduced physical activity, food intake, aging, smoking and administration of drugs including glucocorticoids, that can cause or participate to insulin resistance **(83)**.

The most important factor that causes insulin resistance is obesity which is usually resulted from polygenetic and/or environmental factors **(84)**. The amount and quality of fat in the diet could be of importance for the development of insulin resistance and related inflammatory activity. A high proportion of long-chain unsaturated fatty acids (FAs) and a low proportion of saturated FAs in the diet have been associated with improved insulin action **(85)** .

1.5.6 Mechanism of Insulin Resistance

In insulin resistance, the reduction of insulin sensitivity by insulin responsive tissues lead to decrease the ability of insulin to inhibit the production of glucose by the liver and decreased peripheral glucose utilization. consequently, blood glucose level may be raised in insulin resistance with increased secretion of insulin to overcome insulin resistance **(86)**. the subnormal biological response could be due to the inability of plasma insulin to bind to its receptor (insulin receptor defect) or the presence of a post receptor binding defect (insulin signaling defect) **(87)** .

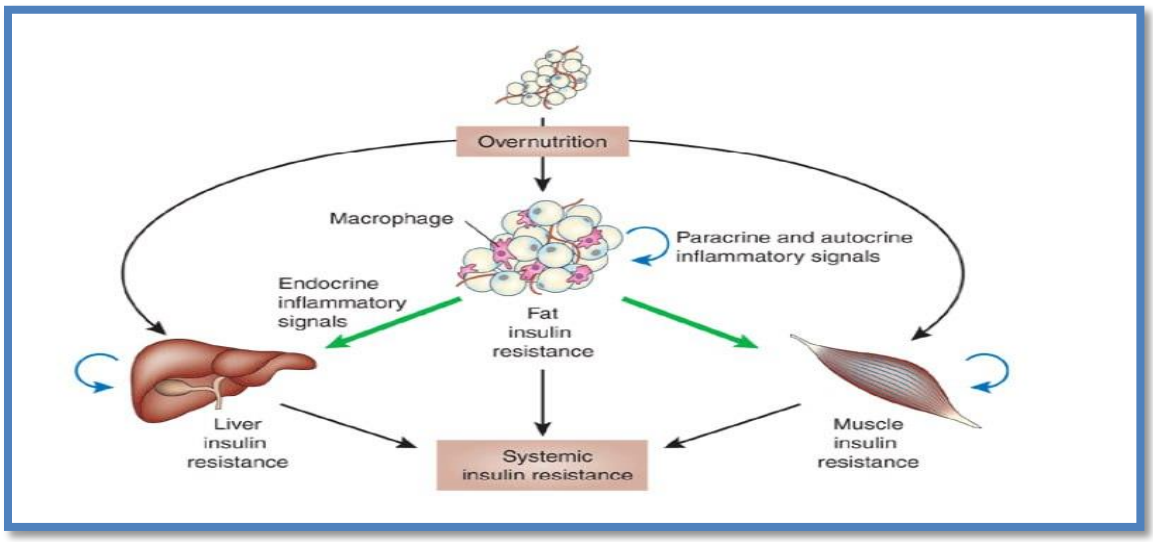

Figure 1-8: insulin resistance **(86)**

1.7. Genetic variation

Genetic variation for any given gene in the human population is called alleles **(88)**.

There are several types of DNA variation, one of these variants is a mutation which can be defined as a variation in the DNA sequence that occurs in lower than one percent of the population whereas the most common variant is called single nucleotide polymorphism (SNP) **(89)** .

A single nucleotide polymorphism or SNP (pronounced "snip") is a variation of a single position in the DNA sequence between individuals. also, the DNA sequence consists of four nucleotide base chains:

(A, C, G, and T). if more than 1% of the population does not have the same nucleotide at a certain position in the DNA sequence in this case it is SNP. Its effect is related to the sites involved in the gene such as the promoter region or the exon region **(90)**. genetic research is a special opportunity for new treatment options, since the genetic components of type 1 and type 2 diabetes are known, genomics provides the opportunity to discover new high-quality targets for these diseases **(91)**.

The evidence for the genetic component of type 2 diabetes is largely undisputed, facts above, it is now estimated that the sibling risk for type 2 diabetes is about 3.5 times the general population although it varies from group to group. compared with the relatively mild genetic component of type 2 diabetes, the risk of type 1 diabetes is more obviously affected by genetic factors **(92)**.

1.7.1 Genetics basis of T2DM

Family history of patient with diabetes is associated with a variety of metabolic disorders and is a significant risk factor for developing type 2 diabetes **(93)**. Although it is likely that this increased risk of patient with T2DM is mediated in part by genetic and common environmental factors among family members. the exact factors underlying this risk increase remain unknown. risk factors for patient with T2DM include lifestyle factors such as BMI, waist circumference, and physical inactivity **(94)**.

Numerous studies indicate that the majority of USA adults are aware of and capable of disclosing patient with T2DM in their first-degree relatives (88–95) %, depending on the relative type to a lesser extent, in their second-degree relatives (70–77) %, depending on the relative type **(95)**.

Given this knowledge of family history and the importance of this chronic disease to public health, family history can be an effective screening method for detecting and preventing patient with T2DM and its complications **(96)**.

Numerous studies have shown that genetic factors play an important role in the pathogenesis of patient with T2DM, also numerous prospective and cross-sectional studies have shown that a healthy family history of type 2 diabetes confers an increased risk on first degree members with the risk being greatest when both parents are affected **(97)**.

The diabetes incidence ranges significantly across ethnic groups that supporting the notion that genetic factors lead to disease predisposition **(98)**.

Multiple laboratories data support the hypothesis that genetic factors contribute to the development of patient with type 2 diabetes through lowering insulin sensitivity and secretion, both of which deteriorate concurrently in the majority of human type 2 diabetes cases **(99)**.

The effective identification of many single nucleotide polymorphisms (SNPs) associated with diabetes susceptibility that allowed by advances in molecular biology was associated with diabetes susceptibility **(100)**.

Genome-wide approaches such as genome-wide association studies (GWAS), have been effective in establishing statistically significant associations between particular genomic loci and susceptibility to patient with T2DM **(101)**.

1.7.1.1 Genome –wide association studies (GWAs) associated with T2DM

Until now, genome-wide association studies (GWAS) have identified approximately 70 gene loci associated with patient with type 2 diabetes

(102).The advent of GWAS in the mid-2000s accelerated the development of gene discovery in T2DM with a large number of loci determined to date. Despite the fact that all of the loci associated with patient with T2DM have been identified information for less than 10% of the genetic heritability of T2DM is available **(103)**. The most recent findings have identified over 240 genetic risk loci (with ∼400 independent association signals) for type 2 diabetes, but thus far only 25 risk loci have been replicated in Arab populations **(104)**.

1.7.1.2 Polymorphism

Between unrelated people, around 99.9 % of human DNA is the same between two chromosomal strands, only 0.1 % of coded DNA is unique **(105)**. these distinctions help to explain the range of human phenotypes and their susceptibility to environmental factors **(106)**. mutations are sequence variations that affect less than 1% of the population, while polymorphisms are more common variations **(107)**. Single nucleotide polymorphisms are the most prevalent genetic variants (SNPs).

Other types of polymorphisms include (inversions, insertions, deletions).

A total of approximately 12 million SNPs has been recorded , around 90% of genetic variation between individuals is believed to be due to SNPs **(108)**.

The classification of SNPs is based on their genomic position.

Coding SNPS (CSNP) are located in exon sequences (translated sequences) of DNA, while noncoding SNPs are located in (untranslated sequences) of DNA (introns) **(108)**.

1.7.2.2 Function insulin like growth factor 2 mRNA binding protein2 (IGF2BP2)

IGF2BP2 is located on chromosome 3q27 , IGF2BP2 highly expressed in pancreatic islets, belongs to a family of insulin-like growth factor 2 (IGF2) mRNA-binding proteins, which play roles in normal embryonic growth and development **(109)**.

IGF2BP2 belongs to an mRNA-binding protein family that plays roles in RNA localization, stability and translation, This gene encodes a protein that binds the 5' UTR of insulin-like growth factor 2 (IGF2) mRNA and regulates its translation. It plays an important role in metabolism and variation in this gene is associated with susceptibility to diabetes **(110)**.

IGF2BP2 is associated with decreased insulin secretion which plays a role in T2DM and important growth and insulin signalling molecule also plays a role in glucose homeostasis through increasing peripheral glucose uptake in different tissues as well as inhibition of hepatic gluconeogenesis **(111)**.

Several variants of IGF2BP2 gene were identified and investigated for association with T2DM. Intron-2 single nucleotide polymorphisms (SNPs) rs4402960 and rs1470579 were the most studied and have been considered strong candidate variants for T2DM susceptibility ,However, due to the ethnic difference in risk-alleles frequency, the contribution of these common variants to T2DM appears to be race dependent which makes them highly controversial candidates for T2DM **(112)**.

T2DM patients with different IGF2BP2 genotypes showed various levels of insulin secretion, It has been demonstrated that variants in IGF2BP2 affect first-phase insulin secretion and the disposition index detected by hyperglycemic clamps **(113)**.

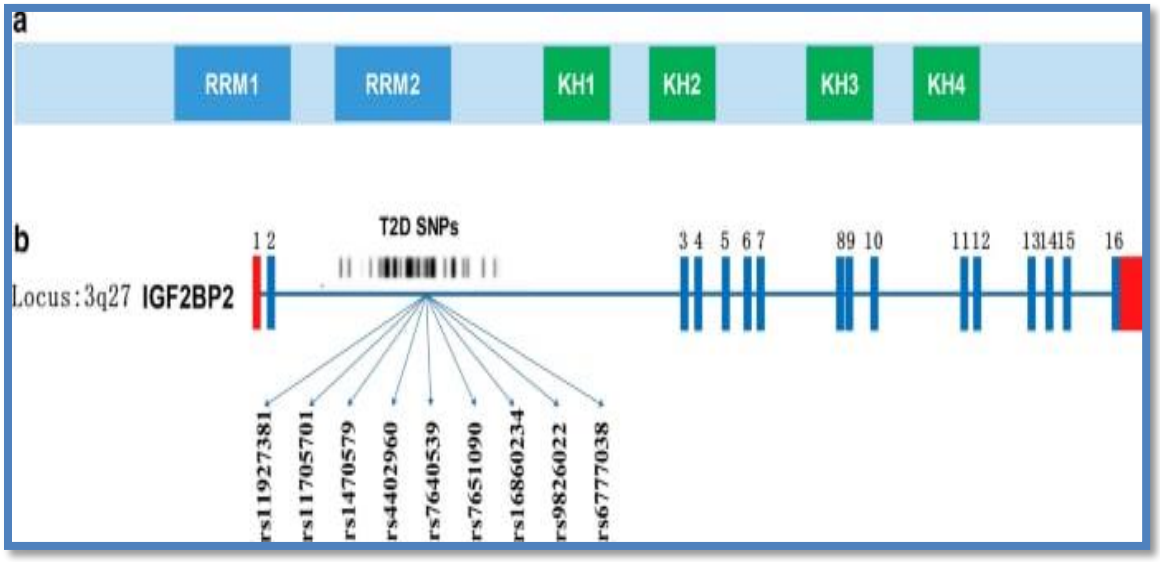

Figure 1-9: location of gene in chromosome and SNPs are located with in second intron of IMP2 gene and chromosome 3 **(114).**

Aims of the study

The main objectives of the present study can be summarized as follows:

1-To find the possibility of correlation between Protein Kinase A and Insulin, HbA1c and HOMA-IR in T2DM patients.

2-To evaluate the role of *IGF2BP2 gene* polymorphism as risk of T2DM development .

Chapter Two

Materials and Methods

2.1 Materials and method

2. Materials and Kits

2.1. Chemicals

All the kits used in this study were shown in table (2-1).

Table 2-1: Kits used in this study

NO.	Kit.	Origin
1	Agarose LE	Intron/Korea
2	Absolute ethanol	Germany
3	DNA extraction kit (blood)	SIM Bio Lab,Iran
4	G2 Master Mix	Denmark
5	Glucose kit	England
6	Human protein kinase A ELISA kit	China
7	Insulin ELISA kit	China
8	HbA1c	England
9	Primers	Macrogen
10	Proteinase K	Promega
11	Nuclease free water	Bioneer (Korea)
12	Red safe	Intron (korea)
13	Safe-Green 100bp Opti-DNA Marker	Abm/Canada
14	TBE buffer	Intron (korea)

2.2. Instruments and Equipment

The instruments used in this study were shown in table 2-2

Table 2-2: Instruments and Equipment used in this study

NO.	Instruments and Materials	Origin
1	Agarose	Spain
2	Afias	England
3	Balance	Germany
4	Deep freeze	GFL / Germany
5	Disposable syringe (5 ml)	China
6	Distiller	GFL / Germany
7	Ethanol	Chia
8	ELISA reader and washer	Biotek / USA
9	Eppendorf tube (1.5 µl)	China
10	Ethidium bromide	Korea
11	Incubator	Fisher cient. / German
12	Micropipettes (5-50 µl), (2-20 µl), (20-200 µl), (100-1000 µl) Slamed / Germany	Slamed / Germany
13	Multichannel micropipette (0-250 µl)	Slamed / Germany
14	Master mix kit	Russia
15	Pipette tips 1 ml	China

16	Pipette tips 0.2 ml	China
17	PCR	Biometra (Germany)
18	Shaker-centrifuge	Korea
19	Spectrophotometer	UK
20	Test tube with separation gel	AFCO, Jordan
21	Vortex (Electronic)	Bionex (Korea)
22	Water bath	Memmert / Germany

2.3. Subjects

There are two groups in this a case-control study design: the first one includes patients with type 2 diabetes mellitus, and the second one is the control group includes apparently healthy individuals. This research was carried out at the laboratory of the College of Medicine at the University of Babylon. The collecting of samples carried out from 1st of October 2022 till 1st of January 2023. Questionnaires were created to collect data from the control and patients group.

2.3.1. Patients group

The patients group that consisted of 60 patients with type 2 diabetes The patients' ages between (35-60) years. Physicians diagnosed patients. They were obtained at Al-Sader Teaching Hospital in Najaf city.

2.3.2. Control Group

The control group consists of 60 individuals who seemed to be healthy. This group's age between 35 to 60 years old, and with FBG < 100 mg/dl. They were devoid of any illness symptoms and indicators.

2.3.3 Inclusion Criteria

The following conditions have been used to choose the patients:

1-A patient who has been diagnosed with Type 2 diabetes mellitus.

2- Patients without chronic complications of Type 2 diabetes melltius.

3- All subscribers must be 35 years or more.

2.3.4. Criteria for Exclusion:

1-Immune disease.

2-Pregnant or lactation.

3-Any patient with chronic liver disease.

4-Any patient with thyroid problem.

5- Any patient with nephropathy.

6-Any patient with hypertension.

7-Any patient with cardiovascular disease.

8-Any patient who take insulin as treatment.

9-Any patient with type1 (DM).

10-Cancer.

11-Patient on corticosteroid or thyroxin treatment.

2.3.5. Ethical Approval and Consent

Before collecting samples, all subjects participated in this study were informed and verbal consent was obtained from each of them. The committee on publishing ethics of the College of Medicine, University of Babylon, Iraq, accepted this study under the reference number BMS/0248/016.

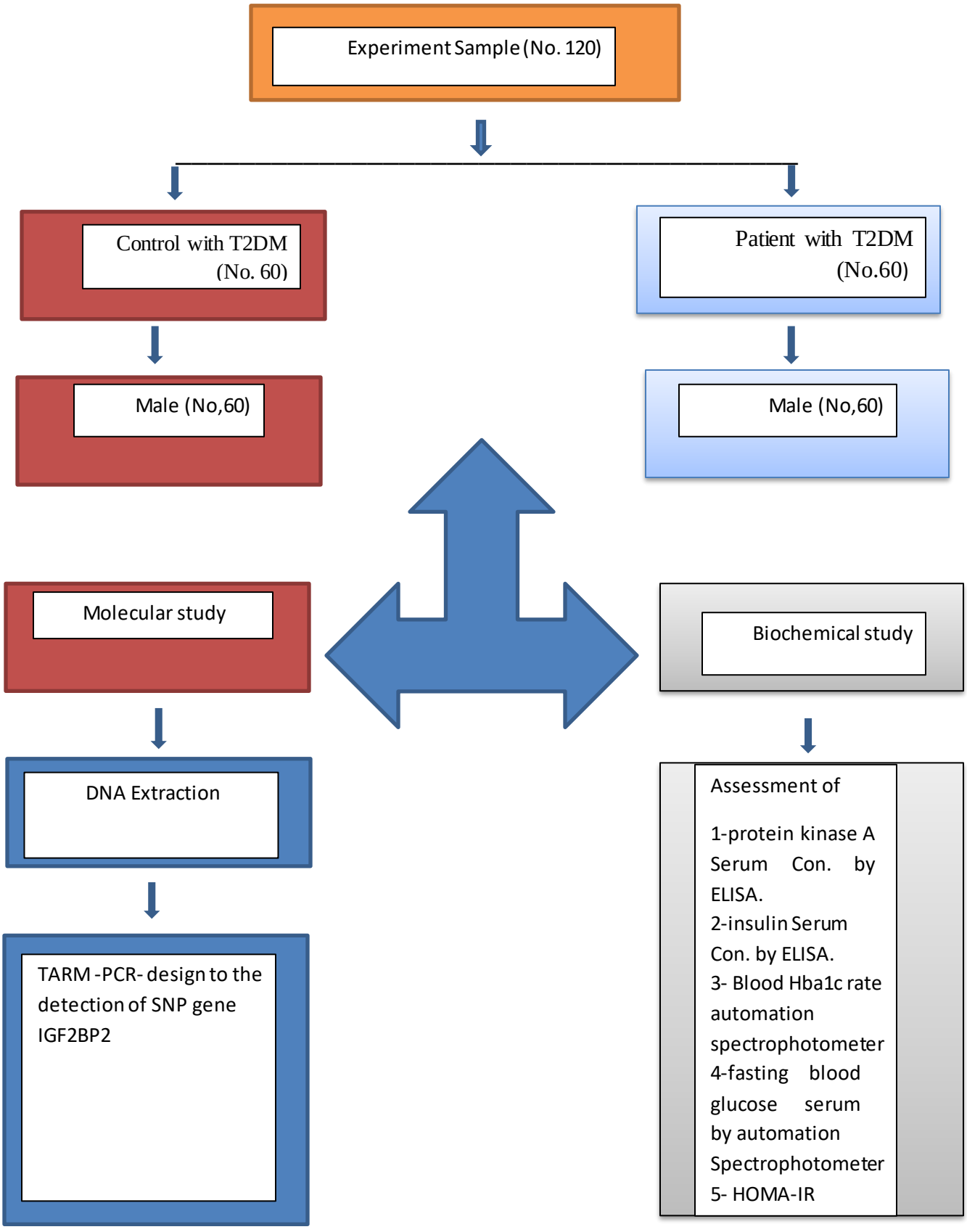

Figure 2-1: Experimental Design

2.4. Methodologies

2.4.1 Collection of The Blood Samples:

In the sitting posture, venous blood samples were taken from control and patients using a disposable syringe (5 ml). a venous puncture was used to extract five ml of blood, which was progressively pumped into disposable tubes containing separating gel and EDTA tube. The blood in the EDTA-containing tubes was to avoid clot at room temperature. The blood in the gel-containing tubes was allowed to clot at room temperature for 10 minutes before being centrifuged at 2000 ×g for 10 minutes, then divided into small aliquots and stored in a deep freezer (-20 C) for further analysis.

2.4.2. Measurement of Body Mass Index (BMI).

The BMI is defined as the body weight divided the square of the height of the body in meters and is expressed worldwide in kg/m^2 units, resulting from kilograms of mass and meters of height .

The BMI number and classifications are listed below according WHO **(115)**.

Severely underweight - BMI less than 16.5k kg / m^2.

Underweight - BMI under 18.5 kg/ m^2.

Normal weight - BMI greater than or equal to 18.5 to 24.9 kg/ m^2.

Overweight – BMI greater than or equal to 25 to 29.9 kg/ m^2.

Obesity – BMI greater than or equal to 30 kg/ m^2.

Obesity class I – BMI 30 to 34.9 kg/ m^2.

Obesity class II – BMI 35 to 39.9 kg/ m^2.

Obesity class III – BMI greater than or equal to 40 kg/ m^2.

2.4.3. Biochemical Parameters

2.4.3.1. Determination of Serum Protein Kinase A (PKA)

Level was measured by enzyme linked immunosorbent assay kit (melson/china).

Principle

Sandwich-ELISA is the technique used in this ELISA kit. This kit includes a Microelisa stripplate that has been pre-coated with a PKA specific antibody. The relevant Microelisa stripplate wells are filled with standards or samples, which are then mixed with the relevant antibody. Then, in each Microelisa stripplate well, a Horseradish Peroxidase Chapter two Materials and methods 43 (HRP)-conjugated antibody specific for PKA is applied and incubated. the components that aren't needed are rinsed away. Each well receives the TMB substrate solution.

Only the wells containing PKA and HRP conjugated PKA antibody will show blue before becoming yellow once the stop solution is added.

At a wavelength of 450 nm,The absorbance is determined spectrophotometrically. the absorbance value is related to the amount of PKA present. By comparing the absorbance of the samples to the standard curve, the level of PKA in the samples was determined **(116)**.

Procedure

1- All reagents were prepared before starting assay procedure.

2- The standard solution was added set standard wells, testing sampling wells. avolume of 50µl was added from standrads to standard wells.

3- A volume of 10µl from tesing samples was added. then, a volume of 40µl from samples diluent to testing samples well .blank well doesn’t add anything .

4-A volume of 100µl from HRP-conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60 minutes at 37c.

5- The plate well was aspirated and washed, repeating the process four time for a total of five washes. washing by filling each we wash solution 400 µl using a squirt bottle, manifold dispenser. complete removal of liquid at each step is essential to good performance .after the last wash ,removed any remaining wash solution by aspiratring or decanting invert the plate and blot it against clean paper towels.

6- A volume of 50µl from chromogen solution A and 50µl from chromogen B was added to each well**.** gently mixed and incubated for 15 minutes at 37c protect from light**.**

7- A volume of 50µl from stop solution was added to each well . the color in the wells were changed from blue to yellow.

8- The optical density were read in each well at 450 nm in microtiter plate reader within 15 minutes.

Calculation

Known levels of human PKA standard and its corresponding reading absorbance is plotted on the scale (x-axis) and the scale (y-axis) respectively. The level of human PKA in sample is determined by plotting the sample's absorbance. on the Y-axis as shown in figure (2-2). The original level is calculated by multiplying the dilution factor.

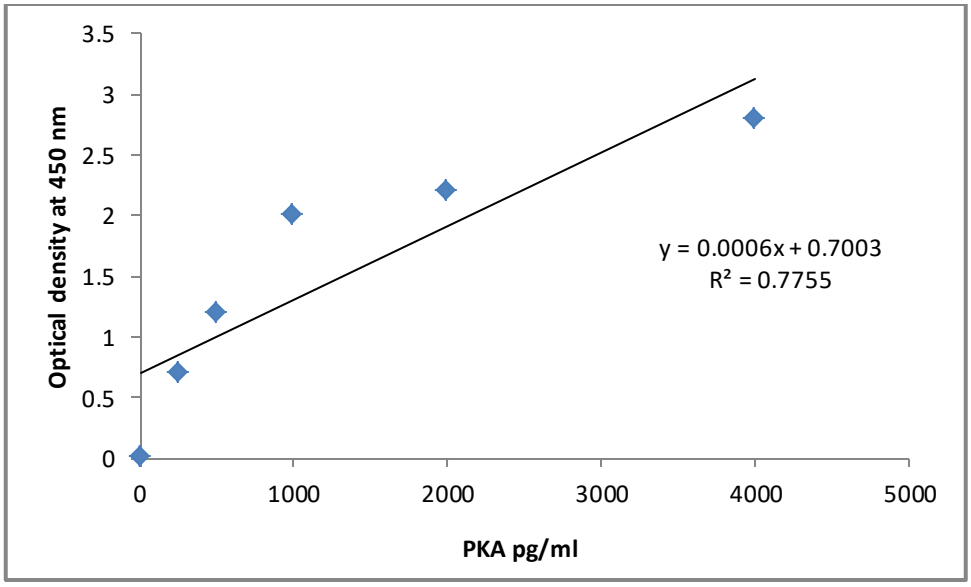

Figure 2-2: Standard curve for determination of protein kinase A level

2.4.3.2. Determination of Serum Insulin Level

Level was measured by enzyme linked immunosorbent assay kit (melson/china).

Principle:

The essential reagents required for an immune-enzymometric assay include high affinity and specificity antibodies (Ab),(enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen (Ag). in this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal Insulin antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. after equilibrium is attained, the antibody-bound fraction is separated from

unbound antigen by decantation or aspiration. the enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. by utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be as certained **(117)**.

Reagent Preparation

1. Buffer washing: the contents of the concentrated wash were diluted to 1000 ml with distilled water in a suitable storage container.

2. Substrate working solution: the contents of the amber vial labeled Solution A were poured into the transparent bottle labeled Solution .

Procedure

1- All reagents were prepared before starting assay procedure. it is recommended that all standared and sample be added in dublicate to microelisa stripplate.

2- The standard solution was added set standard wells, testing sampling wells. avolume of 50µl was added from standrads to standard wells.

3- A volume of 10µl from tesing samples was added. then, a volume of 40µl from samples diluent to testing samples well .

4- A volume of 100µl from HRP-conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60 minutes at 37^0C.

5- The plate well was aspirated and washed, repeating the process four time for a total of five washes. washing by filling each we wash solution 400 µl using a squirt bottle, manifold dispenser or auto washer .complete removal of liquid at each step is essential to good performance .after the last wash ,removed any remaining wash solution by aspiratring or decanting invert the plate and blot it against clean paper towels.

6- A volume of 50µl from chromogen solution A and 50µl from chromogen B was added to each well**.** gently mixed and incubated for 15 minutes at 37c .protect from light**.**

7- A volume of 50µl from stop solution was added to each well . the color in the wells shoud change from blue to yellow. if the color in the wells is green or the color change does not appear uniform ,gently tap the plate to ensure through mixing .

8- The optical density were read in each well at 450 nm in microtiter plate reader within 15 minutes.

Calculation

A standard curve was constructed by plotting the OD of each standard on the vertical axis (Y) versus its concentration on the horizontal axis (X) and plotting a better fit curve through the points on the graph as seen in figure 2-3. these calculations were performed with Microsoft Excel software to obtain the straight-line equation through regression analysis.

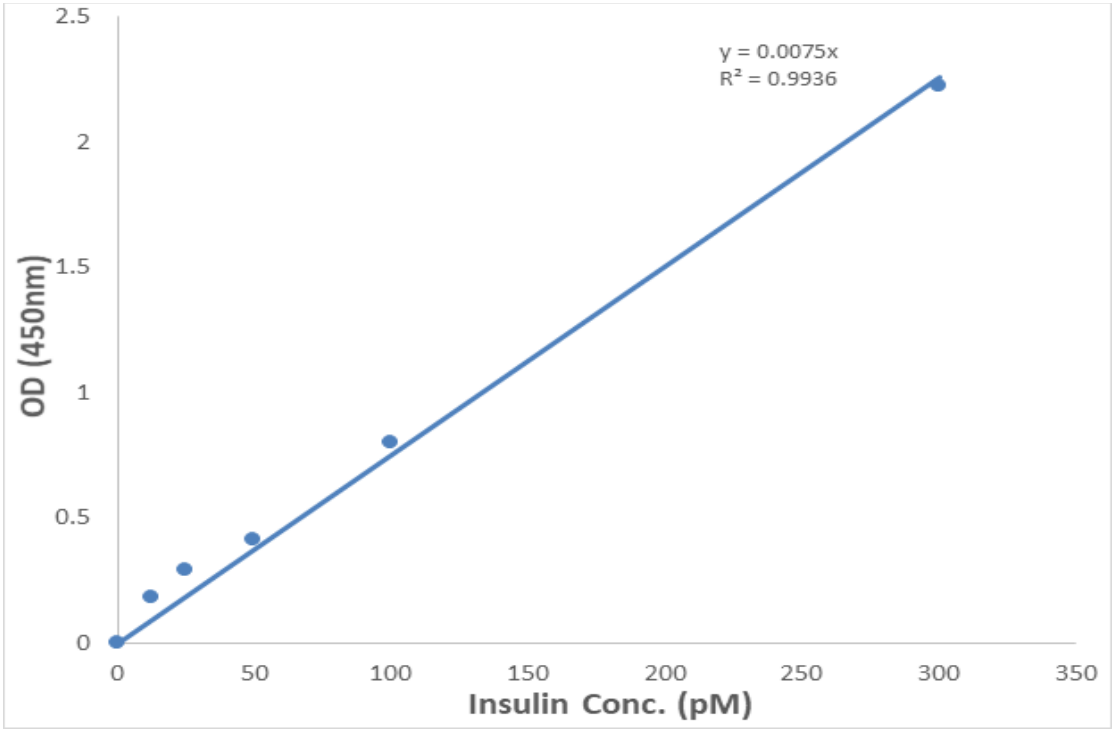

Figure 2-3: Calibration curve of Insulin

2.4.3.3. Determination of HbA1c Level

Serum samples were analyzed by AFIAS instrument based on antigen-antibody reaction and fluorescence technology (Fluorescence Immuno-Assay) **(118)** .

A-AFIAS Instrument

Afias (automated fluorescent immunoassay system) is an automated fluorescent immunoassay system that uses blood, urine, and other samples to measure quantitatively or semi-quantitatively the concentration of the targeted analyte. afias all in one cartridges are designed to optimize the structure and operating principle of the reader.

Figure 2-4: Strip of AFIAS instrument **(118)**

B-Procedure:

1. A volume of 150 µl of the sample was taked with a pipette and dispense into the sample well on the specific cartridge on each parameter.
2. The cartridge was inserted into the cartridge holder.
3. The tip was inserted into the tip hole of the cartridge.
4. The start icon was Tap on the screen.
5. The test result will be displayed on the screen after 28 minutes.

C-Expected values

The American Diabetes Association's (ADA‟s) Clinical Practice Recommendation for diabetes specifies a treatment goal of less than 7% HbA1c **(119)**.

2.4.3.4 Estimation of insulin resistance in patients with type 2 diabetic.

HOMA-IR has been the most frequently employed technique both in clinical practice and in epidemiological studies **(120)**.

Insulin resistance was evaluated by as follows:

2.4.3.5 Calculation of Insulin Resistance (IR)

The quantification of β-cell function and resistance of insulin were determined by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) process HOMA-IR = [fasting plasma glucose (mg/ dl) * fasting insulin(µU/ml)] / 405 **(121)**.

2.4.3.6 Calculation of Insulin sensitivity

The quantification of β-cell function and sensitivity of insulin were determined by Quantitative Insulin Sensitivity Check Index (QIUCKI) as the equation QUICKI= 1/log(fasting insulin µU/ml) + log(fasting glucose mg/dl) **(121)**.

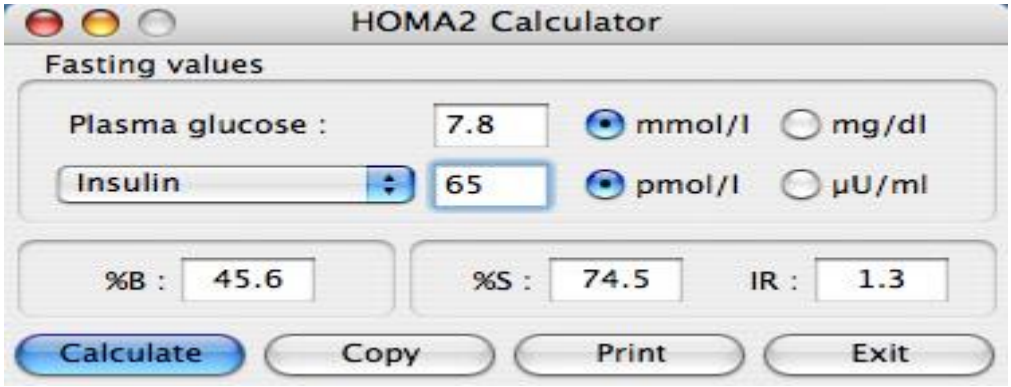

Figure 2-5: The window of HOMA2-calculator **(122)**

2.4.3.7 Determination of fasting glucose

Serum samples were analyzed by AFIAS instrument based on antigen-antibody reaction and fluorescence technology (Fluorescence Immuno-Assay) **(123)** .

Procedure:

1- A volume of 150 µl of the sample was taked with a pipette and dispense into the sample well on the specific cartridge on each parameter.

2- The cartridge inserted was into the cartridge holder.

3- The tip inserted was into the tip hole of the cartridge.

4- The start icon was tap on the screen.

5- The test result will be displayed on the screen after 28 minutes.

2.5. SNPs Genotyping

Genomic DNA was extracted from peripheral white blood cells using blood genome DNA extraction kits according to the manufacturer's instructions (SIM Bio Lab, Iran).

Procedure:

Isolated of was DNA from whole blood, buffy coat, and cultured cells.

A) Cell lysis

1- A volume of 20µl of proteinase k was added to a clean 105 ml tube.

2- 200 µ of whole blood, buffy coat or 10^4-10^8 cultured cells was applied to the tube containing proteinase k.

3- A volume of 200µl of lysis buffer(SLB) was added to sample and mix immediately by vortex mixer.

4-Incubated was at 57c^o for 20 min.

B) Removing contaminations.

1- A volume of 200µl of ethanol(96%) was adding and mix well by pipetting. don't vortex, this might reduced DNA yield.

2- The lysate was transferred in to the upper reservoir of the binding column tube (fit in a 2ml tube).

3- The tube and centerfuge were closed at 13000 rpm for 1 min.

4- The collection tube was discard collection solution and added 500 µ of washing buffer 1(SBW1) and centerfuged at 13000 rpm for 1 min.

5- The solution poured from the 2 ml tube into adisposal bottle.

6- The washing buffer 2 (SBW2) was added 700 µ and centerfuged at 13000 rpm for 1 min.

7- Centerfuged was once more at 13000 rmp for 1min to completely remove ethanol .

C) Elution.

1- The binding column the tube was transferred to a new 1.5ml tube for elution (supplied), added 200 µ of elution buffer (SBE, or nuclease –free water) on to column tube, and waited for at least1 min at RT(15-25c^{o}) until SBE is completely absorbed into the glass fibre of column tube.

2- Eluted to centerfuged at 13000 rpm for 1 min. about 200 µ of eluting can be obtain when using 200 µ of elution buffer.

2.5.1 Agarose gel electrophoresis

principle

When charged molecules are placed in an electric field, they migrate towards either the positive or negative pole according to their charge **(124)**.

Requirements:

1. Electrophoresis unit
2. Casting tray
3. Combs
4. Boat
5. Micropipette

6. Microwave

procedure:

Step 1: Mixing gel

1.Weight 1% of agarose on to a piece of weighing paper was on the scale.

2. The agarose was added to conical flask.

3. 1X TBE was added 100mL of to conical flask.

4. Swirl vigorously was to thoroughly mix agarose.

Step 2: melting gel

1.The agarose and 1x TBE slurry was putted into microwave.

2.The mixture was heated for 160°C for 3 minutes.

3.Clear was swirl until mixture.

4. Add 2µl of EtBr and mix properly.

5.The EtBr and mix properly was Added 2µl**.**

Step 3: **pouring the gel**

1.The casting chamber was placed gel tray.

2.The casting comb(s) was added into the appropriate slot(s).

3.The gel tray was pouring agarose to about 5-7mm.

4.Let sit for at least 30 min, until gel was cool to touch and was opaque in appearance.

5. Carefully combs were removed by pulling them upwards firmly and smoothly in a continuous motion. the remaining depressions were the wells into which your samples will be loaded.

6. placed gel and trayed into gel rig, with wells on the left (cathode) side. Then, fill gel rig with 1X TBE sufficient to cover the entire gel.

Step 4: Loading the Gel

1. A piece of paraffin was cutted and placed it flat on the bench top.

2.The placed small dots of 6X loading dye (About 1µL) on to paraffin,by using a pipette, in rows of 8, 1 dot for each DNA or PCR sample that

would be loaded on the gel. It is not necessary to be exact, and it is not necessary to change the tip.

3.Taked 3 µl of product and pipette it onto its corresponding dye dot,by Using a pipette, then mix the sample and dye by pipetting up and down. Then, pipette up the dye/sample solution and release it into the proper well.

4.Putted gel box cover into placed (this step is essential for your gel to run and to minimize the risk of electric shock). turn on power supply.

Run the gel for 75 minutes at 80 volts.

Step 5: visualizing the gel

1.The power supply was Turn off ,used gloves, , removed the cover from the gel box.

2.Gel was then taken out for the results.

3.The gel was placed inside the gel documentation system.

4.The gel observed for the required bands.

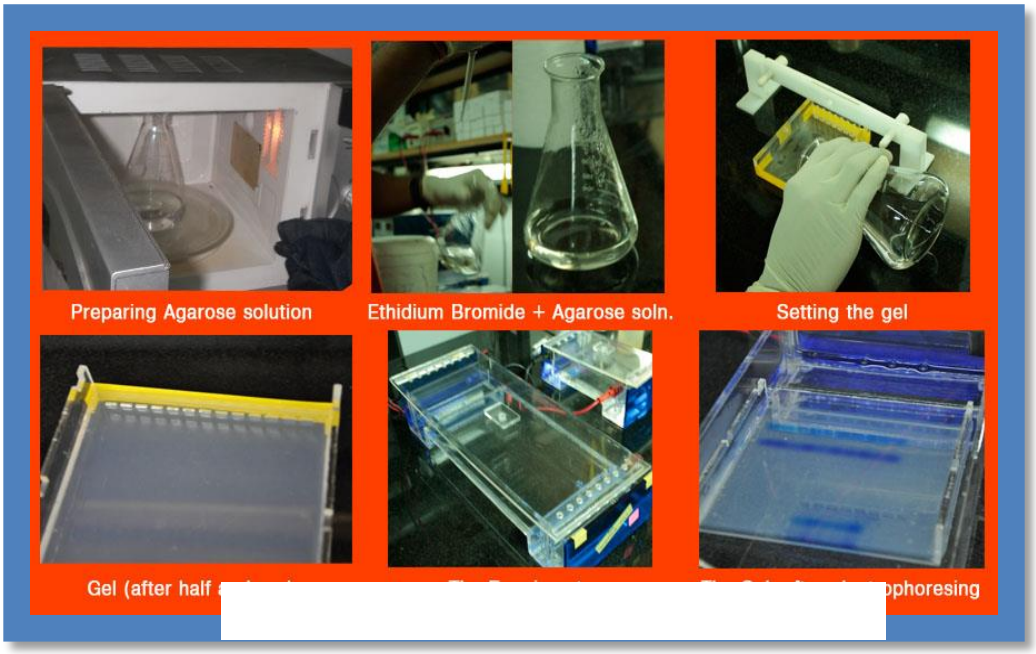

Figure 2-6: Preparation of agarose gel electrophoresis **(125)**

2.5.2 Polymerase chain reaction (PCR).

principle:

PCR is used to amplify a specific region of a DNA strand (the DNA target). most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size. A basic PCR set up requires several components and reagents **(126)**.

These components include:

a) DNA template that contains the DNA region (target) to be amplified.

b) Two primers that are complementary to the 3' (three prime) ends of each of the sense an anti-sense strand of the DNA target.

c) Taq DNA polymerase master mix red **(127)**.

DNA melting: The process of separating the two strands of DNA double helix by employing high temperature.

The two separated strands can now be used as templates for the synthesis of new strands. the specificity of PCR products depends on the primers

Procedure:

To carry out a PCR experiments the targeted DNA is mixed with a pair of gene specific oligonucleotides primers deoxynucleotide and Taq DNA polymerase.

Ingredients:

Table 2-3: Components of materials of PCR

Forward Primers 5 Pico moles	2µl
Reverse Primer 5 Pico moles	2µl
Master mix	20µl
DNA sample	1µl

These ingredients were needed to amplify one DNA sample ERβ exon 8. Total volume 25µl was taken in each microfuge tube. these tubes were kept in the DNA thermo cycler the first end into the holder.

Start the machine and observe the capillary tube extruding from the holder carefully. close the hot top lid slightly, but not so tight to crush the tubes.

Start the programme of PCR:

Step 1: Initialization: heat the mixture at 95^0C for 5 minutes to ensure that the DNA strands as well as the primers have melted. the DNA-Polymerase can be present at initialization, or it can be added after this step.

Step 2: Denaturation: heat at 94 ^{0}C for 1 minute.

Step 3: **Annealing:** heat at 51 ^{0}C for 1 minute.

Step 4: Extension: heat at 72 ^{0}C for 1 minute.

Step 5:**Step 2-4** are repeated to 29 cycles until the target DNA is amplified to target level.

Step 6: Final extension: heat at 720 ^{0}C for 7 minutes to make sure all extensions is completed.

Step 7: 4^0C for 30 minutes.

Step 8: end

The machine will hold the tubes at 4^0C until the reaction tubes are removed **(128)**.

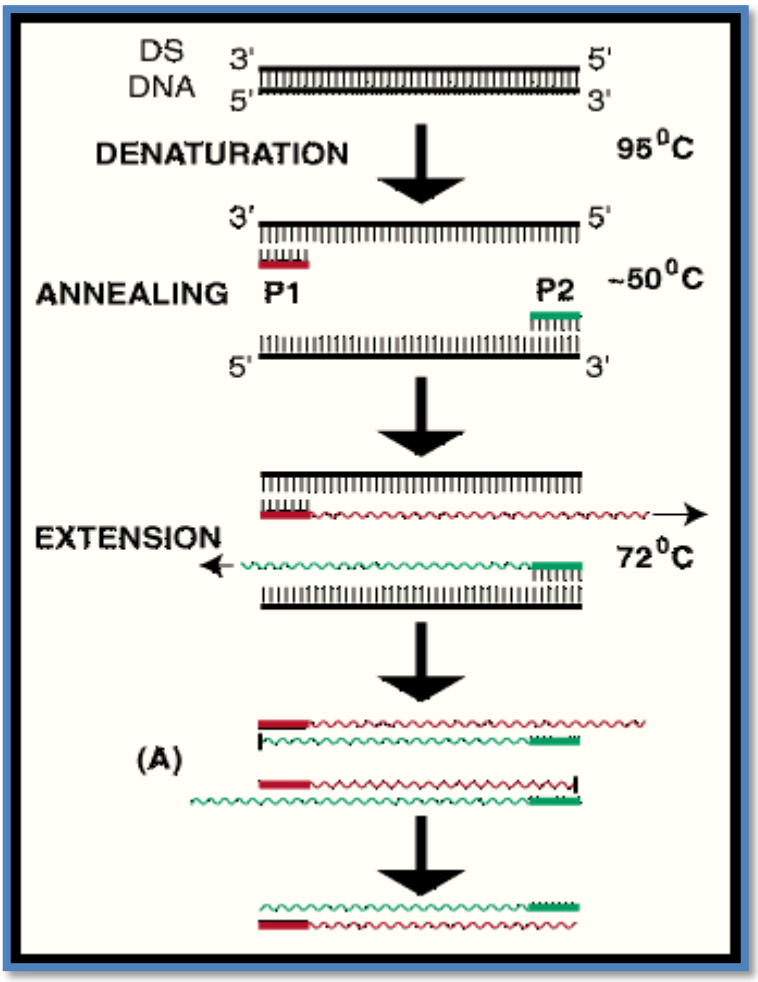

Figure: 2-7 Steps of PCR (128)

Table 2-4: Steps of PCR

Steps	Cycle	Tempreture	Time/Min
One cycle	Denaturation	95 ℃	5
35 cycle	Denaturation	95 ℃	30
	Anneling	59 ℃	30
	Extention	72 ℃	35
	Final extention	72 ℃	5

2.5.3. Preparation of primers

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH2O to obtain a final concentration of 100 µM/µl which served as a stock solution that stored at -20°C. a concentration of 10 µM/µl was prepared from the stock primers to be used as a work primer.

Primers Used in This Study Primers of Nested T-ARMS P The primer sequence for PCR amplification of IGF2BP2 gene polymorphism (rs4402960) G/T and (rs11705701) G/A.

		SNP rs4402960 of IGF2BP2 gene (G/T)			
Target gene		**Sequence (5'-3')**	**GC%**	**Ta**	**Product size**
Outer	**F**	GGAAAACTTGAGAGGAACAGTTACG	**44**	**56**	**380 bp**
	R	GAGGTTGAGACTGCAGTGAGTTTGTTT	**44.4**	**55.6**	
AlleleC	**T**	AGCAGTAAGGTAGGATGGACAGTAGACTT	**44.8**	**55.2**	**280 bp**
AlleleT	**G**	TTGCAAACACAATCAGTATCCTC	**39.1**	**60.9**	**180 bp**
		SNP rs11705701 of IGF2BP2 gene			
Target gene		**Sequence (5'-3')**	**GC%**	**Ta**	**Product size**
Outer	**F**	CCGCACTGAGAATGGTGTTA	**50**	**50**	**403bp**
	R	CTCTCTTCTTCTCCAGCCCAT	**52.9**	**54**	
AlleleG		ACCCTGGTGGCCAGCGTGAAC	**66.6**	**48**	**260 bp**
AlleleA		TTCCCACGCATGTCATTCGCA	**52.9**	**49**	**184 bp**

2.5.5. Principle of T-ARMS- PCR

The principle of the present technique relies on the modification of primers to amplify a specific allele. the 3' end of the primer is modified in such a way that one primer can amplify a mutant allele while the other can amplify the normal allele. to do this, researchers modify a few bases from the primer 3' OH end **(129)**.

2.5.6. Optimization of T-ARMS PCR Conditions

A slight modification of T-ARMS PCR technique was implemented in which an outer PCR product was amplified prior to the T-ARMS PCR. This outer-PCR would serve as a DNA template for the T-ARMS PCR. In brief, it is consisted of Taq polymerase and four primers with two outer primers. the ratio of the outer primer to the allele-specific primer is 1:10 in a final reaction volume of 25 µl. the reaction condition is consisted of pre-heating at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min. Scoring was done by running the PCR products on a 1.5% agarose gel electrophoresis at 3–5 volts/cm for 40 min. the genotypes are differentiated by checking the amplicon sizes in reference to molecular size markers .

2.5.7-Detection of pcr product by agarose gel electrophoresis:

The PCR products should be fragments of DNA of defined length, the simplest way to check for the presence of the fragments is to load a sample taken from the reaction product, along with the appropriate molecular weight markers, on to an 2% agarose gel which contains 0.8-4.0% ethidium bromide. DNA bands on the gel can then be visualized under UV gel doc. by comparing product bands with bands from the know molecular weight markers, we should be able to identify any product fragments, which are of appropriate molecular weight **(130)**.

Procedure:

Step 1: Mixing gel

1.Weight 1.5% of agarose onto a piece of weighing paper was on the scale.

2. The agarose was added to conical flask.

3. 1X TBE was added 100mL of to conical flask.

4. Mix agarose was swirl vigorously.

Step 2: melting gel

1.The agarose and 1x TBE slurry was putted into microwave.

2.The mixture was heated for 160°C for 3 minutes.

3. The mixture was swirl until clear.

4. Added 3µl of EtBr and mixed properly.

Step 3: **pouring the gel**

1.The casting chamber was placed gel tray.

2.The casting comb(s) was added into the appropriate slot(s).

3.The gel tray was pouring agarose to about 5-7mm.

4.Let sit for at least 30 min, until gel was cool to touch and was opaque in appearance.

5.Carefully removed combs by pulling them upwards firmly and smoothly in a continuous motion. the remaining depressions were the wells into which your samples will be loaded.

6.Placed gel and trayed into gel rig, with wells on the left (cathode) side. Then, fill gel rig with 1X TBE sufficient to cover the entire gel.

Step 4: **loading the gel**

1. A piece of paraffin was cutted of paraffin and placed it flat on the bench top.

2. The place small dots of 6X loading dye onto paraffin, Load the sample (8µl of PCR product +2µl of Bromophenol blue) was into wells using a micropipette, by Using a pipette.

3. The lid on the gel box and the electrode was placed.

4. The supply was Powerd should be sent at 50v.

5. The DNA was traveled from negative end to positive end.

6.The electrophoresis continued until the tracking dye move at least 2/3rd. of the gel length.

7. The bands Compared with 100bp DNA ladder.

Step 5: visualizing the gel

1.The power supply was turn off ,used gloves, removed the cover from the gel box.

2.Gel was then taken out for the results.

3.The gel was placed inside the gel documentation system.

4.The gel observed for the required bands. **(131)**.

2.6. Statistical Analysis:

All numerical data were reported as mean ± standard deviation.

Descriptive statistics and graphs were created using SPSS 20. the mean and SD of the parameters were also compared using the independent

t test.

Data were analyzed statistically using SPSS 20 program, P-value was used for comparison **(132)** .

The relationship between genotypes and allele frequency was mathematically determined using the Hardy Weinberg equation, which was estimated using an online program (www.ekstoem.com). The genetic power of the polymorphisms under investigation was calculated using software available online (osse.bii.a-star.edu.sg).The output data were expressed as odds ratios (OR), confidence intervals (CI 95 percent), and a P 0.05 value.

p2+2pq+q2=1 Where ‘p’ and ‘q’ represent the frequencies of alleles **(133)**.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Demographic Feature of the Study Groups

The number of subjects that participated in this study was 60 as patients' group with T2DM and 60 apparently healthy individual as a control group.

3.1.1. Age

The min-max of age in this study was about (35-60) years, the result showed that the control group matches patient group in age p-value>0.05, mean value and SD of age for patients with T2DM and control .

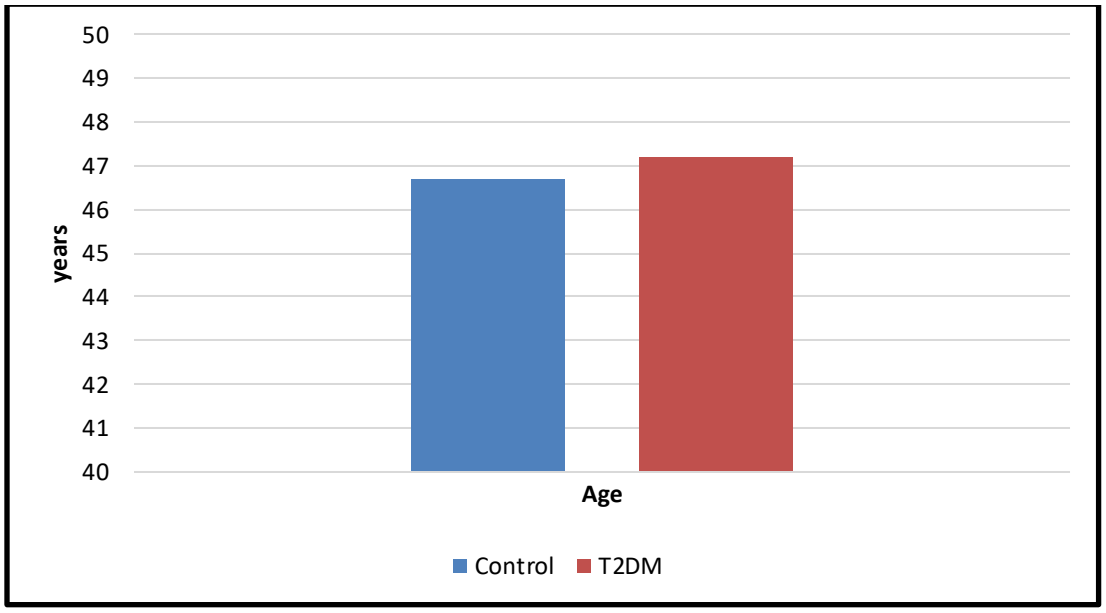

Figure 3.1: Mean of age in type 2 diabetic mellitus compared with control group.

The patients and control were showed that there were no significant differences in the age between them (p-value>0.05). this maching between control group with patient group to elimant any factor that effect on the result as shown in figure 3.1

Age of onset also has some effects on the overall health status of T2DM patients. accumulating data suggest that T2DM has a more rapid deterioration of β-cell function than is seen in later-onset T2DM **(134)**. There is also associations of young-onset T2DM with premature mortality and morbidity, Patients with early-onset T2DM (defined here

as a diagnosis at <40 years) have an increased risk of vascular complications ,It is suggested that younger-onset T2DM increases mortality risk and that this is mainly through earlier cardio vascular disease mortality. Therefore, efforts to delay the onset of T2DM might reduce mortality **(135)**.

The frequency distribution of patients with T2DM according to family history was as following: (70 %) have family history of T2DM, (30%) cases no family history of T2DM.

3.1.2 Family history

Family history and life style are the risk factors for early-onset factor for T2DM disease , the presence of a family history of diabetes and being obese was found to increase the chances of acquiring T2DM, though family history increases the risk of getting T2DM, in particular, a family history of disease in both parents is most strongly associated with the impaired residual β-cell function ,First-degree relatives of people with T2DM especially relatives of those with young-onset diabetes, are at high risk for diabetes **(136)**.

3.1.3 smoker

In This study , the percentage of smoker T2DM were (60%) and percentage of non- smoker T2DM were (40 %) for patients group.

Although this study didn't examine the relationship of smoking with type 2 diabetes in our research, some studies say the people who smoke cigarettes are 30%–40% more likely to develop type 2 diabetes than people who don’t smoke, it is believed that the link between smoking and the increased risk of developing insulin resistance is the high level of the hormone "cortisol" caused by smoking. cortisol is one of the hormones that is secreted when the body is exposed to stress or psychological tension, and it leads to many effects in the body, including raising the level of blood sugar, **(137)**.

Smoking also affects people who already have prediabetes, bringing them one step closer to developing type 2 diabetes ,nicotine also raises the level of triglycerides in the blood which in turn can contribute to insulin resistance **(138)**.

Song and Hardisty showed that in cohorts with type 2 diabetes with disease onset before 40 years of age, by the sixth decade of life, a substantially higher risk of cardiovascular disease is seen in those with the earlier onset of diabetes **(139)**.

3.1.4 Body Mass Index

The results revealed that there are no significant differences in the BMI between T2DM and control P-value >0.05, the means were 25.31 and 24.7 kg\m^2 of both groups respectively.

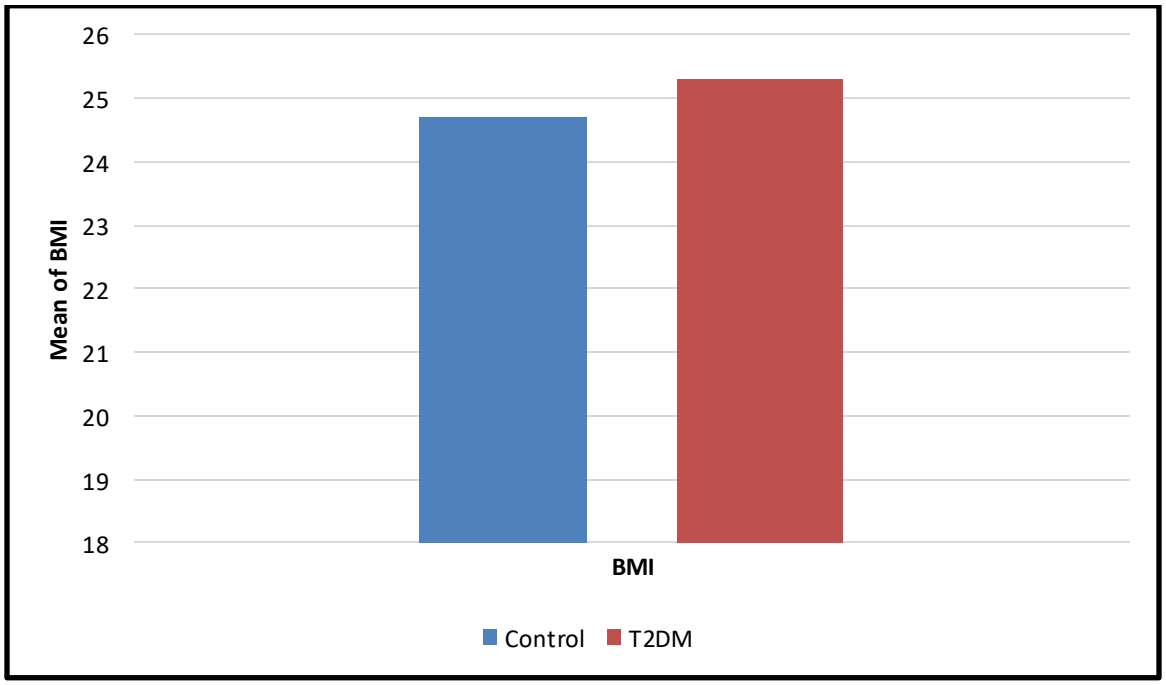

Figure 3.2: Mean of body mass index in patient with T2DM compared with control group.

A major cause for that increase in the percentage of overweight and obesity is particularly due to the sedentary lifestyle with inactivity and unhealthy with too much fat and carbohydrate in diet, as well as excessive sodium consumption favor the development of overweight

and obesity ,also the obese subjects generally have higher blood pressure, higher serum total cholesterol, lower HDL cholesterol, higher serum triglycerides, higher blood glucose, and higher plasma insulin levels than thin persons **(140)**.

As a result of obesity and insulin resistance and another problems such as hyperglycemia, dyslipidemia and hypertension, patients with type 2 diabetes develop metabolic syndrome .also the obesity may induce oxidative stress and that increased oxidative stress in accumulated fat and development of metabolic syndrome that effect to patient with diabetes type 2 mellits **(141)**.

3.2.Biochemical parameter:

3.2.1 serum human Protein Kinase A among patient and control group.

The results revealed that there was significant difference in PKA level between patient with T2DM and their control ($p<0.001$). The means, standard devation, and statistical parameters presented .

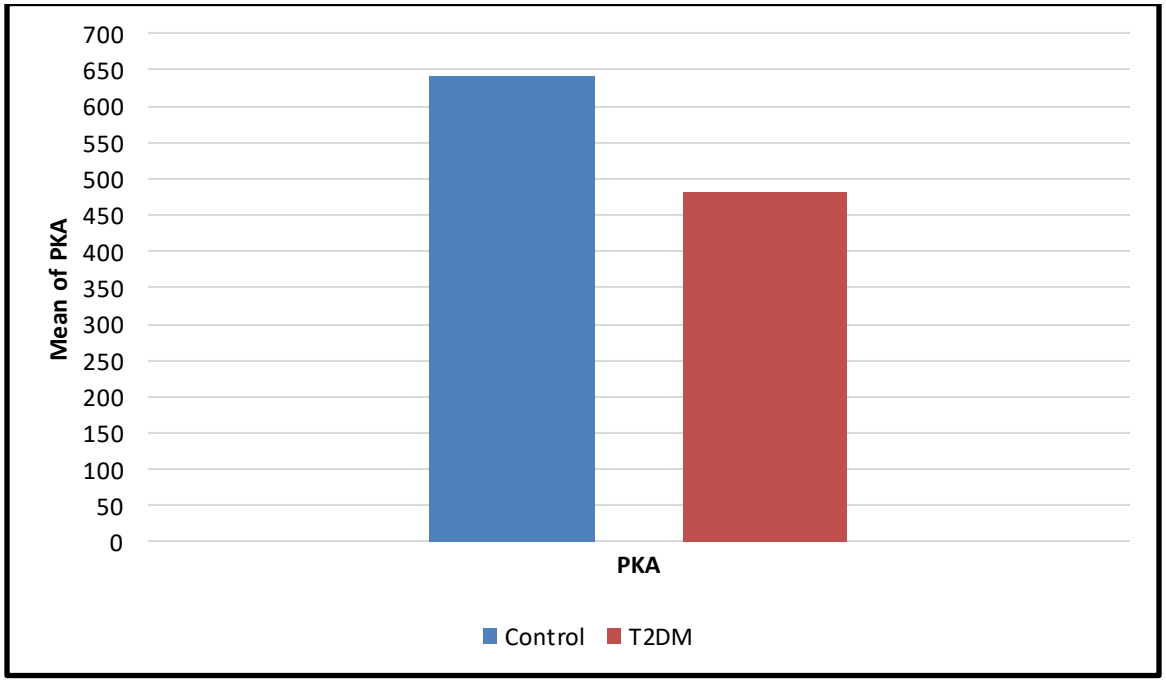

Figure 3.1 Mean of protein kinase A in patient with type 2 diabetic mellitus compared with control group.

In this study the result revealed that there were significant differences in PKA levels between patients and their control**.**

The current study that discovered decrease of PKA level in patients with type 2 diabetes mellitus is due to hyperglycemia, which leads to inhibition of the proteins synthesis, and as a result decrement PKA level. in addition, hyperglycemia leads to the glycated of this enzyme molecule and a decrease in its activity. our results are in agreement with the results of the study of Pernicova and Korbonits, **(142)**, they concluded that metformin works by decreasing hepatic gluconeogenesis and blocking the action of glucagon to reduce blood glucose levels results have indicated that metformin improves glucose metabolisms predominantly in the liver, muscle, fat, pancreas, and intestine. The anti hyper glycemic effect of metformin is closely related to its capabilities in suppression of hepatic glucose production and intestinal glucose absorption, and promotion of β-cell functions and insulin sensitivity **(143)**.

Inhibition of mitochondrial complex I causes cAMP and protein kinase A signaling to be disrupted in response to glucagon on the same direction, cAMP/PKA signaling in different tissues has pleiotropic effects on whole-body glucose homeostasis and regulates glucose metabolism at multiple levels. He *et al.*, Pernicova & Korbonits were strongly supported this signaling cascade as a target for the development of new T2DM therapeutics **(144)** .The insulin secretion potentiated by PKA activity does not result in chronic hyperinsulinemia, but rather it delivers acute enhancement of insulin secretion to rapidly lower glucose levels.

3.2.2 Serum human insulin among patient and control group.

The results revealed that there was significant difference in insulin level in serum between patients and their control ($p< 0.001$). The means, standard deviation, and statistical parameter .

Insulin resistance increase in patients with type2 because the cells in muscle, adipose tissue and the liver becomed more resistant to insulin .

The majority of patients with type2 diabetes have weight gain ,and their becomes gluconeogenesis ,and don't lipolysis because the activity of insulin is sufficient to enter for gluconeogenesis ,but not sufficient to do lipolysis. apposite type 1 diabetics ,they have lipolysis and become thin **(145)**.

Metabolic syndrome would be the most common clinical syndromes associated with insulin resistance. insulin resistance typically predates the development of diabetes and is commonly found in unaffected first-degree relatives of diabetic patients **(146).**

The patient with type 2 becomes more resistant to insulin with decreasing time of diabetes, so, that insulin level is high in the body but the available insulin is insufficient **(147)**.

Insulin is known to enhance glucose uptake across the specific membrane of insulin-sensitive cells, and because of the delayed absorption of glucose, this study in our patients an increase in the level of blood sugar **(148)**.

The current result reveald that there was significant increase of insulin level in patient ,where current study agreed with (A-hakeim,*et al* 2015) **(149)** which indicated that the diabetic patients had a higher mean insulin level than control group indicating a hyper-insulinemia in those patients.

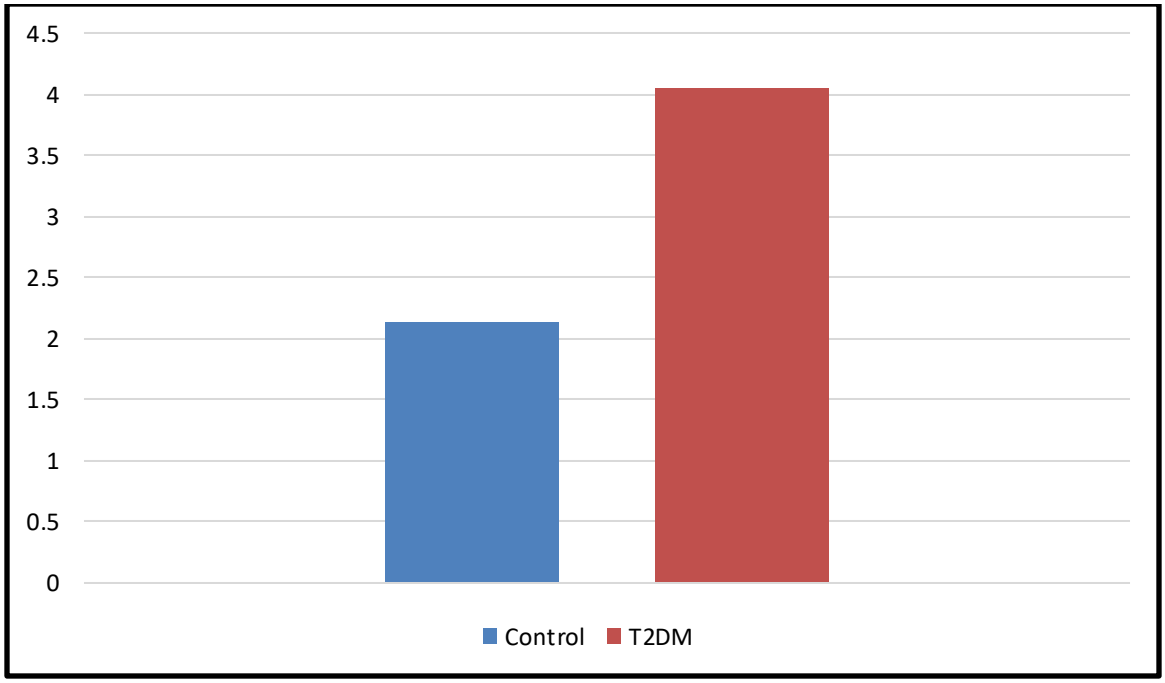

Figure 3.2: Mean of insulin in patient with type 2 diabetic mellitus compared with control group.

3.2.3 Glycated hemoglobin among patient and control group.

The mean of Glycated hemoglobin in patients with type 2 diabetic mellitus patients and their healthy group, were statisticaly significant diffrences between patients and control groups .

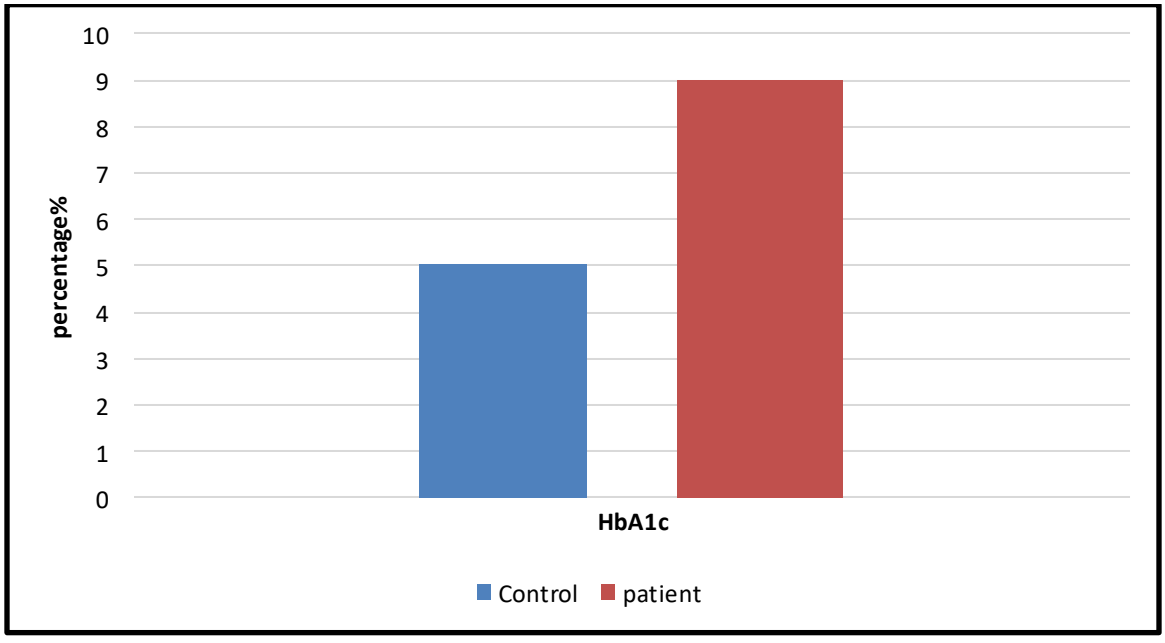

Figure 3.3 Mean of Glycated hemoglobin in patient with type 2 diabetic mellitus compared with control group.

When the blood sugar level increase in a diabetic patient type 2, glucose begins to bind with hemoglobin molecules and protein that makes up red blood cells, to be glycosylated haemoglobin, which we tested in our research.

Its known that HbA1c is increased in patients with diabetes , overweight type2 diabetic patients had increased HbA1c because glycated hemoglobin (non enzymically with glucose), also HbA1c has contact with higher glucose concentration during 120 day life time of these cells **(150)**.

This study used HbA1c to monitoring glycemic control in patient with type 2 diabetes.

Also to avoid problem of glucose values fluctuation occur all the time, and don't require any special preparation such as fasting and unaffected by recent exercise or food ingestion **(151)**.

This study is measure HbA1c in patient with T2DM to accurate knowledge of their own HbA1c level and whether this is associated with better glycaemic control.

These results indicated a poor control of diabetic patients and the treatment is either not adequate or very poor diet control during 2-4 months ago **(152)**. According to the American Diabetes Association (ADA) guidelines 2015, the value of HbA1c should be kept below 7% in all diabetics **(153)**.

3.2.4 Fasting blood glucose among patient and control group.

The mean of fasting blood glucose in in type 2 diabetic mellitus patients and healthy groups, were significant difference P-value is 0.001 .

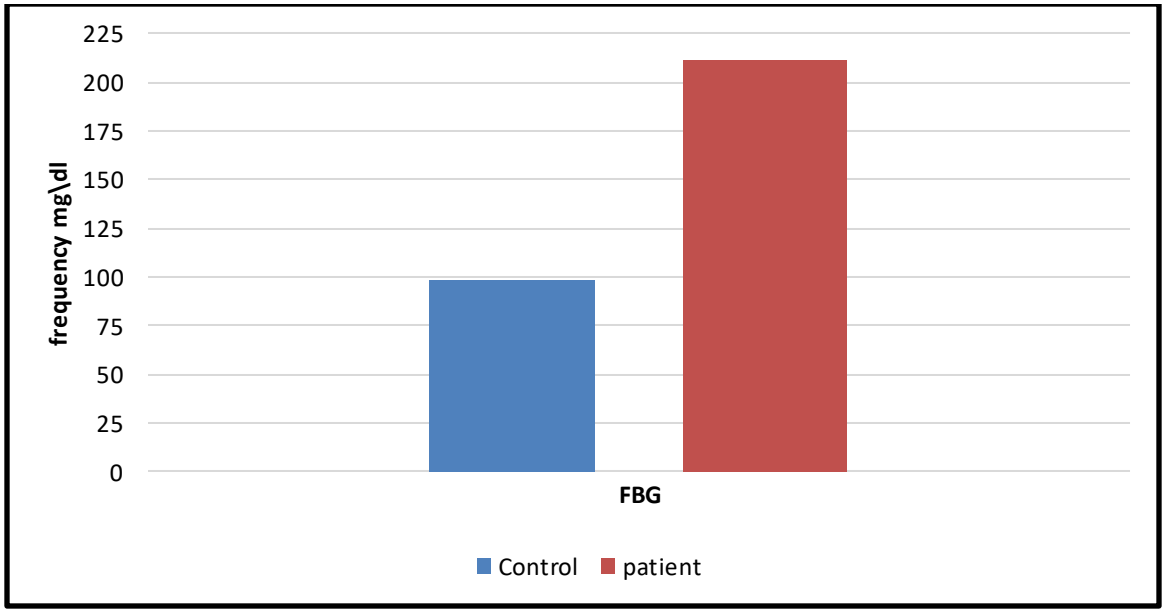

Figure 3.4: Mean of fasting blood glucose in in patient with type 2 diabetic mellitus compared with control group.

The results of this study appeared significant differences (P-value 0.001) in serum level between patients and control, as explained in figure 3-2-4.

The glucose level in serum increase in current result because two main problems ,firstly, the pancreas does not produce enough insulin, a hormone that regulates the movement of sugar into cells. secondly, the cells do not respond properly to the hormone insulin and therefore absorb less sugar. this long-term condition causes too much sugar in the blood. High blood sugar levels can lead to disturbances in the circulatory system, nervous system, and immune system **(154)**.

High blood sugar in patients with type 2 diabetes can lead to oxidative stress, which in turn leads to insulin resistance, abnormal amounts of fats in the blood (dyslipidemia) and impaired glucose tolerance **(155)**.

Also increases in blood glucose level are associated with increases in lipid biosynthesis (lipogenesis) and an increase in weight , these findings were probable as overweight or obesity is known to induce insulin

resistance because of a decrease in insulin-sensitive receptors as the weight increases, So, the majority of type 2 diabetics have excess weights **(156)**. So, current results agreed strongly with (A-hakeim,*et al* 2015) **(149)** which reported these results are expected due to the fact that the main characteristic feature of diabetes millitus is hyperglycemia.

3.2.5 Homeostasis model assessment insulin resistance (HOMA-IR) among patient and control group.

The mean of (HOMA-IR) in patient with T2DM patients and control groups, were significantly different, P-value is 0.001 .

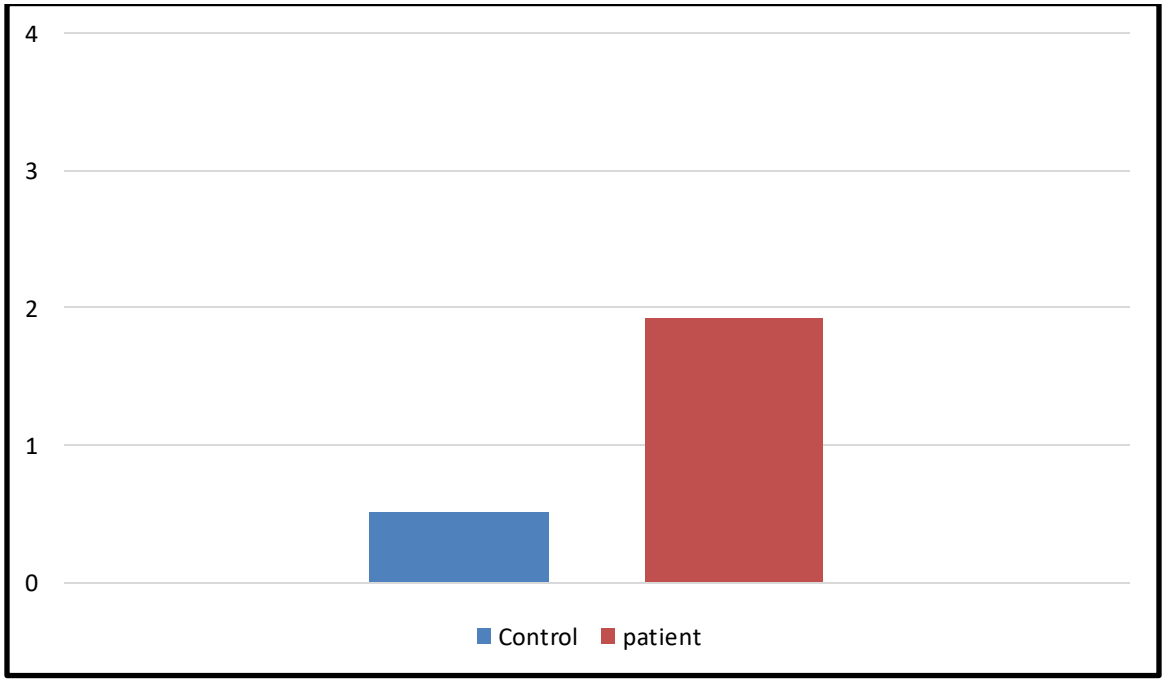

Figure 3.5: Mean of Homeostasis model assessment insulin resistance in patient with type 2 diabetic mellitus compared with control group.

Insulin resistance is decreased ability of target tissues, such as liver, adipose, and muscle to respond properly to normal circulating concentration of insulin. for example, insulin resistance is characterized by uncontrolled hepatic glucose production,and decreased uptake by muscle and adipose tissue **(157)**.

HOMA2 is more predictive than HOMA1 for the progression to diabetes in prediabetes or non-diabetic subjects **(158)**.

The T2DM group had the highest HOMA-IR index and lowest quiki.

Besides obesity and overweight, there is another important habitual factor that affects the development of insulin resistance and patient with T2DM is a sedentary lifestyle. Moreover, a sedentary lifestyle is one of the modifiable risk factors of patient with T2DM and the value of exercise to improve insulin signalling and glucose metabolism cannot be over emphasized **(159)**.

According to the results of the present investigation, the data of the HOMA method was highly suggestive to be used for asses of insulin resistant type 2 diabetic patients **(160)**.

The value of the insulin resistance indices ,i.e. (HOMA-IR) were significant ($p< 0.001$) elevations indices were found in the patients when compared with the control group .

The results of the current study are in agreement with those of Lukshmy *et al.* **(161)** .

3-3 Personal Correlation between PKA, Hba1c, Insulin, HOMA and in T2DM between patients and control.

Table 3-1: The correlation of PKA with other parameters in the patients group with T2DM.

Parameters	R^2	R	P-value
PKA			
HOMA-IR	0.565	-0.558	*P* <0.01
INSULIN	0.78	-0.71	*P* <0.01
Hba1c	0.453	-0.443	*P* <0.01

Correlation between PKA and insulin.

Serum PKA showed a negative correlation with insulin (r=-0.71, *P* <0.01).

Some studies demonstrated an association between low PKA levels and increase the insulin, similar to our study **(162)**.

Due to the central role played by PKA in glucagon signaling, its disinhibiting by specific depletion of Prkar1a in the liver leads to a significant abrogation of glucose stimulated insulin secretion and increased plasma glucose **(163)**.

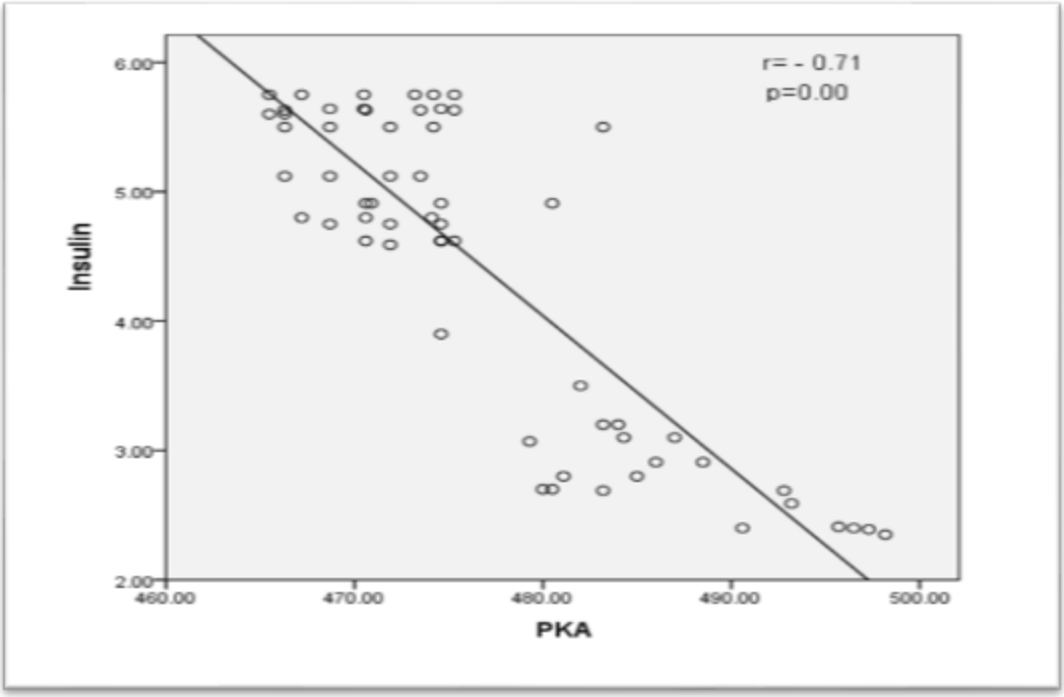

Figure 3.3: correlation between PKA and insulin

Correlation between PKA and HbA1c.

Serum PKA showed a negative correlation with HbA1c (r=-0.443, P <0.01).

Some studies demonstrated an association between low PKA levels and increase the HbA1c, similar to our study **(164)**.

For instance in vitro studies and experiments performed in cells expressing insulin receptor showed that PKA phosphorylate specific serine/threonine residues of insulin receptor and its intracellular substrates belonging to the family of insulin receptor substrates (IRSs), causing impairment of insulin signaling **(165)**.

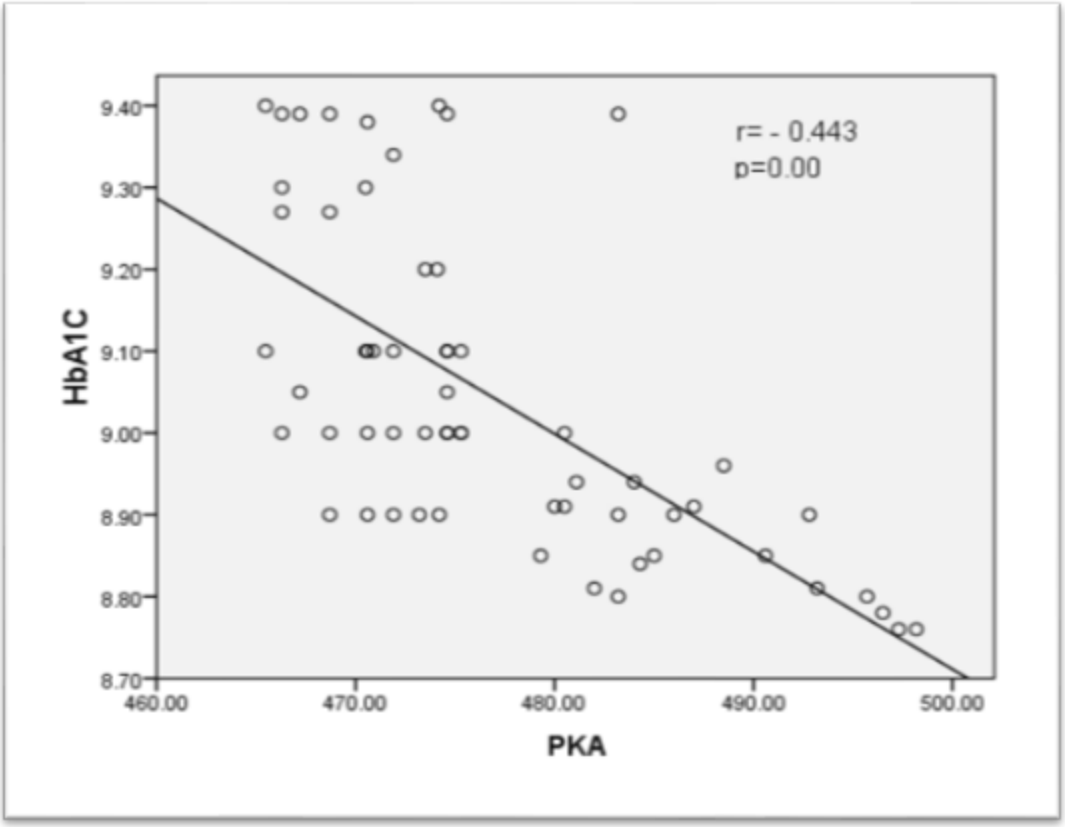

Figure 3.4: correlation between PKA and HbA1

Correlation between PKA and HOMA.

Serum PKA showed a negative correlation with HbA1c (r=-0.558, *P* <0.01).

Some studies demonstrated an association between low PKA levels and increase the HbA1c, similar to our study **(166)**.

Loss of PKA function or inhibition of PKA activity in these cells increases the levels of Ci 155 in both embryos and disks and results in the misregulation of some hh-responsive genes . Ci has four consensus PKA phosphorylation sites in its C terminus that can be phosphorylated by PKA **(167)**.

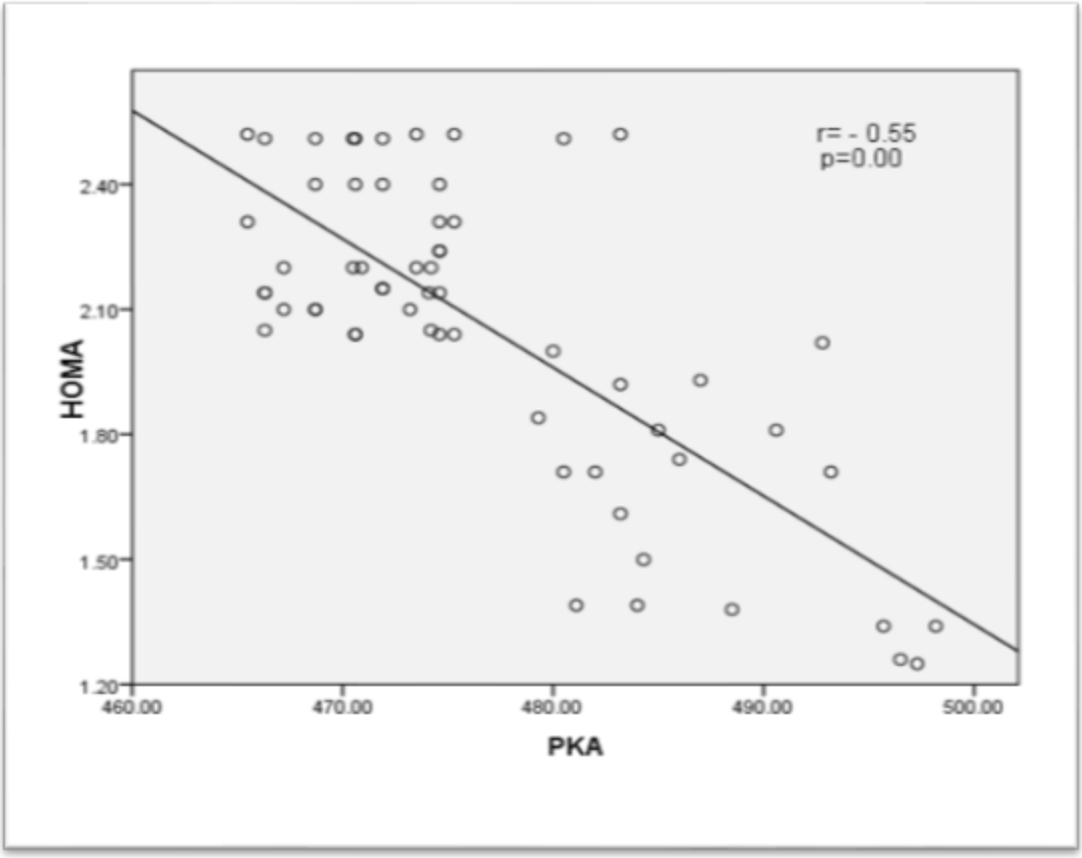

Figure 3.5: Correlation between PKA and HOMA

3.4 Measurement of concentration and purity of deoxyribonucleic acid.

A ratio of absorbance at 260 nm and 280 nm was measured for the estimation of DNA concentration and purity . Data was demonstrated in table (3-4) and figures (3-6), (3-7) respectively.

Table 3-2: DNA concentration and purity.

DNA concentration and purity	**Mean ± SD**
DNA concentration ng/Ml	31.06 ± 7.27
DNA purity (260/280)	1.72 ± 0.4

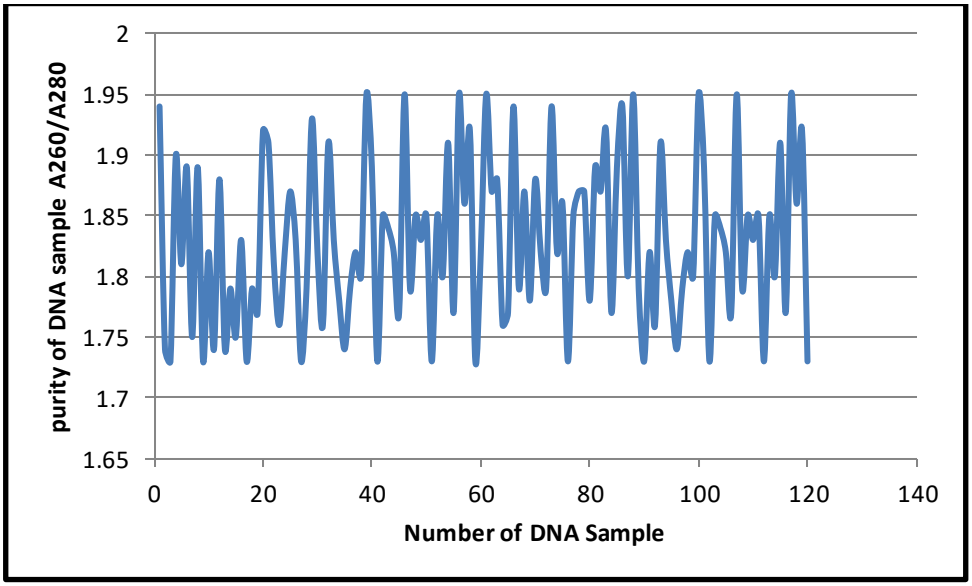

Figure 3-6: Purity of extracted DNA samples

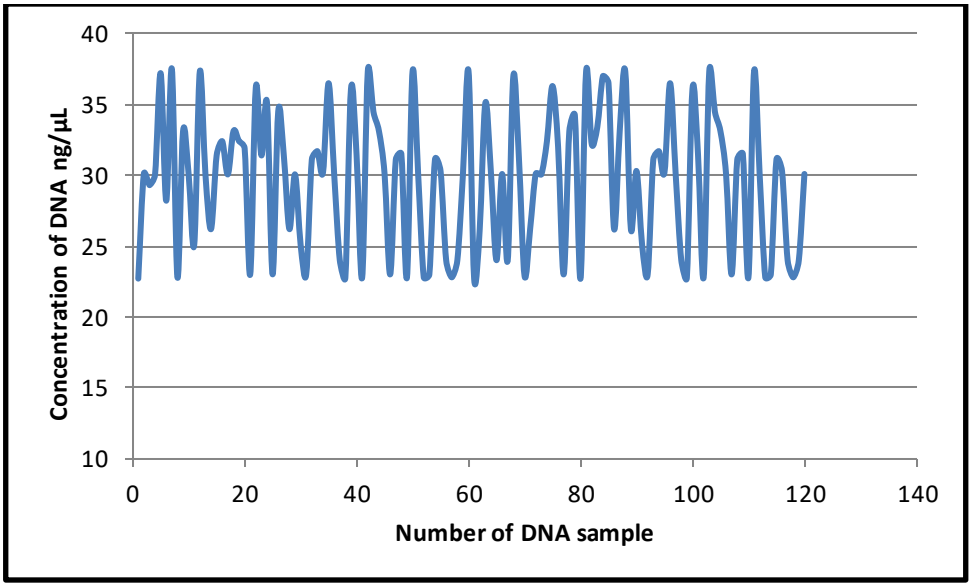

Figure 3-7: Concentration of extracted DNA samples

3.5 Results of amplification reaction:

In current study the gene polymorphism of IGF2BP2 gene was studied in T2DM and control groups. the genotyping were detected by T-ARMS-PCR .

There are two SNPs rs4402960 G/T and rs11705701 G/A within the IGF2BP2 gene included in present study,

The amplification of SNPs of IGF2BP2 gene SNPs rs4402960 G/T and rs11705701 G/A was showed as (380bp, and 403bp ,) respectively

3.5.1- IGF2BP2 Gene (rs4402960 G/T) SNP Polymorphism.

The subjects enrolled in present study were reported into three genotypes, These homozygous for (GG) wild type, heterozygous (GT) and the last one was homozygous for the allele (TT).

* Two band (380 bp) and(180 bp) is the wild genotype (GG).
* Three bands (380 bp), (180 bp) and (280bp) are heterozygous genotype (GT).
* Two bands (380 bp) and (180bp) are the homozygous genotype (TT).

The genetic power was calculated. it represents the power to detect a significant difference at level of 0.05 for IGF2BP2 gene rs4402960 G/T it is found to be (40%). it seemed to be less than the optimal level (80%). mostly, these findings are due to relatively small sample size .

3.5.2- The Hardy–Weinberg Equilibrium (WHE) model for (rs4402960 G/T).

The result from Hardy-Weinberg equilibrium (WHE) exact test revealed patient with T2DM group the genotype frequency not follow the Hardy-Weinberg equilibrium , but follow with the Hardy-Weinberg equilibrium in control group indicating that the investigated allele frequencies are constant between generations as in table (3-5).

Table: 3-3 Results of Hardy Weinberg Equilibrium for - IGF2BP2 Gene (rs4402960 G/T).SNP genotypes in patient with T2DM and Control groups.

Group	X2	P-value
T2DM	2.45	**0.29**
Control	5.64	**0.05**

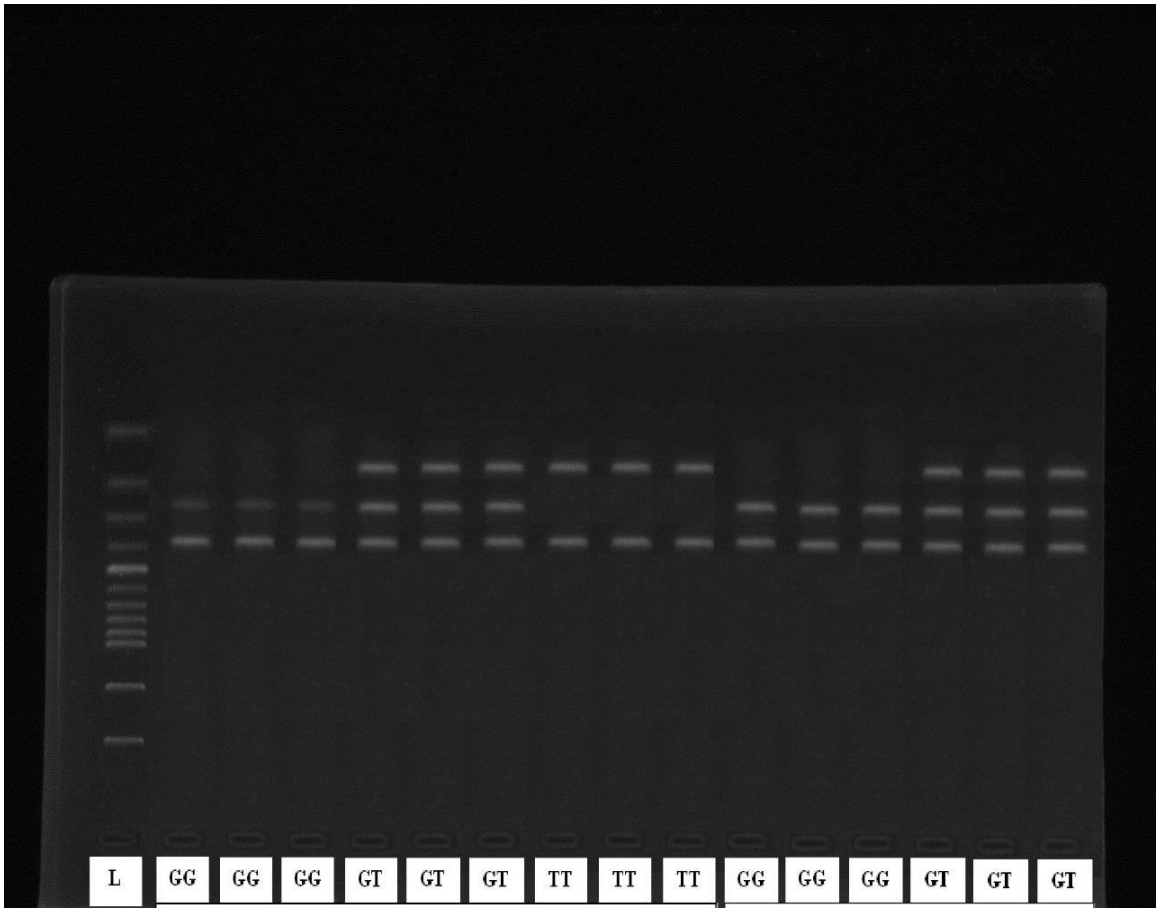

Figure 3-8: T-ARMS-PCR bands of human gene - IGF2BP2 rs4402960 G/T SNP.
M = DNA marker
Lane (4,5,6,13,14 and15) heterozygosity (rs4402960 G/T) (380 bp-280 bp-180bp)
Lane (7,8,and 9) homozygote mutant (rs4402960 G/T) (380bp-180bp).
Lane (1,2,3,10,11 and 12) homozygote wild type (rs4402960 G/T) (380bp-280 bp) .

3.6 Relevance of IGF2BP2 Gene (rs4402960 G/T) Polymorphism

The genotypes distribution and frequency of IGF2BP2 gene (rs4402960 G/T) SNP as shown in Table 3-6. the analysis of results indicated that the IGF2BP2 gene (rs4402960 G/T) SNP genotype frequencies of wild genotype (GG), heterozygous (GT) and homo- zygous genotype (TT) were, (50 %, 35 % and 15 %) and T2DM group, (53.3 %, 30 % and 16.6 %) control group respectively.

The heterozygous genotype (GT) of IGF2BP2 *gene* (rs4402960 G/T) was found to be non significantly difference (OR =0.803 , CI 95% , 0.36-1.79 ,P =0.59) the risk of T2DM with respect to those of the wild genotype (GG) of IGF2BP2 gene.

Also, the homozygous genotype (TT) of IGF2BP2 *gene* (rs4402960 G/T) SNP was found to be non significantly difference (OR =1.04 , CI 95% , 0.37-2.91 ,P =0.93).

Table 3-4: Genotypes distribution and frequency of IGF2BP2 Gene (rs4402960 G/T) SNP in patient with T2DM, and control groups.

Geno-type	T2DM N=60	control N=60	OR	(95% CI)	P-value
GG	30 50%	32 53.3%	Refe	Refe	Refe
GT	21 35%	18 30 %	0.803	(0.36-1.79)	0.59
TT	9 15%	10 16.6%	1.04	(0.37-2.91)	0.93

GG: Wild genotype **GT**: Heterozygous and **TT** : Homozygous genotype

The allele distribution and frequency of G and T of IGF2BP2 gene(rs4402960 G/T) SNP were found to 67.5 % and 32.5 % in the patient with T2DM group respectively and 68.33 % and 31.66 % in the control group respectively. the minor allele frequencies (T) of IGF2BP2 *gene* (rs4402960 G/T) SNP in patient with T2DM and control groups were found to be 32.5% , and 31.6 % respectively. it was non-significantly difference (P >0.05) in patient with T2DM compared with that of the control group.

Table 3-5: Alleles distribution and frequency of IGF2BP2 gene(rs4402960 G/T SNP) in patient with T2DM and control Groups.

Allele		T2DM	control	OR	95% CI	P-value
G	No.	81	82	Refe	Refe	Refe
	%	67.5	68.33			
T	No.	39	38	0.96	(0.55-1.65)	0.89
	%	32.5	31.66			
Total	NO	120	120			

The results of present study revealed that significant decrease ($P < 0.05$) in serum PKA concentration in the homozygous genotype_(TT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patient with T2DM .

Also, significant decrease ($P < 0.05$) of serum PKA concentration in the heterozygous genotype (GT) of IGF2BP2 gene (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 gene (rs4402960 G/T SNP) in patient with T2DM.

Table 3-6 : Mean Serum PKA Concentrations for IGF2BP2 *gene* (rs4402960 G/T) in p-value patients with T2DM.

	Genotype	Mean ± SD
PKA Pg/ml	**GG**	489.2 ± 4.1
	GT	483.21 ± 7.8
	TT	471.1 ± 5.5

GG: Wild genotype, **GT**: Heterozygous and **TT**: Homozygous genotype

The results of present study revealed that significant increase ($P < 0.05$) of serum insulin concentration in the homozygous genotype (TT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM

Also, significant increase ($P < 0.05$) of serum Insulin concentration in the heterozygous genotype_(GT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM as shown in table 3-8.

Table 3-7: Mean serum insulin concentrations for IGF2BP2 *gene* (rs4402960 G/T) in patients with T2DM.

	Genotype	Mean ± SD
Insulin µu/ml	**GG**	3.1 ± 0.9
	GT	4.2 ± 0.3
	TT	5.13 ± 0.5

GG: Wild genotype, **GT**: Heterozygous and **TT**: Homozygous genotype

The results of present study revealed that non-significant increase ($P > 0.05$) of serum HbA1C concentration in the homozygous genotype (TT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM ,while significant increase ($P<0.05$) of serum HbA1C concentration in the heterozygous genotype (GT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM.

Table 3-8: Mean serum HbA1C concentrations for IGF2BP2 *gene* (rs4402960 G/T) in patients with T2DM.

	Genotype	Mean ± SD
HbA1C	**GG**	8.91 ± 0.2
	GT	9.1 ± 0.1
	TT	9.25 ± 0.15

GG: Wild genotype, **GT**: Heterozygous and **TT**: Homozygous genotype

The results of present study revealed that significant increase ($P < 0.05$) of serum FBG concentration in the homozygous genotype (TT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM, Also, significant increase ($P < 0.05$) of FBG concentration in the heterozygous genotype (GT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM as shown in table 3- 10

Table 3-9: Mean serum of FBG concentrations for IGF2BP2 *gene* (rs4402960 G/T) in patients with T2DM.

	Genotype	Mean ± SD
FBG	**GG**	207.2 ± 4.1
	GT	210.7 ± 3.2
	TT	216.01 ± 2.7

GG: Wild genotype, **GT**: Heterozygous and **TT**: Homozygous genotype

The results of present study revealed that no significant increase ($P > 0.05$) of HOMA-IR concentration in the homozygous genotype (TT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM, Also, no significant increase ($P > 0.05$) of HOMA-IR concentration in the heterozygous genotype (GT) of IGF2BP2 *gene* (rs4402960 G/T SNP) when compared with wild genotype (GG) of IGF2BP2 *gene* (rs4402960 G/T SNP) in patients with T2DM .

Table 3-10: Mean of HOMA-IR for IGF2BP2 *gene* (rs4402960 G/T) in patients with T2DM.

	Genotype	Mean ± SD
HOMA-IR	**GG**	1.7 ± 0.33
	GT	2.1 ± 0.5
	TT	2.3 ± 0.2

GG: Wild genotype, **GT**: Heterozygous and **TT**: Homozygous genotype

Table 3-11: Show the wild genotype (G/G) (rs11705701 G/A) for patients and control

Genotype	rs11705701 G/A			P- value
	Case (n=60)	Control(n=60)	OR(95% CI)	
GG Wild type	60 (100%)	60 (100%)	Reference	1.000
GA Mutant heterozygous	0	0	1(0.019-51.38)	
AA Mutant homozygous	0	0	1(0.019-51.38)	

Although patient with T2DM's etiology is complex and requires more research, genetic factors have been identified as a major cause and were identified in recent years by an increasing number of candidate genes. The investigation of candidate genes that have been implicated in initiation and progression of patient with T2DM will also be shedding the light on fundamental molecular mechanisms of this disease **(168)**.

IGF2BP2 is located on chromosome 3q27. IGF2BP2 highly expressed in pancreatic islets, belongs to a family of insulin-like growth factor 2

(IGF2) mRNA-binding proteins, which play roles in normal embryonic growth and development **(169)** .

It has been demonstrated that variants in IGF2BP2 affect first-phase insulin secretion and the disposition index detected by hyperglycemic clamps **(170)**.

The IGF2BP2 was found to transport the target m-RNAs to the surface of the mitochondria and inhibiting it prevents assembly and activation of the respiratory complexes I and IV in mitochondria . the functional role of the IGF2BP2 in mitochondrial assemblies, metabolism, and activity is supported by some studies . IGF2BP2 is necessary for the development of the embryos and neuronal differentiations at the physiological level. IGF2BP2 dysregulation causes a range of illnesses, including T2DM **(171,172)**.

IGF2BP2 is a signaling molecule important for insulin action and growth and has an effect on the development of the pancreas in animal models . Furthermore, the increased levels of FPG, and serum insulin detected among . The gene diacylglycerol kinase g-1 (DGKG) located in the region close to IGF2BP2 was reported to associate with the regulation of insulin secretion **(173)**. Therefore, additional functional studies are required for IGF2BP2 pathophysiological mechanisms.

IGF2BP2 is one of the genes identified though GWAS (Genome-Wide Association Studies) to be associated with T2DM, which has been repeatedly confirmed among different ethnic populations . the most extensively studied variants polymorphisms rs4402960 and rs11705701 G/A . while many studies confirmed the association **(174)** , other studies reported no association **(175)** . more over, subsequent replication studies in different populations yielded inconsistent results **(176)**.

In the present study, we investigated rs4402960 and rs11705701 in IGF2BP2 gene with the development of patient with T2DM in the Iraq

population, We also focused on the implication of these single nucleotide polymorphisms (SNPs) in different clinical phenotypes related to Type 2 diabetes .

This results showed an no association of rs4402960 SNP in patients with T2DM. also, no association of rs11705701 SNP patient with T2DM . In a previous study in Lebanese Arab population **(177)**, both IGF2BP2 variants represented common patient with T2DM susceptibility genes with the strongest association at rs4402960.

In contrast to our results, several previous studies in the Chinese Han population showed that rs11705701 G/A mutant variants were inversely correlated patient with T2DM susceptibility, where GA and AA genotypes had lower frequencies in patients with T2DM compared with healthy controls **(178)**.

Conclusions

1- Insulin,HbA1c and HOMA were negatively correlated with Protein Kinase A in patients with T2DM

2-(TT) SNPs of IFG2BP2 is association with increase insulin resistance,FBG,HbA1c,insulin and decrease protein kinase A.

3- The IGF2BP2 gene SNP (rs4402960 G/T) and (rs11705701 G/A**)** don't associate with T2DM patients .

3-7 Recommendations

1- Study of the gene expression another SNPs.

2-Determine the genotyping of IGF2BP2 gene in other provinces in Iraq to give a complete picture about genotype distribution in Iraqi population.

3-Conduct whole IGF2BP2 gene sequencing to detect the possible SNPs related to patient with T2DM.

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References

Appendix

Questionnaires

Date of sample:

Hospital name:

Name of the patient:

Age of the patient:

Duration of disease:

Male/Female:

City:

Type of medicine:

Blood pressure:

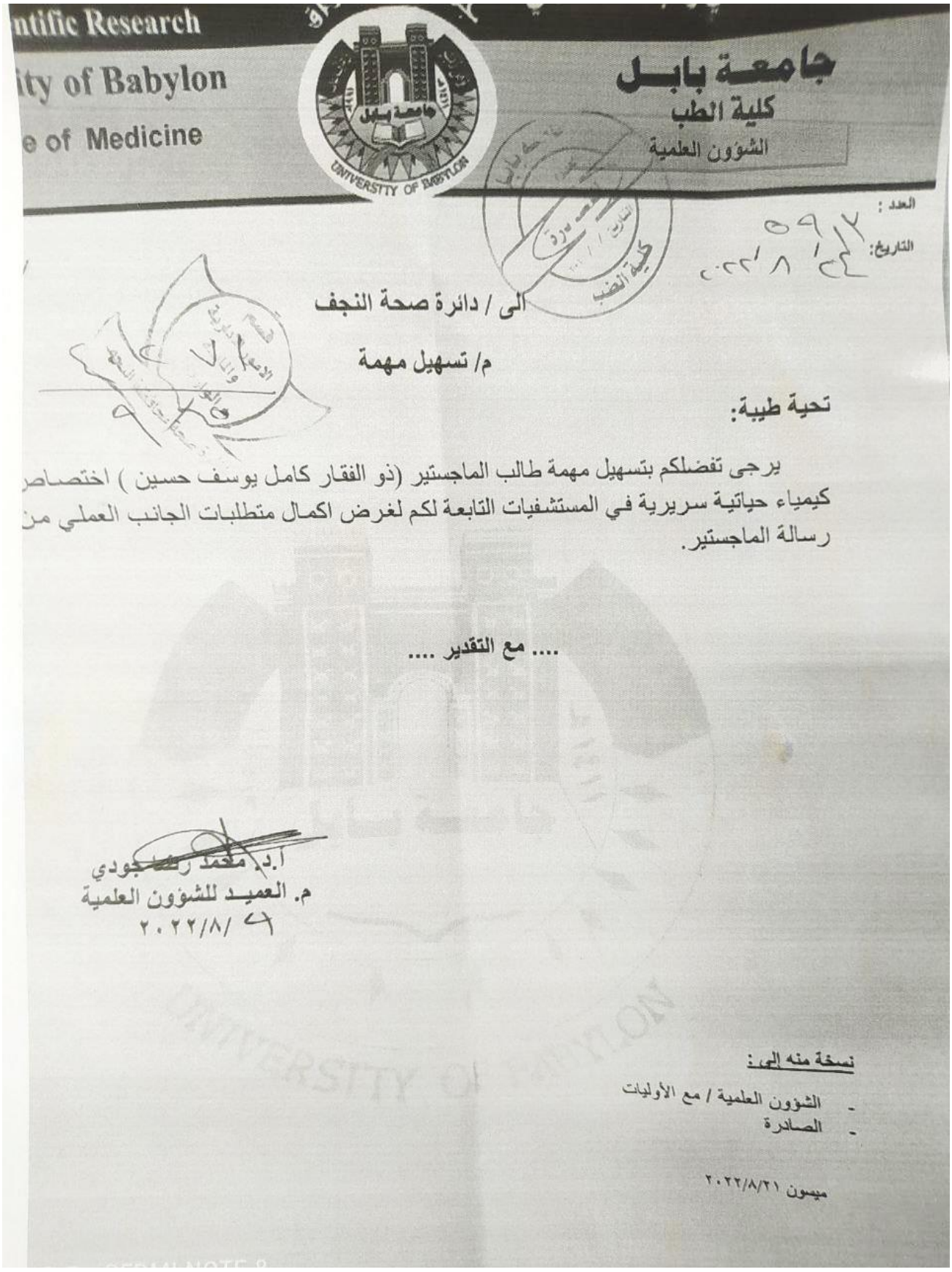
You have any complication disease of DM:

Weight:

Length:

Note:

Appendix



ntific Research
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e of Medicine

جامعة بابل
كلية الطب
الشؤون العلمية

العدد : ٥٩٧٢
التاريخ: ٢٠٢٢/٨/٢٤

الى / دائرة صحة النجف

م/ تسهيل مهمة

تحية طيبة:

يرجى تفضلكم بتسهيل مهمة طالب الماجستير (ذو الفقار كامل يوسف حسين) اختصاص كيمياء حياتية سريرية في المستشفيات التابعة لكم لغرض اكمال متطلبات الجانب العملي من رسالة الماجستير.

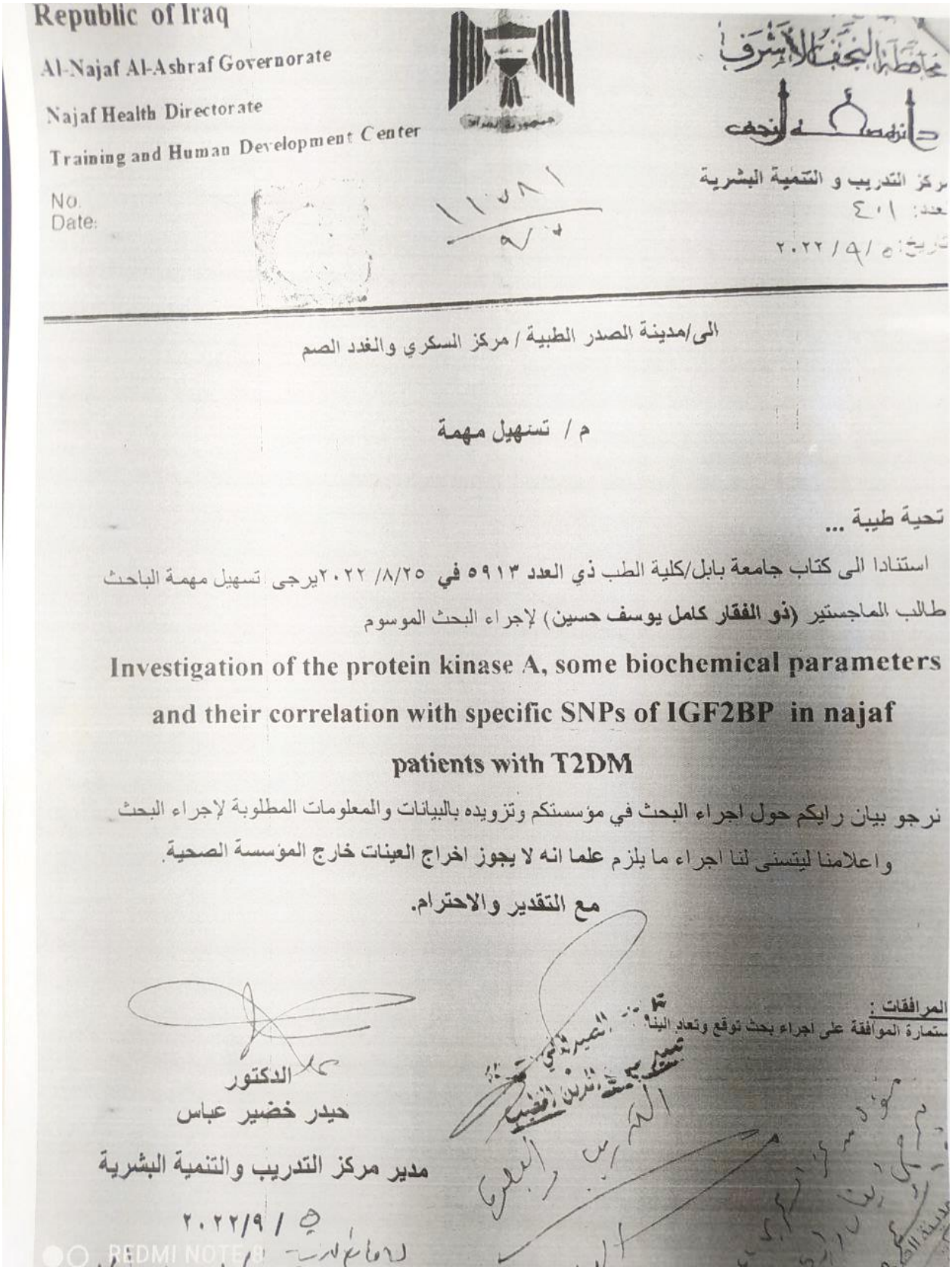
.... مع التقدير ....

أ.د. محمد [illegible] جودي
م. العميد للشؤون العلمية
٢٠٢٢/٨/ ٢٤

نسخة منه إلى:
- الشؤون العلمية / مع الأوليات
- الصادرة

ميسون ٢٠٢٢/٨/٢١

Appendix



Republic of Iraq
Al-Najaf Al-Ashraf Governorate
Najaf Health Directorate
Training and Human Development Center

محافظة النجف الأشرف
مديرية صحة النجف
مركز التدريب و التنمية البشرية

No.
Date:

العدد: ٤٠١
التاريخ: ٢٠٢٢/٩/٥

الى/مدينة الصدر الطبية / مركز السكري والغدد الصم

م / تسهيل مهمة

تحية طيبة ...

استنادا الى كتاب جامعة بابل/كلية الطب ذي العدد ٥٩١٣ في ٢٠٢٢/٨/٢٥ يرجى تسهيل مهمة الباحث طالب الماجستير (ذو الفقار كامل يوسف حسين) لإجراء البحث الموسوم

Investigation of the protein kinase A, some biochemical parameters and their correlation with specific SNPs of IGF2BP in najaf patients with T2DM

نرجو بيان رايكم حول اجراء البحث في مؤسستكم وتزويده بالبيانات والمعلومات المطلوبة لإجراء البحث واعلامنا ليتسنى لنا اجراء ما يلزم علما انه لا يجوز اخراج العينات خارج المؤسسة الصحية.

مع التقدير والاحترام.

المرافقات :
استمارة الموافقة على اجراء بحث توقع وتعاد الينا

الدكتور
حيدر خضير عباس
مدير مركز التدريب والتنمية البشرية
٢٠٢٢/٩/٥

Appendix

Republic of Iraq
Al-Najaf Al-Ashraf Governorate
Najaf Health Directorate
Training and Human Development Center
No.
Date:

جمهورية العراق
محافظة النجف الأشرف
مديرية صحة النجف
مركز التدريب و التنمية البشرية
العدد: ٢٨٤٤٦
التاريخ: ٢٠٢٢/٩/٧

إلى/جامعة بابل / كلية الطب

م / تسهيل مهمة

تحية طيبة ...

إشارة إلى كتابكم ذي العدد 5913 في ٢٠٢٢/٩/٦ بخصوص تسهيل مهمة الباحث طالب الماجستير (ذو الفقار كامل يوسف حسين) للحصول على الموافقة الاخلاقية لإجراء البحث العلمي الموسوم:

Investigation of the protein kinase A, some biochemical parameters and their correlation with specific SNPs of IGF2BP in najaf patients with T2DM

حصلت موافقة اللجنة العلمية للبحوث في مركز دائرتنا على إجراء البحث في (مدينة الصدر الطبية / مركز السكري والغدد الصم) في دائرتنا مع التأكيد على الالتزام الكامل بتعليمات السلامة الحيوية والضوابط الاخلاقية والحصول على موافقة المشاركين قبل الشروع بالبحث والحفاظ على خصوصيتهم وعدم افشاء البيانات او استخدام العينات لغير اغراض البحث العلمي ... على أن لا تتحمل دائرتنا أية تبعات مادية ولا يسمح بإخراج العينات خارج مختبرات المؤسسة.

للتفضل بالاطلاع .....مع الاحترام

الدكتور
احمد عباس طاهر الاسدي
المدير العام/وكالة
٢٠٢٢/٩/٧

دائرة صحة النجف الصادر قسم الامور الادارية والمالية والقانونية

الخلاصة

يصف مصطلح السكري مجموعة من الاضطرابات الأيضية التي تتميز بوجود فرط سكر الدم. يمكن العثور على مرضى السكري من النوع 2 في كل سكان العالم تقريبًا.

لدراسة تأثير داء السكري من النوع 2 على مستويات البروتين كيناز A والأنسولين وسكر التراكمي وسكر الدم الصائم ومقاومة الانسولين ودراسة الارتباط بين بروتين كيناز أ ومستويات الأنسولين وسكر التراكمي ومقاومة الانسولين في مرضى السكري من النوع 2. ولتحقيق هذا الهدف ، تم دراسة 60 مريضًا يعانون من مرض السكري من النوع 2، مقارنتا ب 60 شخص يتمتعون بصحة جيدة كمجموعة ضابطة. تراوحت أعمار المرضى بين(35-60) سنة ، وكان متوسط ± الانحراف المعياري (SD) (13.1 ± 47.2) سنة ، مع ± الانحراف المعياري (SD) للموشر كتلة الجسم كان(2.59 ±25.31) كغم / م^2. تراوح عمر المجموعة الضابطة بين (35-60) سنة بمتوسط ± الانحراف المعياري (SD) كان(12.9 ± 46.7) سنة ، مع المتوسط ± الانحراف المعياري (SD) لموشر كتلة الجسم كانت (1.11 ± 24.7) كغم / م^2. كان هناك تطابق في العمر ومؤشر كتلة الجسم بين المرضى والمجموعة الضابطة (القيمة الاحتمالية> 0.05). تم استخدام عينة المصل لجميع المجموعات لقياس مستوى بروتين كيناز أ، الأنسولين باستخدام طريقة ELISA، تم حساب معاملات مقاومة الأنسولين باستخدام HOMA2-Calculator. بينما، تم قياس سكر الدم الصائم و سكر التراكمي بواسطة جهاز AFIAS.

أظهرت نتائج الدراسة الحالية وجود فروق ذات دلالة إحصائية في مستويات بروتين كيناز أ بين مرضى السكري من النوع 2 ومجموعة المراقبة ($p <0.05$)، وكانت هناك فروق ذات دلالة إحصائية في مستويات الأنسولين بين المرضى والمجموعة الضابطة. ($P<0.001$)، كما كان هناك فرق معنوي في مستويات سكر التراكمي بين مرضى السكري من النوع 2 ومجموعة التحكم ($p <0.001$) ، وكان هناك ارتباط معنوي بين بروتين كيناز A والأنسولين، سكر التراكمي ، وHOMA على التوالي. تم العثور على النمط الجيني متغاير الزيجوت (GT) لجين SNP (rs4402960 G / T) IGF2BP2 في المرضى الذين يعانون من مجموعة داء السكري من النوع 2 مقارنة بمجموعة التحكم ليكون هناك فرق غير مهم أيضًا في النمط الوراثي متماثل (TT) لجين (rs4402960 G / T) IGF2BP2. تم العثور على اختلاف غير معنوي في SNP في مجموعة مرضى السكر مقارنة مع مجموعة التحكم. تم العثور على ترددات الأليل الصغرى (T) لجين SNP (rs4402960 G / T)) IGF2BP2 في مرضى السكري

ومجموعة التحكم لتكون 67.5٪ و 32.5٪ على التوالي لم يكن هناك فرق معنوي (P> 0.05) في مرضى السكر بالمقارنة مع مجموعة السيطرة

أخيرًا، لم يكن تعدد الأشكال لجين IGF2BP2 (rs11705701 G / A) مرتبطًا بمريض مصاب بداء السكري من النوع 2، (اكتشفنا فقط النمط الوراثي من النوع GG في جميع المشاركين المدروسين، ولم يقدم أي منهم الأنماط الجينية GA أو AA الطافرة).

وزارة التعليم العالي
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بروتين كينازأ ، و موشرات مقاومة الانسولين وعلاقتها مع التعبير الجيني SNPs لجين IGF2BP2 بين المرضى المصابين بداء السكري من النوع الثاني في مدينة النجف الاشرف.

رسالة مقدمة الى
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كجزء من متطلبات درجة ماجستير في العلوم الكيمياء الحياتية السريرية

من قبل الطالب
ذو الفقار كامل يوسف عجينة
بكالوريوس علوم كيمياء / كلية العلوم / جامعة الكوفة /2012

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