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Diagnostic Utility of Leucine Rich Alpha-2 Glycoprotein1 and Insulin Like Growth Factor Binding Protein 7 in Serum of Iraqi Patients with Type 2 Diabetes mellitus

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Master Degree in

[Clinical Chemistry]

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ وَأَن لَّيْسَ لِلْإِنسَٰنِ إِلَّا مَا سَعَىٰ (39) وَأَنَّ سَعْيَهُۥ سَوْفَ يُرَىٰ (40) ثُمَّ يُجْزَىٰهُ ٱلْجَزَآءَ ٱلْأَوْفَىٰ (41)﴾

صدق الله العلي العظيم

[سورة النجم]

Supervisor - Certification

we certify that this thesis entitled

Diagnostic Utility of Leucine Rich Alpha-2 Glycoprotein 1 and Insulin Like Growth Factor Binding Protein 7 in Serum of Iraqi Patients with Type 2 Diabetes mellitus.

Was prepared by **(Khalid Mohsen Nasih)** under our supervision at the college of medicine, university of kerbala , as a partial fulfillment of the requirement for the Degree of Master in **(Clinical Chemistry)**.

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We, the examing committee cetrtify that we have read this M.Sc. thesis entitled (**Diagnostic Utility of Leucine Rich Alpha-2 Glycoprotein1 and Insulin Like Growth Factor Binding Protein 7 in Serum of Iraqi Patients with Type 2 Diabetes mellitus).** We have examined the postgraduate student **(Khalid Mohsen Nasih)** in it M. Sc.Thesis content and in our opinion, it meets the standard for the degree of master in **(Clinical Chemistry)**.

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Dedication

With love, I dedicate this work to the soul of

(my halal sakban uncle)

and to my biggest supporter (my brother), and

the one who alleviated my difficulties with her

prayers (my mother) and all my family and

friends who have supported me along the way

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Khalid

Summary

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin resistance or both. Two main forms of diabetes were identified; type 1 and type 2. The more prevalent form, type 2 diabetes mellitus (T2DM), accounts for more than 90% of cases **(**T2DM) which usually begins as insulin resistance, a disturbance in which the cells do not utilize insulin properly or shortage in insulin secretion from the pancreas cells. This study aimed to find the relationship between Leucine Rich Alpha-2 Glycoprotein 1 (LRG1) and Insulin Like Growth Factor Binding Protein 7 (IGFBP7) with occurrence and development of T2DM.The current study is a case control study included (90) individuals aged between (20-70) year where conducted on (60) of patients group with Type2 diabetes mellitus from Imam hassan center for endocrinology and diabetes in Holy kerbala governorate and also on (30) of control group (apparently health) during the period from (November-2024) to (march-2025) and collected fasted blood samples for all participants in this the study. Took medical history of all participants and measured blood pressure, body mass index , Fasting blood Sugar, Glycated hemoglobin, insulin and lipid profile in addition to measuring Leucine Rich Alpha 2 Glycoprotein1 (LRG1) by (competitive immune assay technique) by using Enzyme Liked Immuno Sorbent assay (ELISA) device and Insulin like Growth Factor Binding Protein 7 (IGFBP7) was measured by

(sandwich immune assay technique) by using Enzyme Linked immuno Sorbent assay (ELISA) device. The current study showed increased levels of fasting blood sugar, glycated hemoglobin in patients group more than control group while insulin values were lower compared to control group that was within normal level. The present study showed increased levels of (cholesterol) , triglyceride, low density lipoprotein and very low density lipoprotein in patient group more than control group. While high density lipoprotein levels increased in control group more than patients group. In the present study observed that Leucine Rich Alpha 2 Glycoprotein 1 (LRG1) and Insulin like Growth Factor Binding Protein 7 (IGFBP7) levels are raised in patients group more than control Group with p value (< 0.001) and then it was concluded that both biomarkers Leucine Rich Alpha 2 Glycoprotein1 (LRG1) and Insulin like Growth Factor Binding Protein 7 (IGFBP7) are predictive and risk factors for development of Type 2 diabetes mellitus (T2DM).

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List of Abbreviations

Abbreviations	Word
ADA	American Diabetes Association
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
AUC	Area Under Curve
ANOVA	Analysis Of Variance
BMI	Body Mass index
CHE	Cholesterol esterase
CHOD	Cholesterol oxidase
CVD	Cardiovascular Disease
CI	Confidence Interval
DKA	Diabetic Keto Acidosis
DNA	Deoxy ribonucleic Acid
DR	Diabetic retinopathy
ELISA	Enzyme-Linked Immuno Sorbent Assay
EDTA	Ethylene Diamine Tetra Acetic Acid
FBS	Fasting Blood Sugar
GDM	Gestational Diabetes Mellitus
GOD	Glucose Oxidase
GK	Glycerol Kinase
GTT	Glucose Tolerance Test
GPO	Glycerol Phosphate Oxidase
GSvs	GLUT4 storage vesicles
HHS	Hyperosmolar Hyperglycemic State

HbA1c	Glycated Hemoglobin
HOMA	Homeostatic Model Assessment
HDL	High density lipoprotein
H_2O_2	Hydrogen Peroxide
HDAOS	[N-(2-hydroxy-3-sulfopropyl)- 3,5-dimethoxyaniline]
HRP	Horse Radish Peroxidase
IDF	International Diabetes Federation
IGF-1	Insulin- Like growth factor-1
IL	Interleukin
IGFBP7	Insulin-Like Growth Factor – Binding Protein 7
IBM	International Business Machine
IRS-1	Insulin receptor substrate-1
LDL	Low Density Lipoprotein
LRG1	Leucine rich alpha-2 glycoprotine1
LRC	Leucine Rich C-terminal domain
LRR	Leucine Rich Repeat
MENA	Middle East and North Africa
Ng/ML	Nano grams per Milliliter
OR	Odds Ratio
OGTT	Oral Glucose Tolerance Test
OD	Optical Density
POD	Peroxidase
PAD	Peripheral Artery Vascular Disease
PAK1	P21-Activated Kinase1
P Value	Probability Value
PIP2	Phosphatidylinositolbisphosphate

PDK	PIP3-Dependent kinase
RLUs	Relative Optical Units
RTK	Receptor Tyrosine- Kinase
R_1	Anti-insulin-AB-biotin
R_2	Anti-insulin-AB-Ru(bpy)
r	Pearson Correlation Coefficient
ROC	Receiver Operating Characteristics
SPSS	Statistical Package for Social Science
TC	Total- Cholesterol
TG	Triglyceride
TGF-β	Transforming Growth Factor-β
T1DM	Type 1 Diabetes mellitus
T2DM	Type 2 Diabetes Mellitus
µg/ML	Micro grams per Milliliter
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization
4-AAP	4-aminoantipyrine

CHAPTER ONE
Introduction and Literature Review

1.Introduction

1.1.Diabetes Mellitus (DM)

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Two main forms of diabetes were identified; type 1 and type 2. shortage of or severe decreasing in insulin secretion due to autoimmune or vital destructions of cells is responsible for type 1 diabetes mellitus (T1DM) which accounts for 5-10% of diabetic patients. The more prevalent form, type 2 diabetes mellitus (T2DM), accounts for more than 90% of cases **[Olefsky et al .,2021]** T2DM usually begins as insulin resistance, a disturbance in which the cells do not utilize insulin properly. As the need for insulin elevation, the pancreas gradually loses its ability to produce it **[Cohen et al ., 2019]**.

In the human body, the liver is the major site of gluconeogenesis. Excess gluconeogenesis in the liver of patients with T2DM is considered a main contributor to hyperglycemia and subsequent diabetic organ damage. Insulin is a key hormone that inhibits gluconeogenesis, and insulin resistance is a hallmark of T2DM. Understanding the regulation of gluconeogenesis and the role of insulin signaling in this pathway is important to developing new remedies for T2DM **[Hatting et al ., 2023]**.Genetic factors and lifestyle play a critical role in the development of T2DM **[Vazquez et al ., 2015]**. Currently the episode of T2DM has reached epidemic levels in Asia .Despite knowledge of the critical role of genetic factors; these have not been confederated into the clinical appraisement of T2DM risk **[Ahmed et al ., 2016]**.

1.1.1. Definition:

The word, diabetes comes from the excessive amount of urination in the disturbance and mellitus refers to the diabetic person's carbohydrates in the urine, in Latin meaning 'sweetened by honey' or sugar cane urine **[Ritz E et al ., 2018]**. T2DM contributed to 14.5% of all human deaths, of which half happen in people less than 60 years old **[Cho et al ., 2017].** Generally, the type 2 diabetes mellitus (T2DM) may be combination between genetics and environmental factors **[Kasper DL et al ., 2015]**.

1.1.2. Classification of Diabetes

There are different types of DM, triggered by genetic or environmental considerations and by decisions regarding lifestyles.

1.1.2.1. Type 1 Diabetes Mellitus (T1DM)

Is a recurrent case of young adulthood that is one of the most common. It is caused by pancreatic B-cell autoimmune damage and includes daily observation of blood glucose levels (BGLs) and lifetime need for administration exogenous insulin **[mirazi et al ., 2015 & puchulu et al ., 2018]**. Both sexes are similarly affected in infancy, but males are most greatly affected in early adult life **[STEDMAN et al ., 2020]**. Patients with type 1 diabetes mellitus, with polyuria, polydipsia, polyphagia (excessive hunger) and loss of weight, lethargy, vision loss and ketoacidosis remain dependent on lifelong insulin supplementation lifelong **[Livingstone SJ et al.,2015 & Jakobsen et al.,2018].**

1.1.2.2 Type 2 Diabetes Mellitus (T2DM)

Is the most common type, which accounts for about 85% to 95% of all diabetes cases **[Li Y et al.,2015]**. Mostly, T2DM occur after the age of 40 year **[BURTIS et al ., 2021]**. The decrease in islet secretive activity may be followed by the reduction in the peripheral insulin tolerance and substitutionary hyper secretion **[Forbes et al ., 2023].** Skeletal muscle, liver and adipose tissue are the tissues most preeminently demonstrate less insulin sensitivity because of the specific needs of glucose and metabolism at these locations **[Forbes et al ., 2023]..** These people do not need insulin therapy to survive, at least initially, and sometimes during their lives **[Association AD ., 2018]**. Patients of T2DM are at 2-4 times more likely than the general population to suffer mortality and cardiovascular events **[Rawshani et al ., 2017].** Food is a very significant element in regulating T2DM. Recent researches have demonstrated that.

DM can be managed without need for hypoglycemic treatment by diet and physical activity **[Astrup et al ., 2017].** Also, shown the different in the table (1.1) **[ORAM et al ., 2016]**.

1.1.2.3. Gestational Diabetes Mellitus (GDM)

It is classified as various levels of glucose intolerance first found during pregnancy, affecting 4 to 18 % of pregnant women with different diagnostic and ethnic parameters **[puchulu et al ., 2018 & simmons et al .,2015]**. The GDM pathophysiological pathway is similitude to T2DM due to insulin resistance, oxidizing stress and systemic inflammation, diagnosed during the second or third trimester of pregnancy that is not clearly overt diabetes **[Allalou et al ., 2016 & Azad et al ., 2017]**.

1.1.2.4. Other Specific Types of Diabetes Mellitus

A-Monogenic Diabetes syndrome

B-Exocrine pancrease of Diabetes

It is included pancreatitis, cushing 's syndrome, haemochromatosis and drugs induced of diabetes **[Solis-Herrera et al ., 2018]**.

Table (1.1) Differentiate between Type 2 DM and Type 1 DM [ORAM et al ., 2016].

Clinical features	Type 1	Type 2
Age of onset	Most 25 but can occur at any age (not before 6 month)	Usually 30 years
Weight	Usually thin	90% overweight
Islet autoantibody	Usually presents	Absent
C-peptide	Undetectable low	Normal/high
Insulin production	Absent	Present
First line treatment	Insulin	Non-insulin – antihyperglycemic agent
Family history of DM	Infrequent (5%-10%)	frequent (75-90%)
DKA	Common	Rare

* DKA diabetic ketoacidosis

1.1.3. Type 2 Diabetes Mellitus

Is the most common type, which accounts for about 85% to 95% of all diabetes cases **[Li Y etal ., 2015]**. Mostly, T2DM occur after the age of 40 year **[BURTIS et al ., 2021]**. The decrease in islet secretive activity may be followed by the reduction in the peripheral insulin tolerance and substitutionary hyper secretion **[Forbes et al ., 2023].** Skeletal muscle, liver and adipose tissue are the tissues most preeminently demonstrate less insulin sensitivity because of the specific needs of glucose and metabolism at these locations **[Forbes et al ., 2023].**

1.1.3.1. Epidemiology

Type 2 diabetes mellitus is one of the most prevalent chronic diseases **[Goldman et al ., 2016].** Accumulated studies proved that the incidence of T2DM is forecasted to rise over the next two decades, especially among those aged 45 to 64 **[Prakash et al ., 2019** & **GUJRAL et al ., 2021]**. Nearly 463 million people in the world suffer with diabetes according to International Diabetes Federation in 2020 shown in Figure (1.1) **[IDF, 2020].**

In Iraq three million residents, or 13.4 % of the total population, were expected to be affected **[Ali NSM et al ., 2024]**. In 2024 (2,669,400) cases diabetes were registered in Iraq, 425 million people have diabetes in the world and more than 39 million people in Middle East and North Africa (MENA) region; by 2045 this will raise to 67 million, Iraq is among the 19 countries and territories of the MENA region of International Diabetes Federation (IDF) **[IDF, 2017].**

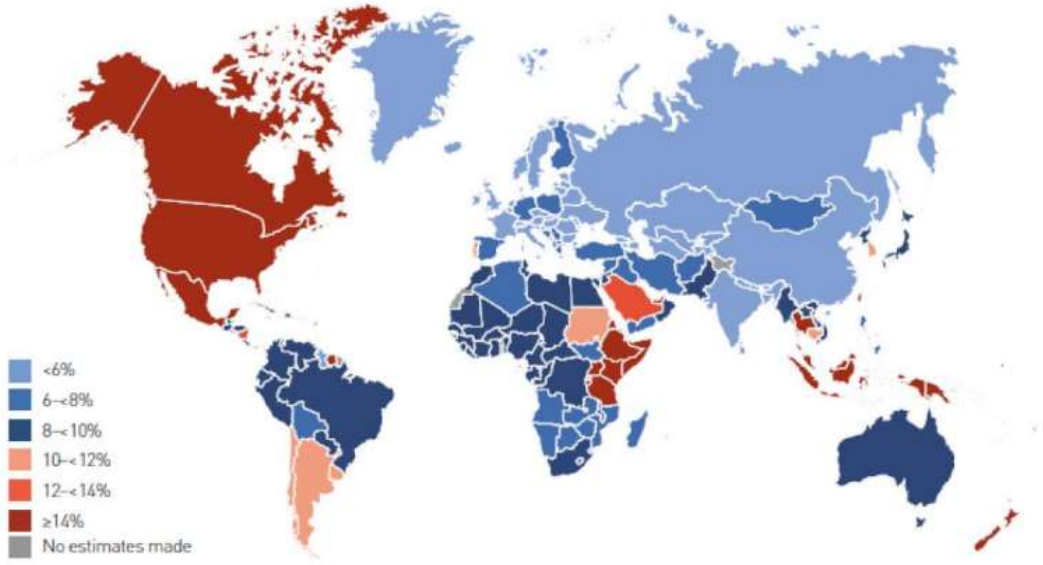


Figure (1.1) Prevalence of Impaired Glucose Tolerance in Adults (20–79 years) in 2019, Acclimatized from International Diabetes Federation [IDF,2020].

1.1.4. Risk, Factors of Type 2 Diabetes Mellitus

1.1.4.1. Obesity

Obesity is the most important cause in predisposition to T2DM in particular, genetic predisposition. Several studies have shown that mild grade of chronic inflammation is an important factor in DM and obesity **[Kwon et al .,2021, Shield et al ., 2015 & Saltiel et al .,2017].** Obesity patients a diposites contain large amounts of pro-inflammatory cytokines such as interleukin 6 and interleukin 1 **beta [Vielma et al .,2023 & Basanta et al ., 2016]**. In 2014 the total rate of overweight and obesity was

52%, while the estimated incidence was nearly 90% **[WHO, obesity & overweight,2017].** 5 – 10 % weight loss is linked to major health effects, including improved lipid parameters, glycemic regulation and blood pressure **[BRAY et al.,2022].**

1.1.4.1.1. Body Mass Index (BMI)

Definition:

Body mass index (BMI), defined as weight divided by height squared, is the most commonly utilized measure of adiposity, with individuals exceeding a certain BMI threshold classed as obese , BMI, such as obesity ,is positively jointed with metabolic abnormalities, many common diseases and all-cause mortality **[Young et al ., 2016]**.

The (BMI) is the metric presently in utilize for defining anthropometric weight/height characteristics in adults and for classifying (categorizing) them into groups **[Nuttall et al ., 2015]**.

Diabetes and high (BMI), defined as a BMI greater than or equal to 25 kg/m², are leading causes of mortality and morbidity global, high BMI is an important risk factor for diabetes **[Pearson et al ., 2018]**.

Baseline BMI, calculated as the weight in kilograms divided by the square of the height in meters, was classified at baseline into six categories according to the World Health Organization classification: underweight (<18.5), normal weight (≥18.5 to <25), overweight (≥25 to <30), and obesity grade 1 (≥30 to <35), grade 2 (≥35 to <40), and grade 3 (≥40 kg/m2) **[Mohammedi et al ., 2018]**.

1.1.4.2. Age

T2DM is expected to affect 10% to 15% of the population over the age of 65 years, and 20% of the population the ages of 65 -80 years **[Nanayakkara et al ., 2018]** or more than eight times greater than the frequency among those aged 18 - 44 years (2.4 % prevalence) **[Diaz Ana et al ., 2019].** This appears to have occurred because aging causes an increase in FBS of (1 to 2 mg/dl) per year. These alterations would be linked to changes in insulin sensitivity in peripheral or alterations in pancreatic islet function **[Huang et al ., 2018]**.

1.1.4.3. Nutrition and Physical Activity

The American Diabetes Association advises strict glycemic control as an effective method to avoid microvascular complications of T2DM by adjustment of drug and lifestyle **[ADA,2017]** Obesity and T2DM advances are linked with low physical activity and poor eating patterns. The use of a friendly, diet and regular physical exercise are necessary not only to averted and therapy DM, but, also for physical and mental health maintenance **[khemayanto et al ., 2023]**.

1.1.4.4. Hypertension

Diabetic and hypertensive cohabitation affects clinical results in both microvascular and macrovascular illness. Hypertension pathophysiology for diabetes includes maladaptive alterations and complicated interactions between the autonomous neuronal system and mechanical forces **[Frimponig et al ., 2020]**. The main causes for atherosclerosis and T2DM are hypertension, involving heart attacks and strokes. DM and high blood pressure overlap significantly, suggesting a

considerable interpenetration in their etiology and disease processes **[Cheung et al ., 2016]**.

1.1.4.5. Genetic Factor

It has also been shown that T2DM has a clear genetic base. In monozygous twins, T2DM concordance is around 70 % to 20-30% in dizygous twins **[Kaperio et al ., 2022].** In research, they appeared that first grade family history is linked with risks for future T2DM **[Lyssenko et al ., 2021]**.

The interplay of environmental considerations with a high inherited component outcomes in (T2DM). Examine the heritability of T2DM and the history of genomic and genetic research in this field. The introduction of genome-wide association analyses has resulted in the discovery of many genes. Several of them were previously unknown to have any function in T2DM **[Ali et al ., 2021]** as exhibited in figure (1-2) the relationship between genetics, epigenetics and environment **[Hu et al ., 2018]** as in the following figure (1-2).

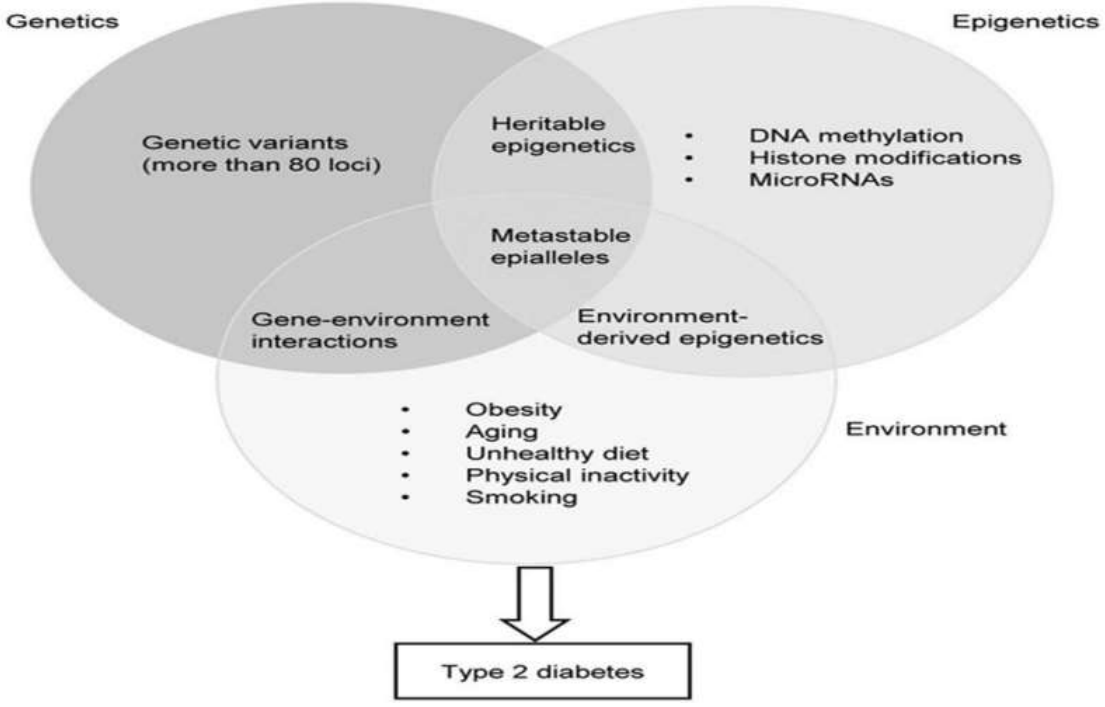


Figure (1-2) Roles of Genetics, Epigenetics and The Environment of T2DM [Hu et al ., 2018].

1.1.4.6. Smoking

The high levels of nicotine from smoking cigarettes can make the cells in the body less responsive to insulin which makes the blood sugar levels higher people with diabetes mellitus type 2 which are exposed to high amounts of nicotine may need to regulate their blood sugar levels **[US. Department of Health and Human services ,2021]**.

1.1.4.7. Ethnicity

Ethnicity is associated with incidence of (type 2) diabetes mellitus because it lead to increased in fats and decreased in body mass index (BMI) for the patients and this is lead to excess of blood glucose levels in the human body **[ADA,2022]**.

1.1.5. Insulin Resistance

Insulin resistance (IR) is biologically impaired reaction to target tissue, particularly in liver, adipose tissue, and skeletal muscle, has lower sensitivity to insulin and thus glucose tolerance **[Deacon et al ., 2019& Seong et al ., 2019]**. This has important outcomes for patients because they cannot obtain the energy they need from glucose to maintain cell metabolism processes. IR is of global interest because many chronic disturbances like T2DM, obesity , Cardiovascular disease and hepatic cirrhosis **[Hoglund et al ., 2017]**. Although any insulin impairment on target tissues may be classified as IR, in clinical practice, the latter.

usually decreased insulin activity on glucose metabolism **[Williams et al., 2016]**. Excessive insulin resistance is a common cause of T2DM, where body cells do not respond suitably to insulin **[Boucher et al .,2022]**. The major insulin is a reduction in the number of receptors and their catalytic function due to many factors, involving mutations, modifications or translations in the insulin receptor and a deminish in the amount of metabolic rate **[Olivares et al ., 2023]**. Even so, insulin resistance does not happen in all obese individuals and even in non-obese populations, the genetic history is highly responsible for insulin resistance **[Graham Timothy et al ., 2021]**.

1.1.5.1. Determination of Insulin Resistance

The appraisement of insulin resistance may be directed by the Homeostatic model assessment (HOMA). In 1985 , David Matthews et al. publicized the model, where they presented it **[Nakell et al ., 2022]**. The HOMA model was mathematically derive by Searching for the relationship between FBS and Fasting insulin **[International congress of nutrition,2016]**. These association can be utilized to measure beta-cells activity and insulin resistance **[Wikstrom et al ., 2021]**.The following formula are.

used:

HOMA-IR = (fasting blood insulin × fasting blood glucose)/405

= Kg/m^2 **[Mathews David et al ., 2017]**.

1.1.6. Relationship between Inflammation and T2DM

The inflammation plays essential role in the development of insulin resistance and development T2DM **[Khan steeven et al ., 2018]** and **[Green field et al .,2022]**. Several evidences have displayed that cytokines involving IL-1β IL-6, TNFα IFN-γ play a role in pathogenesis of T2DM **[Wang et al ., 2016, Mirza et al .,2019 & Moradi N et al .,2021]**. Different genetic and environmental factors have a significant role in the raised glucose. Obesity is consider as one of the most important stimulator of low-grade inflammation for long time that lead to insulin resistance **[Esser et al ., 2020]**.

1.1.7. Obesity and Diabetes Mellitus

Obesity is a complex and multifactorial disease resulting from the interactions among genetics, metabolic, behavioral, sociocultural and environmental factors **[Goni et al ., 2018]**.

Obesity and (T2DM) are the leading worldwide risk factors for mortality. The complicatedly interrelated pathological progression from increased weight gain, obesity, and hyperglycemia to T2DM, usually commencing from obesity, typically originates from overconsumption of sugar and high-fat diets **[Hossain et al ., 2015]**.

Obesity may precede the onset of (T2DM) and is linked with an excess risk of developing T2DM. Furthermore, obesity has been associated to metabolic dysfunction and may further exacerbate T2DM –related metabolic abnormalities. Obesity-linked metabolic dysfunction is also independently related with brain alterations **[Yoon et al ., 2017].**

Obesity and T2DM are also independent risk factors for several disturbances, including arterial hypertension, dyslipidemia, and macro angiopathy.

1.1.8. The pathological Relationship between Aging, Obesity and T2DM.

T2DM is characterized by hyperglycaemia, which results from a progressive deterioration of insulin secretory β-cell function, usually associated with varying degrees of insulin resistance. These two key pathogenetic mechanism are usually accompanied by other glucoregulatory disturbances such as unsuitable hyperglucagonaemia

and an inhibited incretin response **Figure (1-3) [ADA,2021& Defronzo et al ., 2022]**. Insulin resistance alone is rarely sufficient to trigger the development of T2DM as the pancreas can primarily compensate by propbably excess insulin secretion. However, long-term hyperinsulinaemia incurs a stress on β-cells that obstruct the acute (first phase) insulin secretoryresponseto a glycaemic catalyst and eventually inhibitingthe later (second phase) insulin response **[ADA,2021&Defronzo et al ., 2022]**. Hence, insufficient insulin secretion is an essential pathogenetic component for most patients with T2DM **[ADA, 2021& Defronzo et al ., 2022]**.

Ageing contributes to the pathogenesis of T2DM both directly through the reduced β-cell function that accentuates the shortage of insulin secretion and indirectly by excess insulin resistance through obesity and other risk factors **Figure (1-3) [Lee et al ., 2017& Egan et al ., 2018]**. For example, β-cell senescence and reduced β-cell sensitivity to glucose during ageing excess susceptibility to T2DM .

Through inadequate compensation for insulin resistance **[Chang et al .,2016 & Li et al ., 2019]** .The detrimental effects of ageing on cellular pathways of insulin action and glucose metabolism are modest when age-linked changes in body composition are considered **[Ferrannini et al ., 2017& Karakelides et al ., 2018]**. For example,the effects of ageing that lead to increased insulin resistance are initially related with the increase adiposity and reduced muscle mass and function (sarcopenia) that are common in older adults, which can be worsened by a sedentary life style **Figure (1-3)[Chia et al ., 2018**, **Amati et al .,2016 & Shou et al .,2020]**. Increased adiposity in older adults usually involved an absolute or relative

excess in visceral adipose tissue depots compared with subcutaneous adipose tissue, which is often decreased **[Buffa et al .,2021& Kyrou et al .,2023]**.

Moreover, ageing is related with ectopic deposition of lipids in the liver as well as with intracellular lipids and extra adipose tissue in cardiac and skeletal muscles **[Kyrou et al ., 2023 & Al-sofiani et al ., 2019]**. These changes further increase the risk of insulin resistance, with intramuscular adipose tissue being a key factor contributing to insulin resistance in lean older people **[Shou et al ., 2019 & Alsofiani et al ., 2019]**.

As well as, unfavourable age-linked changes in body composition can be exacerbated by physical inactivity and poor dietary habits as well as by the effects of comorbidities and their medication **[Tsia et al ., 2015** & **Conn et al ., 2016]** increased visceral and ectopic (intramuscular and hepatic). Adiposity reduces insulin sensitivity by producing the a dipokines and cytokines that distrupt the pathways of necrosis factor, and low-grade inflammatory factors such as C-reactive protein **[Peterson et al ., 2021]**. Furthermore, both ageing and obesity are related with the excess

Production of pro-inflammatory cytokines From adipose tissue **[Mancuso et al ., 2019]**. As well as, both ageing and obesity are linked with an increased population of Macrophages within adipose tissue with an increased population of Macrophages within adipose tissue.

A reduced number of regulatory T cells and a decreased self-renewal of Mesenchymal progenitor stem cells, thereby promoting Metabolic dysregulation and inflammation **[Martyniak et al ., 2018].** Impeded nutrient metabolism and an age-linked decrease in mitochondrial function

also related ageing and insulin resistance, although the mechanistic details remain to be elucidate **[Lee et al ., 2017, Peterson et al ., 2021 & Peterson et al ., 2023].**

Age-related blunting of insulin-mediated glucose Uptake is associated with the progressive deterioration of the structure and function of skeletal muscles. Specific age-linked changes include a decreased skeletal muscle mass with smaller and fewer type II fibres.

In addition a reduced density of capillaries in skeletal muscle **[Greon et al ., 2016 & Ahima et al ., 2018]** Underlying mechanisms involded mitochondrial dysfunction, reduced low-grade inflammation, intramyocellular lipid accumulation and oxidative stress as well As the accumulation of senescent cells and shortage in autophagic capacity and enzymatic activity **[Shou et al ., 2020, Crescioli et al .,2020 & Jiao et al .,2017].** During skeletal muscle ageing, pro-inflammatory pathways become activated. Furthermore, the number of Mitochondria is decreased and their oxidative capacity is reduced due to the decrease activity of antioxidant enzymes, which leads to the intracellular accumulation of reactive oxygen species and excess levels of oxidative stress in skeletal muscle.**[Shou et al ., 2020 & Crescioli et al ., 2020]** . Although the complete spectrum of the underlying mechanisms has not been fully elucidated, all of the processes that characterize skeletal muscle ageing induce insulin resistance and, accordingly, excess the risk of T2DM **[Shou et al ., 2020& Crescioli et al ., 2020]**.

Evidence suggests that a relationship finds between ageing and T2DM at a biological level a number of studies in humans have demonstrated that both diabetes mellitus and ageing shorten telomere length **[Wang et**

al ., 2016] Figure (1-3). And that T2DM induces premature cellular senescence. **[Burton et al .,2018]**. However, the nature of this relationship requires further study to understand if the biological processes included in ageing drive T2DM pathology or if diabetes excess the rate of biological ageing. Ageing can indirectly increase insulin resistance and precipitate T2DM through several comorbidities that are predominant among older adults, notably vascular diseases, chronic stress and poor psychological health **[Lee et al ., 2017, Tsai and Lee et al ., 2015& Kyrou et al ., 2023]** those who have some comorbidities and mild disabilities. Finally, the third group involved those who have a high number of comorbidities and/or dis abilities and a shorter life expectancy (for example, <5 years) **[Munshi et al ., 2020]**. These factors can modify the disease process contrast to that in younger adults and therefore affect the management of both T2DM an any comorbidities **[Godino et al ., 2017]**. Several common clinical aspects are considered here, namely the specific care needs relating to frailty and sarcopenia, multimorbidity, and the susceptibility to hypoglycaemia as in the following figure (1-3).

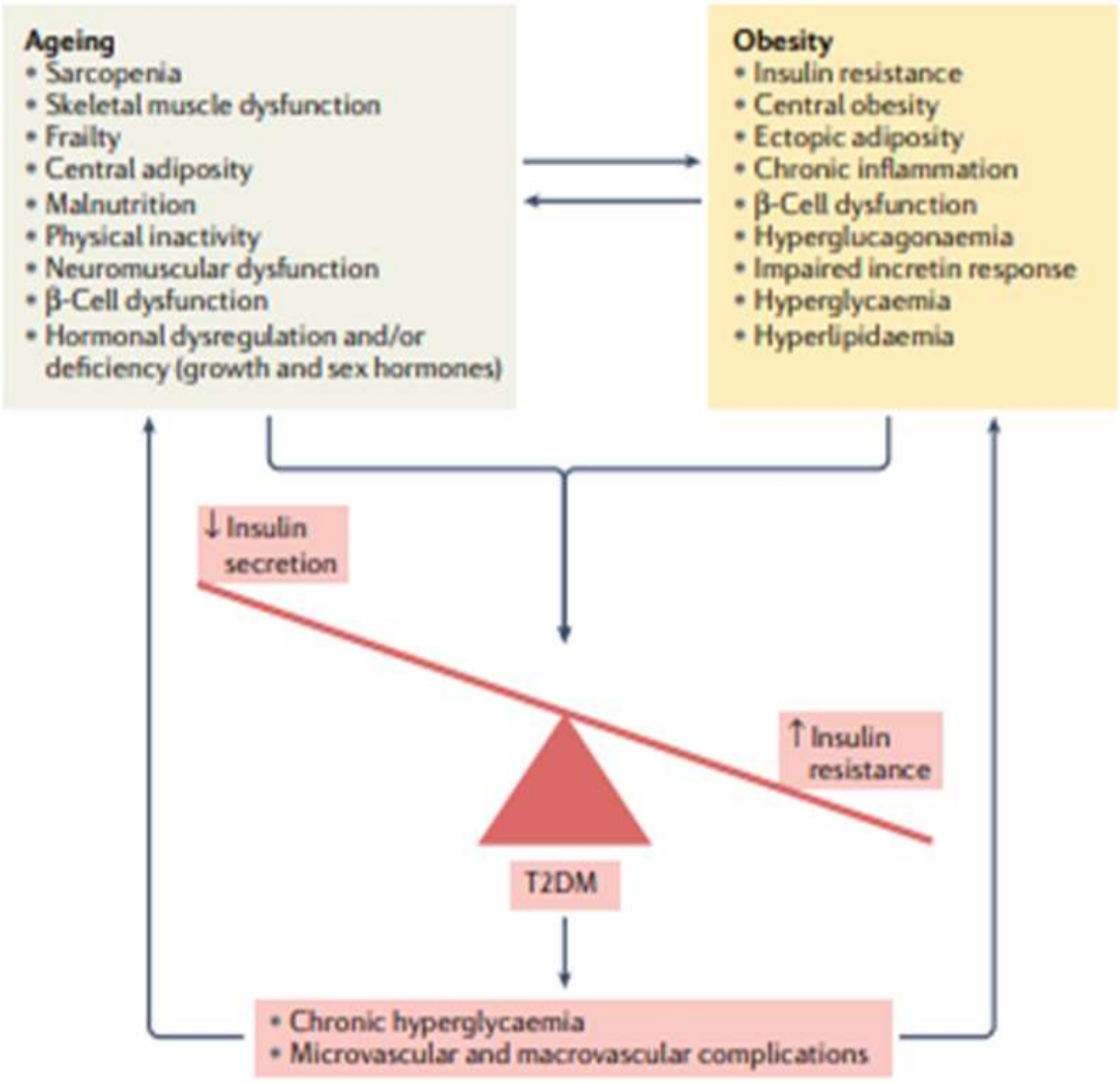


Figure (1.3) Pathophysiological Links between Ageing, Obesity and T2DM [ADA, 2021].

Type 2 diabetes mellitus (T2DM) with overt chronic hyperglycaemia typically represents the result of an imbalance between excess insulin resistance and the deterioration of insulin secretory function**[Sinclair et al**

.,2023] a combination of potential contributing factors due to both ageing and obesity can directly lead to this imbalance, which results in the development and progressive worsening of T2DM. as well as, obesity-linked factors and hyperglycaemia can also contribute to premature or accelerated biological ageing **[center for disease control,2020]** Furthermore, ageing via cellular senescence and dysfunction in various organs or tissues (for example, adipose tissue, skeletal muscles and pancreas) might heighten and/or accelerate the pathophysiological outcome of excess adiposity, particularly of ectopic adiposity and central obesity **[Cruz et al ., 2022]**. Excess insulin resistance and the activation of pro inflammatory pathways in both adipose tissue and skeletal muscles, skeletal muscle loss (sarcopenia) and dysfunction (for example, mitochondrial dysfunction, accumulation of reactive oxygen species and increased oxidative stress levels in skeletal muscles), and pancreatic β-cell dysfunction (for example, decreased insulin secretion due to glucotoxicity, lipotoxicity and/or β-cell senescence) are key parameters in the pathophysiology of this vicious cycle . As T2DM progresses overtime, an increasing disease burden in older adults from chronic hyperglycaemia, macrovascular and/or microvascular complications, and co-morbidity can further promote the adverse effects of the risk factors associated to ageing and/or obesity**[Rodriguez et al ., 2024]**.

1.1.9. Complications of T2DM

1.1.9.1. Acute Complications

1-Diabetic Ketoacidosis:

It is a grave condition which can progress to diabetic shock or even death If the glucose does not get to inside the cells, it starts burning fat for energy production generating ketones. The body can be intoxicated by increased amounts of ketones leading to develop of DKA. Each T2DM patient may development DKA but its less common in T2DM **[Umpierrez et al ., 2016]**.

2- Hyperosmolar Hyperglycemic State (HHS)

In patients with diagnostic parameters of glucose levels >600 mg/dl and raised plasma osmolality >320 mosm/kg, without ketoacidosis, acute hyperglycemic hyperosmolar status is considered **[umpierrez et al .,2016]**. The prevalence of HHS in patients with diabetes is estimated at <1% of their hospitalizations and this is 10-20 % mortality [**steenkamp et al ., 2022]**.

3-Hypoglycemia

This is probably the most common cause of coma seen in diabetic patients. Hypoglycemia is most commonly caused by accident over a dminstration of insulin or sulphonyureas or meglitinides. Precipitating causes .include too high adose of insulin or hypoglycemic drug conversely the patient may have lost ameal or taken excessive exercise after the usual a dose of insulin or oral hypoglycemic drugs**[Haghnazari et al ., 2021].**

1.1.9.2 .Chronic Complications

1.1.9.2.1. Microvascular Complications

1-Diabetic Retinopathy (DR)

This microvascular complication is common with DM, which is really the main cause of blindness and vision disability .The worldwide prevalence of DR is measured at nearly 93 million **[Yau et al ., 2016 &WHO/diabetic-retinopathy, 2020]**. This complication , can be determined after five years of occurring by diabetes, and after 20, years of diabetes roughly all cases show different stages of retinopathy [**Solomon et al.,2017]**. The main risk factors , for DR (rise in DM duration, the control blood glucose is ineffective, ineffective control of blood pressure and BMI)**[Thomas et al ., 2021]**.

2-Diabetic Nephropathy (DN)

Kidney damage done by chronic hyperglycemia, and is a substantial complication of diabetes that affects up to 50% of patients **[YANG et al., 2017& Merjaaneh et al ., 2017]**. Generally, DN has been one of the world's leading causes of progressive kidney disease, and one of the main complications of morbidity and fatality among patients with T2DM **[Singh et al ., 2022]**.

Several of the biological processes at molecular level, such glomerular cell death , metabolic replenishment, and interstitial fibrosis, are affected by the elevation glucose **[Gillbert et al ., 2019]**. This kidney damage is reversible mostly during preliminary stage but becomes irreversible when a reverse nephropathy progresses to final diseases **[Nielson et al.,2023, Ye et al ., 2021&Callaghan et al ., 2020]**.

3-Diabetic Neuropathy

In people with type 1 diabetes, glycemic management is effective in averting neuropathy, but not in people with T2DM. It is the presence of peripheral nerve disease symptoms, and/or signs in diabetic patients with the exception of other factors **[Boulton et al.,2016 & Papanas et al ., 2015]**. Some of studies showed that in hospitalized diabetic patients , the worldwide published of diabetes neuropathy stands at approximately 30% and in community diabetic disturbances 20 to 30% **[Chatterjee et al.,2015 & WHO/CVD, 2021]**.

4-Diabetic Angiopathy

This defect involves damadge to the cells in the blood vessels caused by high levels of glucose. this usually presents as diabetic retinopathy or diabetic nephropathy damadge to the eyes or kidneys. **[Luqk et al ., 2017]**.

1.1.9.2.2. Macrovascular complications

1- Cardiovascular Disease (CVD)

The name of CVD is used to describe for determination of cardiovascular diseases and vessel disturbances, involving coronary heart, disease (heart, attack) and Hypertension (high pressure of blood) **[Yahagi et al.,2017&WHO/CVD,2021]**. Diabetes can cause heart disease by many possible pathophysiological pathways particularly in T2DM patients. Generally, CVD incidence may be risen in association with hypertension and dyslipidemias **[Fowkes et al.,2022]**. Accumulation of these multiple effects contributes decreased cellular function,pro-

inflammatory reaction amplification, endothelial cell apoptosis and general cardiovascular dysfunction **[Moxon et al.,2015]**.

2-Peripheral Artery Vascular Disease (PAD)

It is a vast term that includes a wide range of a therosclerotic and aneurysmal diseases in the extra-coronary circulation **[Heart disease and stroke statistics , 2019 & Jude et al.,2023]**. Decreased blood flow, narrower artery and plaque formation are the major differences between a normal arterial and an atherosclerotic artery **[Kohan Aimee et al.,2023]**. The frequency of PAD in people with diabetes is 20-30 % compared to 45 % after 20 years the diagnosis of DM [**Akalu et al.,2020]**. Cerebrovascular disease in the form of stroke or transient ischemic attacks **[Rojas et al.,2022]**.

1.1.10. Diagnostic Tests for Diabetes Mellitus

Diabetes mellitus is diagnosis according to the following criteria. According to World Health Organization (WHO) recommendation by utilizing this testing fasting blood sugar(FBS) and HbA1c are more easiness and potent simpler and method for diagnoses T2DM and pre-diabetes.

1-Fasting Blood Sugar (FBS)

It is a test measures sugar (glucose) in the blood . It a simple, safe and common way to diagnose prediabetes ,diabetes ,gestational diabetes. and non diabetes. A healthcare provider will prick a finger or use a needle to draw blood from a vein in the arm of the body .we should not eat or drink anything (except water) which can be between (8-12)

hours before the test (FBS) ≥ 7.0 Mm / r 126m /dl. As displayed in Table **(1.3) [Ding et al.,2017]**.

2-Glycated Hemoglobin (HbA1c%)

The Hemoglobin is the red blood cell protein that transport oxygen from lungs to the tissues and the main component of red blood cell life of red blood cell averages some 120 days. Glycated hemoglobin can be utilized as an indication of the average glucose level over the previous one or two months, molecule of hemoglobin is made up of 4 protein chains 2 alpha and 2 beta chains glucose will react and bound to certain positively charged chemical group on the hemoglobin these are located at the start (N-terminal end) of each the alpha and beta chains and on some amino acid side chains within the protein. Glucose reacts with the ε-amino group of lysine amino acid. **[Owen, 2022]**. The glycation process is irreversible the accumulation of HbA1c within red cell reflects the average glucose concentration over the 3 months. The life of a red cell the main effect on the HbA1c % level is weighted to the most recent 2-4 weeks, and in pregnancy HbA1c% are lower due to the physiological changes therefore diagnosis of gestational diabetes will continue to be done by the OGTT and HbA1c % testing is not suitable **[Kanowski et al.,2021]**. And the elevation of 1% in HbA1c% corresponds to approximate average excess of 36 mg /dl in blood glucose . HbA1c% test is recommended for monitoring blood sugar control in diabetic patients. generally this test is used to diagnose prediabetes , diabetes, gestational diabetes and non diabetes. **[Ahmed et al.,2023 & Diagnosis and classification of diabetes mellitus,2022]**.

3-Oral Glucose Tolerance Test (OGTT)

Defined as a test of the body's ability to metabolize glucose that includes the administration of a measured dose of glucose to the fasting state and the delimitation of glucose levels in the blood and urine at measured intervals thereafter and that is utilized specially to detect diabetes mellitus .generally this test is used to diagnose prediabetes ,diabetes, gestational diabetes and non diabetes. **[Khan HA et al.,2018 , Defronzo ralf A et al ., 2023 & Soni HP et al ., 2021]**.

Table (1.2): - Measures for Diagnosis of Diabetes.

[Soni HP et al.,2021].

Condition	HbA1c	FBS (mg/dl)	GTT mg/dl
Normal	<5.6	(<100)	(<140)
Impaired fasting glucose	5.7-6.4	(100-125)	(140-199)
Impaired glucose tolerance	5.7-6.4	(125>100)	(140-199)
Diabetes mellitus	≥6.5	≥(126)	(≥200)

*HBA1C: - hemoglobin A1C, FBS: - fasting blood sugar, GTT: - Glucose tolerance test

4-Fasting Insulin Test (FIT)

It is a test that calculates amount of insulin in the blood sample. This test take after fasting which can be between (8-12) hours and also used to diagnose prediabetes, diabetes, gestational diabetes and non diabetes which acts side by side with another tests such as (FBS),(HbA1C%) and (OGTT). Insulin is a polypeptides consist of 51 amino acids and comprised of Chain A (21 aa) and Chain B (30 aa) **[Seong et al .,2019]**. It is synthesized by pancreatic beta cell as a single chain of 110 amino acids known as preproinsulin, Preproinsulin drops the amino terminal-end signal peptide which results in proteolytic enzymes cause constituting proinsulin. The result of a splitting of a fragment of internal proinsulin (C-peptide) leads to insulin is combined with two chains (α, β) linked two by disulfide bridges and a single third intra-bridge of the alpha chain, as displayed in **Figure (1-4) [Joshi et al.,2017]** . Half-life of insulin is brief (4 to 6 min) **[Steiner et al.,2018]** . Insulin enters the target cell, it connects a receptor upon on surface of cells with intrinsic tyrosine kinase activity. It the α-subunit which cause phosphorylation of insulin receptor contributes to protein activation in different ways **[Salltiel et al.,2021 & Kohn Aimee et al.,2023]**.

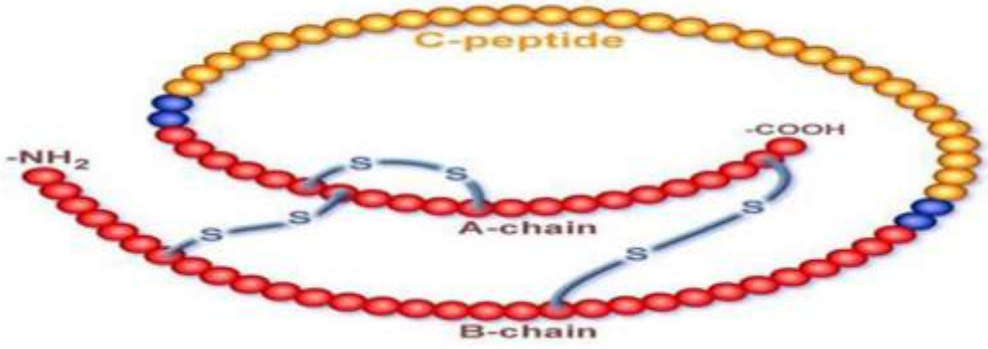


Figure (1-4) Structure of Insulin [Berbudi et al,2020]

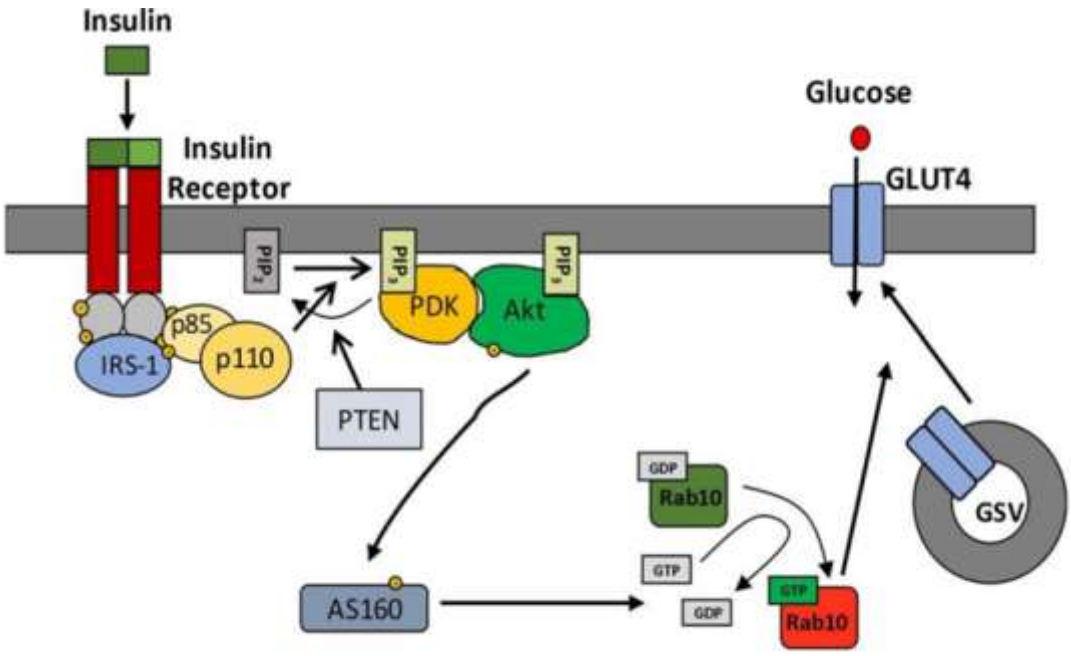


Figure (1.5): Schematic of The Insulin Signalling Pathway and Insulin Resistance [Carmichael et al.,2019].

The insulin receptor is a receptor tyrosine kinase, which undergoes dimerization and autophosphorylation on insulin binding. The phosphorylated receptor conscript and phosphorylate the insulin receptor substrate 1 (IRS-1) on tyrosine residues, which then conscript dimeric PI3 kinase via SH2 domains on the p85 subunit. PI3 kinase catalyses the phosphorylation of phosphatidylinositol bisphosphate (PIP2) at the plasma membrane to PIP3 (reversed by PTEN**)[Doody et al.,2021]** which then recruits PIP3-dependent kinas (PDK) and Akt, allowing PDK to phosphorylate and activate Akt. Activated Akt phosphorylates and inactivates the Rab10 GAP, AS160, allowing fixed Rab10 activation which plays a critical role in trafficking of GLUT4 storage vesicles(GSVs)to the plasma membrane and surface expression of

GLUT4. High levels of `free fatty acids lead to an accumulation of lipid derived second messengers,e.g. diacyglycerol and ceramide which can inhibit the pathway at several different phases **[Carmichael et al.,2019]**.

1.2. Biomarkers

1.2.1. Leucine Rich Alpha-2 Glycoprotein1(LRG1)

LRG1 was first isolated from human serum in 1977 **[Haupt et al.,2017]** and its amino acid sequence was determined in 1985**[Takahashi et al.,2018]** of the family of leucine-rich repeat (LRR). This protein found in many organ cells such as kidney, heart, retina and lung, when glucose levels rise in these organs. This lead to release of the protein from organ cells into the blood **[Ferreira.,2019 & Sharma.,2020].** LRG1 is consist of a single polypeptide chain of 312 amino acid residues and contains 8 LRRs. LRRs are protein–ligand interaction motifs, Each LRR consists of 19–29 amino acids, comprising a well-conserved N terminal stretch of 9–12 amino acids, which is rich in the hydrophobic amino acid leucine, and a C-terminal domain that varies in length, sequence, and structure. Multiple repeats are typically arranged together to form a horseshoe shaped solenoid protein domain with a concave surface providing a platform for protein–protein interactions **[Jemmerson et al.,2021 & Javidet al.,2023]**. The negatively charged leucine-rich N-terminal stretches of the repeats form β-strands located towards the inside of the horseshoe shaped domain **[Dolan et al.,2022]** and represent ideal binding sites for cationic proteins such as TGFβ **[Jemmerson et al.,2022 & Xavier et al., 2016]** Although its crystal structure has not yet been reported, LRG1 has been predicted to contain a leucine rich C-terminal domain (LRC) connected to the LRRs by several loops **[Wang et al.,2019]**. LRG1 is a

glycoprotein with a carbohydrate content of 23% **[Kobe et al.,2021]** and predicted to contain 5 glycosylation sites **[Kallenberg et al ., 2020]** . Indeed, several authors have shown that the exact molecular weight of LRG1 varies due to differences in glycosylation **[Druhang et al.,2017& Kumagai et al.,2016]**. Deglycosylated LRG1 has a molecular weight of about 34–36 kDa, whereas glycosylated LRG1 can reach up to 55–60 kDa. It has been shown that neutrophil-derived LRG1 is glycosylated diferently from serum LRG1 **[Druhang et al.,2017]**, LRG1 is regulated in vivo, nor what impact differential glycosylation patterns may have on function. However, LRG1 from serum samples of pancreatic **[Patwa et al.,2015]** and colorectal cancer patients **[Shinozaki et al.,2023]** shows aberrant glycosylation patterns with regards to content of mannose, fructose and sialic acid suggesting that alterations in sugar chains may influence LRG1 function in cancer. LRG1 is synthesized by hepatocytes ,brown adipocyte and neutrophil **[ODonnell et al .,2022].** LRG1 a circulating protein characterized by marked periodicity in leucine residues, which enables its interaction with other proteins **[Takahashi et al ., 2017]**,has been identified as a modulator of the transforming growth factor-b (TGF-b) signaling pathway **[Wang et al.,2020]** LRG1 binds directly to TGF-b accessory receptor endo glinand enhances activation of downstream signaling pathways, including smad1/5/8, which plays pivotal roles in cellular processes such as fibrosis and angiogenesis **[Wang et al.,2020 & Honda et al.,2019]**. In addition, LRG1 has been considered as a potential acute-phase protein because it is regulated by mediators of acute-phase response, such as interleukin-6 **[Shirai et al.,2018]**. In clinical studies, plasma LRG1 has been associated with pathogenesis of several diseases, including cancer **[Zhong et al.,2019 & Wang et al.,2015],** inflammatory disease **[Serada et al.,2022 & Serada et al.,2023]**, chronic diseases ,obesity and neurodegenerative disease **[Miyajima**

et al.,2021]. we found that the level of plasma LRG1 predicted progressive kidney disease in individuals with type 2 diabetes, **[Hong et al.,2019 & Liujj et al.,2017]**. LRG1 is a protein that carries a negative charge, so it represents an ideal binding site for the positively charged transforming growth factor beta (TGFβ), this binding results in a defect in function of (TGFβ) which is responsible for the growth, regulation and specialization of (β cells) of the pancreas, which are responsible for insulin secretion. This leads to a decrease in insulin secretion and an increase in blood glucose levels **[Camilli et al ., 2022]**.

1.2.2. Insulin Like Growth Factor Binding Protein7 (IGFBP7)

IGFBP7 is a member of structurally homologous protein family which is same for insulin hormone where it is closely linked to insulin hormone.**[Chatterjee et al.,2018 & Tabaky et al.,2019]** and causes decreased insulin secretion and insulin resistance, which lead to increased aggravation of type 2 diabetes, in addition to decreased oxygen consumption and ATP production in the body **[Wendt et al ., 2020 , Sun et al ., 2023 & Rorsman et al ., 2018]**. IGFBP7 proteins are predominantly secreted by the liver and have been studied as regulators of IGF-1 availability and for their potential involvement in the development of metabolic disorders.**[Kaushel et al.,2016 & Heald et al ., 2022]** IGFBP7 stands apart from the other IGFBPs by exhibiting higher binding affinity for insulin than for IGF-1 or IGF-2 **[Yamanaka et al.,2017& Baxter et al.,2021]** and IGFBP7 has been shown to enhance the action of insulin at the insulin receptor in liver **[Morgantini et al.,2019]** as well as interacting with the IGF-1 receptor. **[Evdokimova et al.,2023]** Both IGF-1 and insulin receptor belong to the family of Receptor

tyrosine kinase (RTK**). [Demeyts et al.,2015]**. Interestingly, upregulation of the IGFBP7 gene has also been linked to b-cell maturation**.[Balboa et al.,2022]** Insulin resistance is associated with increased circulating levels of IGFBP7 in diabetic men **[Lobez et al.,2016]** and the IGFBP7 gene in whole blood samples displays differential DNA-methylation in men recently diagnosed with type 2 diabetes **[Gu et al.,2015].** IGFBP7 together with tissue metalloproteinase 2 (TIMP2) has been extensively studied as a putative biomarker of kidney failure secondary to both diabetes and ischemia**,** In addition to, it is associated with pathogenesis of several diseases, including cancer , inflammatory disease, chronic diseases , obesity **[Watanabe et al.,2016 & Esmeijer et al.,2021]** and heart failure **[Zhang et al.,2022]**. IGFBP7 gene expression was overall elevated in islets from donors with type 2 diabetes **[Bascos et al.,2023]** This was true in both female and male donors although male donors had a higher IGFBP7 expression in general. However, IGFBP7 expression was unrelated to glycemic levels **[Hebacker et al.,2020]** as a correlation analysis between IGFBP7 and HbA1c in non-diabetic donors, with glycemic levels within the normal range, showed no association. Donors with type 2 diabetes were excluded from this analysis, as some of them were PrescribedHbA1c-lowering therapies**[Lu et al.,2017 & Zambelli et al .,2018].** Using a general linear model for assessing IGFBP7 and HbA1c correlation in non-diabetic donors with adjustments for age, sex and BMI, IGFBP7 was still not associated with HbA1c (p=0.424). Spearman correlation for age and IGFBP7 in all donors showed no significant association **[Edgar et al.,2021 & Zhou et al.,2023]**. Excess (IGFBP7) level have been found in the plasma of (T2DM) patients and described as statistically significant predictors of type 2 diabetes mellitus (T2DM) due to elevated of (IGFBP7) gene in islets of the pancreas for diabetic group more than control group as well as (IGFBP7)

reduced of insulin secretion through impaired P21-Activated kinase1(PAK1) function which participated in insulin secretion from pancreas cells. Serum (IGFBP7) levels are increases with increasing of insulin resistance for (T2DM) patients **[Westholm et al ., 2024]**.

1.3. Knowledge Gap of the study

Currently, biomarkers with low sensitivity that indicate the risk of rapid disease progression in individuals with type 2 diabetes mellitus (T2DM) to evaluate the disease are expensive, time consuming as well as sensitivity in the procedure pathway therefore, there is need to find a new biomarkers to measure indicators that can evaluate of disease progression such as (LRG1 and IGFBP7) which are considered in a previous study that have relationship with (T2DM) **[Wendit et al.,2020 & Ferreira et al.,2019].** In the present study we noticed possible use biomarkers (LRG1 and IGFBP7) to predict of (T2DM) progression because they are inexpensive, easy to measure and non sensitinve in procedure pathway.

Aims of the study

1-To evaluate serum levels of LRG1 and IGFBP7 in T2DM patients compared with healthy control.

2- To assess correlation between LRG1, IGFBP7 with diabetic parameters (FBS, HbA1c, insulin and lipid profile).

3- To assess association strong between LRG1, IGFBP7 in T2DM patients.

Chapter Two
Patients, Material and Method

2. Patients, Material and Method

2.1. Study Design

The current study is a case-control study conducted on 90 Iraqi participants for average age (20-70) years during the period between (November-2024) to (march-2025) , participants from (Imam hassan center for endocrinology and diabetes) in (Holy kerbala governorate). The practical part was, conducted at laboratories of department chemistry and biochemistry at College of medicine , Kerbala University, Iraq.

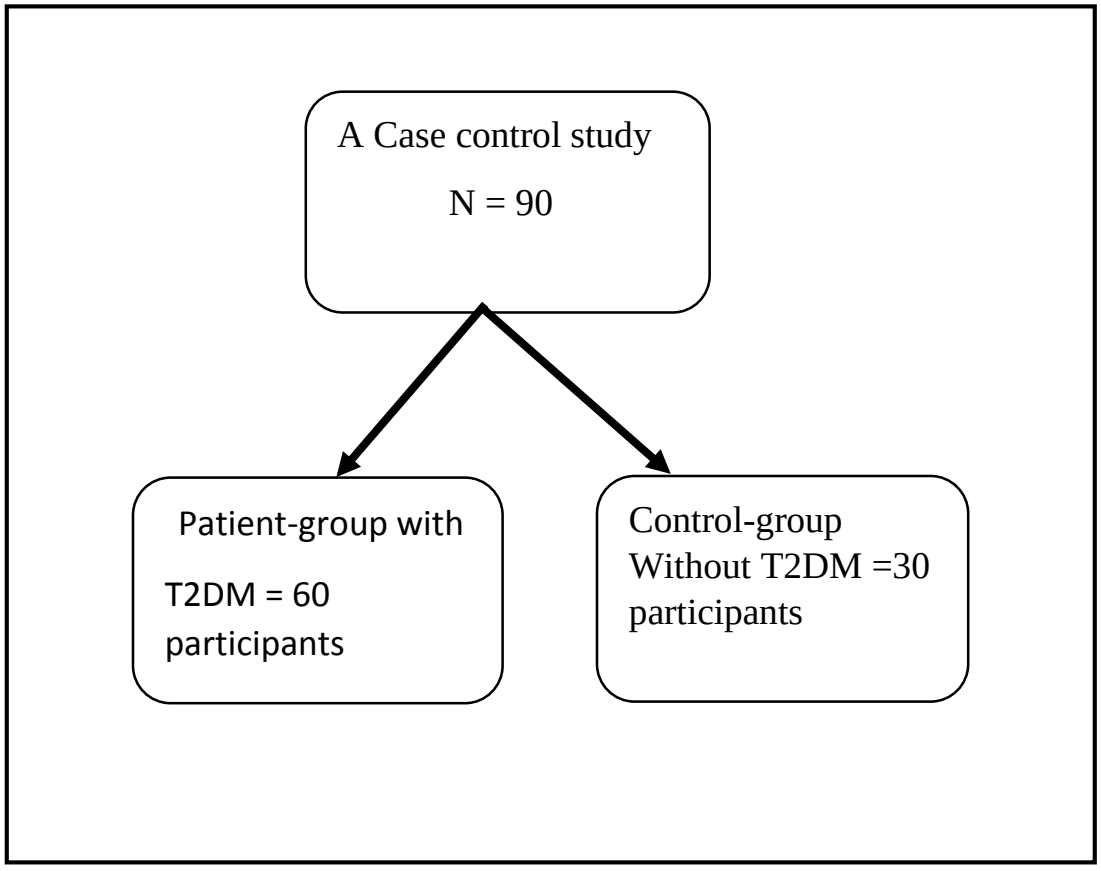


Figure (2-1) Study Design

2.1.1. Study Groups

A- Patients Group

Current study was carried out on 60 patients (30 males and 30 females) of average age (20-70) years with type 2 Diabetes and they were diagnosed by Consultant physician, according to their clinical signs, symptoms and laboratory tests (FBS, HbA1C).

B-Control Group

The control group included 30 volunteers (15males and 15 females) of average age (20-70) years. The control group have neither symptoms and nor signs of diabetes mellitus, so they were apparently healthy. The control group is tested for (FBS, HbA1C).

2.1.2. Inclusion and Exclusion Criteria

- **Inclusion Criteria:**

  Patient group was selected with type 2 diabetes mellitus and then selected control group after show normal results of (FBS and HbA1C).

- **Exclusion Criteria:**

  1. Type 1 Diabetes Mellitus
  2. Chronic Liver diseases
  3. Chronic Renal diseases
  4. Chronic Heart diseases
  5. Chronic Joints diseases
  6. Cancers
  7. Obesity

2.1.3. Collection of The Blood Samples

After a least 8 hours of fasting, blood was collected by the vein puncture with plastic disposable syringes took up to 5mL of venous blood from both control and patient group. Two ml were added to EDTA tube for detection of (HbA1C %) by (Latex turbidity technique), by using (Lifotronic H8) device while the remaining (3 mL) of the blood, is distributed into, gel tube, which, was then left at room temperature for 30 minutes, in order to initiate the, clotting process. The sample was then centrifuged to separate the serum at 3,000 ×g for (15) minutes. The serum were subdivided into aliquot for immediate glucose measurement by (Enzymatic colorimetric technique), by using (Auto-analyzer) device, and stored the leftover serum aliquot (250 µl) two Eppendorf tubes at (20 °c) until assayed concentrations for insulin by (Electrochemiluminescence immunoassay technique),by using cobas device, lipid profile by (Enzymetic colorimetric technique), by using (Auto-analyzer) device, LRG1 by (Competitive immunoassay technique), by using (ELISA) device and IGFBP7 by (Sandwich immunoassay technique), by using (ELISA) device.

2.1.4. Ethical Considerations

The ethical approvals were obtained from the ethical committee team, the college of medicine, the university of kerbala, and the kerbala Health Directorate / kerbala-Iraq by document number (3669) in (21/10/2024).

2.2. Material

2.2.1. Diagnostic kits

Diagnostic kits were used in the current study are describe below as shown in Table (2-1).

Table (2-1) Diagnostic Kits Used in The current Study

No	Kit-Diagnostic	Company	Country
1	Fasting glucose-Kit	GIESSE	Italy
2	HbA1c-Kit	GIESSE	Italy
3	Insulin-Kit	Roche	Germany
4	Total cholesterol-Kit	GIESSE	Italy
5	Triglyceride-Kit	GIESSE	Italy
6	HDL cholesterol-Kit	GIESSE	Italy
7	LDL cholesterol-Kit	GIESSE	Italy
8	LRG1-Kit	Bio-assay Technology laboratory	China
9	IGFBP7-Kit	Bio-assay Technology laboratory	China

2.2.2. Instruments and Equipment

The instruments and equipment were used in the current study are described below, as shown in Table (2.2).

Table (2-2) The instruments and Equipment Used in The current Study

No	Instruments and equipment	Company	country
1	Auto- analyzer (smart-120)	Geon-TEK	Canada
2	ELISA-reader	ELX800	USA
3	DEEP freezer	COOLTECH	USA
4	Centrifuge	Kokusan	Germany
5	Incubator	UKA	Germany
6	Shaker	Taiwan	Taiwan
7	Micropipettes	Bioasic	Canada
8	Filter paper	Slamed	Germany
9	Gloves	DIRUI	Germany
10	Syringe (5ml)	Mhco	China
11	Pipette	DARWEKLL	China
12	Eppendorf tubes	Mhco	China
13	EDTA-tubes	Mhco	China
14	GEL-tubes	Mhco	China
15	Cobas	Roche	Germany
16	Lifotronic H8	MEDI	China

2.3. Laboratory Methods

2.3.1. Measurement of Body Mass Index (BMI)

It is a calculation of the relationship between weight and height, measuring the weight in kilograms divided by the height in meters square (kg/m^2), Weight was measured in kilogram using medical weight scale supplied with the height in meter square to measure the body mass index (BMI).

BMI = Weight in kilogram / (height in meters)^2

Table (2-3) Classification of Body Mass Index (BMI)

[Hanson et al ., 2016]

BMI	Weight status
Below 18.5	Underweight
18.5 – 24.9	Normal
25.0 – 29.9	Overweight
30.0 and above	Obese

2.3.2. Measurement of Serum Glucose Concentration

Principle:

Glucose Oxidase(GOD) catalyses the oxidation of glucose to gluconic acid .The formed hydrogen peroxide (H2O2),it is detected by a chromogenic oxygen acceptor ,phenol–aminophenazone in the presence peroxidase(POD):

ß-D-Glucose + O_2 +H_2O ⟶ Gluconic acid +H_2O_2

H_2O_2+Phenol + Aminophenozone ⟶ Quinone +H_2O_2

The concentration intensity of the color formed is proportional to the glucose in the sample .

Normal values = (74 – 120) mg /dl

Reagents :

- Reagent 1 (Buffer): - Consist of 100 mmol/L of phosphate buffer pH7.5 and 0.75 mmol /L of Phenol.
- Reagent 2 (Enzymes): - Consist of ≥15 KU/Lof glucose oxidase, ≥1.5 KU/L of proxidase, and 0.25 mmol/l of 4-amino-antipyrine.
- Reagent 3 (Standard): - Consist of 100 mg/dL or 5.55 mmol/L of Glucose.

Preparation of The reagent A:

Working reagent A was prepared by adding the substance containing reagent 2 in the vial (enzymes) to the vial of reagent 1 (Buffer) to form mixture containing reagent 1 with reagent 2. To completed the dissolving of all components, the mixture was mixed gently to form reagent A.

Procedure:

wavelength: 510 nm (5oo-520)

Light path: 1cm

Temperature $37c^0$

Reading against blank reagent

Method Increasing End point

Sample/Reagent 1/100

Reagents	Blank	Sample	Standard
Reagent A	1000 µl	1000 µl	1000 µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mixed, incubated at $37c^0$ for 5 minutes, and read against blank reagent the absorbance of the sample (AX) and the standard (AS) at wavelength about (510) nm.

Calculation:

Glucose (mg/dl) = Abs(Assay)/Abs(standard) x standard

2.3.3. Measurement of (HbA1C %) Concentration

Principle:

The method utilized the interaction of the antigen and antibody directly determine the HbA1c % in the whole blood. Total hemoglobin and HbA1c % have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (Reagent B) latex-antibody complex is formed . Agglutination was formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody . The amount

of agglutination is Proportional to the amount of HbA1c absorbed on the surface of latex Particles. The amount of agglutination is measured as absorbance. The HbA1c values is obtained from the calibration curve.

Reagents:

REAGENT (A) Vol-30 ml	Latex o.13% ,Buffer,stabillizer
Reagent(B) Vol = 10ml	Mouse antihuman HbA1c monoclonal antibody 0.05 mg/dl Goat anti-mouse IgG polyclonal antibody 0.08mg/ml stabilizer
Reagent (c) Hemolysis Reagent Vol = 2x100ml	Water and stabilizers
Optional	Calibrator HbA1c-REF.6739 Control HbA1c-REF.6744

Normal values = (4.5 – 6.5) %

Procedure:

Wavelength: 640nm (630-660)

Light path: 1cm

Temperature: $37c^0$

Adjust the instrument to zero with distilled water

Reagent	Reagent(A)	Sample	Standard
Reagent (A)	360 µl		
Sample		10 µl	
Reagent (B)			120 µl

Mixed, incubated at $37c^0$ for (5) minute and read the absorbance of the sample After (5) minute of reagent (B) addition at wavelength about (640) nm

Calculation:

Plot(A) obtained against the HbA1c concentration of each calibrator (1-4) level. HbA1c percentage in the sample is calculated by interpolation of its (A) in the calibration curve.

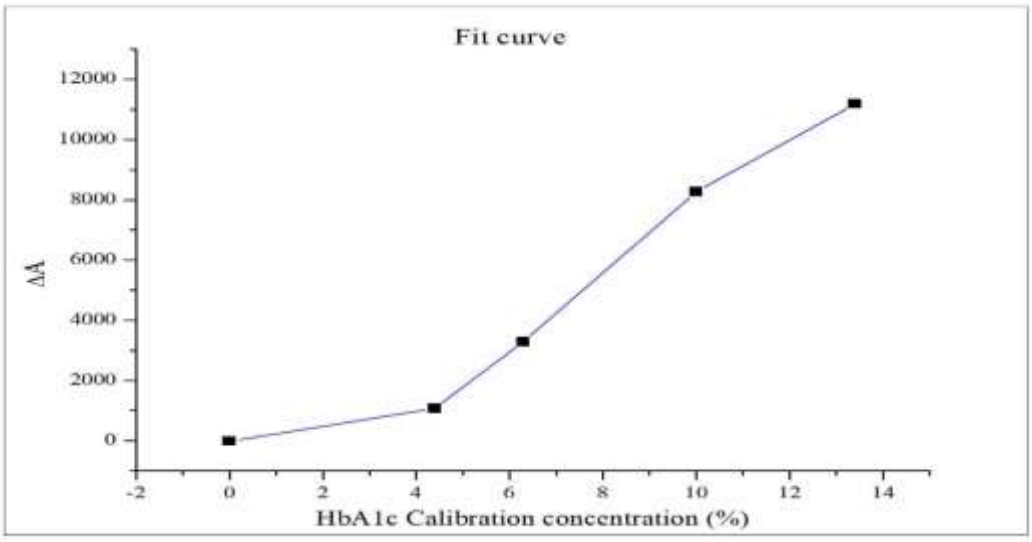
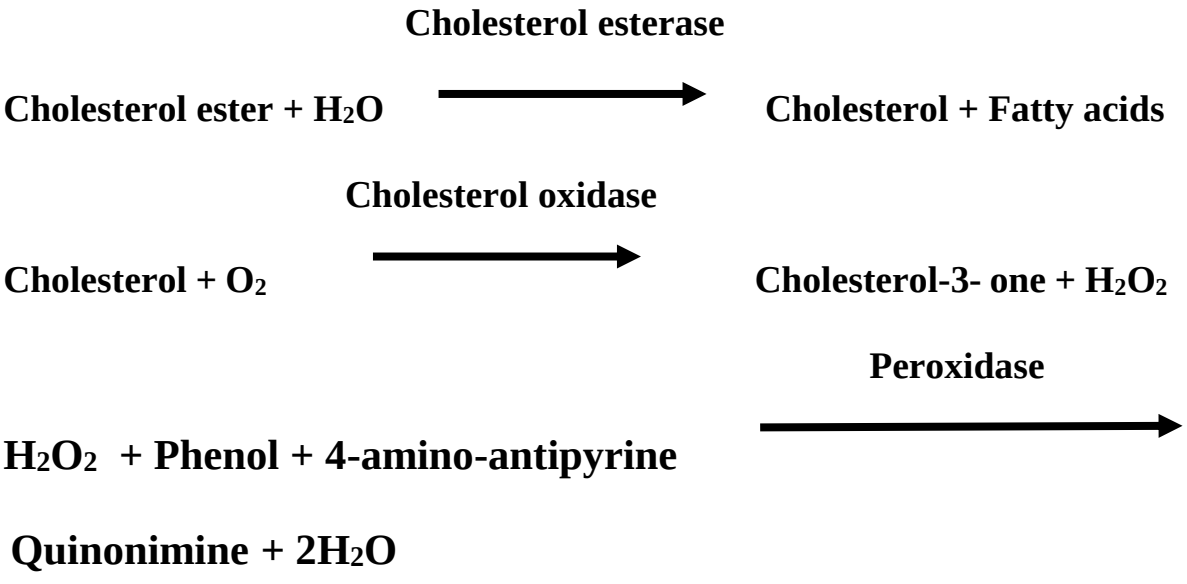


Figure (2-2) Calibration Curve for HbA1c Concentrations

2.3.4. Measurement of Serum Cholesterol Concentration

Principle :

Esterified cholesterol was hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CHE). Cholesterol oxidase (CHOD) oxidizes the free cholesterol into cholesterol-3-one with formation of hydrogen peroxide (H_2O_2). In presence of peroxidase (POD), hydrogen peroxide(H_2O_2) reacts with a derivative of phenol and 4-aminoantipyrine (4-AAP) to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the sample. The principle of determination of cholesterol is based on enzymatic hydrolysis according to the following reactions:



Reagents:

Reagent (A) Volume = 1000 ml	Buffer 4-AAP CHE CHOD POD Derivative of phenol	100 mmol/l 1 mmol/l 300 µ/l 300 µ/l 1500 µ/l 1 mmol/l
Standad Volume = 5 ml	Cholesterol Soduim azide	200 mg/dl 14 mmol/l

Normal values = (150 - 200) mg/dl

Procedure:

Wavelength 510 nm (500-520)

Light path 1cm

Temperature $37c^0$

Reading against blank reagent

Sample/Reagent 1/100

Reagents	**Blank**	**Sample**	**Standard**
Reagent (A)	1000 µl	1000 µl	1000 µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mixed, incubated at $37c^0$ for 5 minutes, and read against blank reagent the absorbance of the sample (Ax) and the standard (As) at the wavelength about (510).

Calculation:

Cholesterol mg/dl = Ax/As x 200(standard value).

2.3.5. Measurement of Serum Triglyceride (TG) Concentration

Principle:

Triglycerides were hydrolized by lipoproteinlipase (LPL) to produce glycerol and free fatty acid. The glycerol participates in a series of coupled enzymatic reactons, in which glycerol kinase (Gk) and glycerol phosphate oxidase (GPO) are involved and H_2O_2 is generated. The hydrogen peroxide reacts with TOOS and 4-AAP to form a colored complex. Whose color intensity is directly proportional to the concentration of triglycerides in the sample. The principle of determination of triglyceride is based on enzymatic hydrolysis according to

The following reactions:

$$\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Glycerol + Free Fatty acid}$$

$$\text{Glycerol + ATP} \underset{}{\overset{\text{Glycerol kinase}}{\rightleftharpoons}} \text{Glycerol – 3 – Phosphate + ADP}$$

Glycerol-3-phosphate oxidase

Glycerol – 3 – Phosphate + O_2 ⟶ Dihydroxyaceto-Phosphate + H_2O_2

H_2O_2 + Parachlorophenol + 4-aminoantipyrine ⟶

Quinonimine + $2H_2O$ + HCL

Note: ADP (Adenosine Diphosphate), ATP (Adenosine Triphosphate)

Reagents:

Reagent (A) Volume =1000 ml	Good buffer Magnesium chloride ATP 4-AAP TOOS LPL (lipoproteinase) POD (peroxidase) GK (glycerol kinase) GPO(glycerolphosphate oxidase)	100 mmol/l 15 mmol/l 4 mmol/l 1 mmol/l 0.1 mmol/l 2500 U/L 1800 U/L 1000 U/L 5500 U/L
Standard Volume = 10 ml	Glycerol	200 mg/dl (2.28mmol/l)

Normal values = (100 – 150) mg/ dl

Procedure:

Wavelength 546 nm (546 – 570)

Light path 1 cm

Temperature: $37c^0$

Reading against blank reaction

Method Increasing End Point

Sample/reagent 1/100

Reagents	**Blank**	**Sample**	**Standard**
Reagent (A)	1000 µl	1000 µl	1000 µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mixed, incubated at $37c^0$ for 5 minutes, read against blank reagent the absorbance of the sample (Ax) and the standard (As) at the wavelength about (546)nm.

Calculation:

Triglycerides mg/dl = Ax/As x 200 (standard value)

2.3.6.Measurement of Serum High Density Lipoprotein Cholesterol (HDL-C) Concentration

Principle:

Specific polianions in the first phase block the interfering lipoproteins (LDL, VLDL and chylomicron) and a specific surface – active agent inhibits the coloration of VLDL, LDL and chylomicron in the second phase. Reaction enzymes were (cholesterol oxidase, cholesterol esterase and peroxidase) interacted with (LDL-VLDL and chylomicron) and reduced them from the reaction . The intensity of color produced is directly proportional to the HDL cholesterol in the sample.

Reagents:

Reagent (A) Volume = 90 ml	Good Buffer Polianions 4-AAP	100 mmol/l 1 mmol/l 4 mmol/l
Reagent (B) Volume = 30 ml	Cholesterol esterase Cholesterol oxidase Peroxidase HDAOS Detergent	800 µ/l 500 µ/l 1500 µ/l 1 mmol/l 4 mmol/l

Normal values = (40 -130) mg/dl

Procedure:

Wavelength 600 nm

Lightpath 1 cm

Temperature $37c^0$

Reading against blank reagent

Method Increasing End point

Reagents	**Blank**	**Sample**	**Calibrator**
Reagent (A)	300 µl	300 µl	300 µl
Water	4 µl		
Sample		4 µl	
Reagent (B)			4 µl

Mixed, incubated at $37c^0$ for 5 minutes, read the absorbance of the sample (Ax) and the calibrator (Ac) against blank reagent at wavelength about 600 nm.

Calculation:

HDL (mg/dl) = (Ax – Abx) / (Ac –Abc) x Calibrator value

mg x 0.02586 = mmol /l (conversion factor)

2.3.7.Measurement of Serum Low Density Lipoprotein Cholesterol (LDL-C) Concentration.

Principle:

When a sample is mixed with reagent (A), the protecting reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase and cholesterol oxidase react with non-LDL lipoproteins (chylomicrons, VLDL and HDL). Hydrogen peroxide produced is decomposed by catalase. When reagent (B) was added, the protecting was reagent which is removed from LDL and catalase inactivated. In this second process, the enzymatic reactions conducted solely on the LDL fraction and the hydrogen peroxide produced yields a color complex upon oxidase condensation with HDAOS [N-(2-hydroxy-3-sulfopropyl)- 3,5-dimethoxyaniline] and 4-AAP in the presence of peroxidase colour intensity is directly proportional to the amount of LDL cholesterol in the sample.

Reagents:

Reagent (A) LDL Volume = 10 ml	Good Buffer HDAOS	20 Mm 1 mM
Reagent (B) LDL Volume = 10 ml	Good Buffer Cholesterol esterase Cholesterol oxidase Peroxidase 4-AAP	20 Mm 5.0 U/ml 1.0 U/ml 15 U/ML 3.0 U/ml

Normal values = (40-130) mg/dl

Procedure:

Wavelength 600 nm

Light path 1 cm

Temperature $37c^0$

Reading against blank reagent

Reagents	**Blank**	**Sample**	**Calibrator**
Reagent (A)	300 µl	300 µl	300 µl
Water	4 µl		
Sample		4 µl	
Reagent (B)			4 µl

Mixed, incubated at $37c^0$ for 5 minutes and read the absorbance of the sample(Ax) and the calibrator (Ac) against blank reagent at wavelength about (600) nm

Calculation:

LDL (mg/dl) = (Ax – Abx)/ (Ac – Abc) x Calibrator value.

And also can calculated of LDL cholesterol by using the following formula:

LDL (mg/dl) = cholesterol – (HDL+ $\frac{\boldsymbol{Triglycerides}}{\mathbf{5}}$).

2.3.8.Calculation of Serum Very Low Density Lipoprotein Cholesterol (VLDL- C) Concentration.

Serum very low density lipoprotein cholesterol (VLDL-C) can be determined by the following equation.

$$\text{VLDL-C (mg/dl)} = \frac{TG}{5}$$

Normal values = (2 – 30) mg/dl

2.3.9. Measurement of Serum Insulin Concentration.

Principle:

The insulin test was an electrochemiluminescence immunoassay. The sample (or calibrator/control), buffer, and magnetic microbeads coated with monoclonal antibody. Beta insulin, and ABEI was bead anti-insulin monoclonal antibody mixed well and incubated, forming a sandwich of immune complexes. After sedimentation in a magnetic field, the supernatant was decanted and then a wash cycle was performed. Next, I added Starter+2 to indicate the chemicals check. The optical signal was measured by a photomultiplier as relative optical units (RLUs), which were proportional to the insulin concentration present in the sample (or calibrator/control). Insulin is a peptide hormone secreted by the B cell of the pancreatic islets of langerhans and maintains normal blood glucose levels by facilitating cellular glucose uptake. Regulating carbohydrate, lipid and protein metabolism and promoting cell division and growth through its mitogenic effects in the human body.

Reagent

Reagents	**Preparation of reagents**
Streptavidin-coated microparticales Volume =5.8 ml	Streptavidin-coated microparticles (0.72 mg/ml) + (50 mmol/L) buffer
Anti-insulin-AB-biotin (R_1) Volume = 10.3	Biotinylated monoclonal anti-insulin antibody(1mg/L)+(50mmol/L) buffer
Anti-insulin-AB-Ru(bpy) (R_2) [Tris(2.2-bipyridyl)ruthenium(II) complex] Volume = 9.5 ml	Monoclonal anti-insulin antibody labeled with ruthenium complex (1.75 mg/L) +(50 mmol/L) buffer

Normal values = (2.6 – 25) µU/ml (Micro units per milliliter)

Procedure:

1. Took (12) µl of sample , added (50) µl of (anti- insulin-AB- biotin) (R_1) and then added (50) µl of [Tris (2.2-bipyridyl ruthenium(II)] (R_2) to form sandwich complex.

2. Mixed, incubated at $37c^0$ for (5) minutes.

3. Added (50) µl of streptavidin-coated microparticles to the mixture.

4. Mixed, incubated at (37) c^0 for (5) minutes and then read the results by instrument automatically.

Calculation:

The sample is calculated automatically by calculating the insulin concentration in each sample via a calibration curve generated by two points.

2.3.10.Measurement of Serum Leucine Rich Alpha-2-Glycoprotein 1 (LRG1) Concentraton

Principle:

The kit is an Enzyme-Linked ImmunoSorbent Assay (ELISA). Added sample to the pre-coated plate. Then added biotinylated antigen. The antigens in the samples compete with the biotinylated antigen to bind to the capture antibody and incubate. Unbound avidin-HRP is washed away during a washing step. TMB substrate is then added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of LRG1 in the sample. The concentration of LRG1 in the sample is then determined by comparing the optical density (OD) of the samples to the standard curve (competitive immunoassay technique).

Reagents and Materials

Components	Quantity
Pre-coated plate	12 * 8 well strips x 1
Human LRG1standard, lyophilized	2 vial
Standard/ Sample Diluent	6 ml x 1 Vial
Biotinylated antigen, lyophilized	1 vial
Avidin- HRP Concentrate	100 µl x1 vial
Biotinylated antigen Diluent	6 ml x 1 vial
Avidin HRP Diluent	5.9 ml x 1 vial
Substrate solution A	6 ml x 1 vial
Substrate solution B	6 ml x 1 vial

Stop solution	6 ml x 1vial
Wash buffer Concentrate(25x)	20 ml x 1 vial
Plate Sealer	2 pcs
Zipper Bag	1
User Instruction	1

Preparation of Reagents

- All reagents will be brought to room temperature before use
- Standard reconstitute one vial of standard with 150 µl of standard/sample Deluent to generate a 96 µg/ml standard stock solution which will be Used within 24 hours. Allowed the standard to sit for 15 minutes with gentle. agitation prior to making dilutions. Prepared duplicate or triplicate standard points by serialy diluting the standard stock solution 1:2 with diluent to produce 48 µg/ml, 12 µg/ml, 6µg/ml and 3µg/ml solutions Added Standard/Sample diluent only as the zero standard (0 µg/ml).

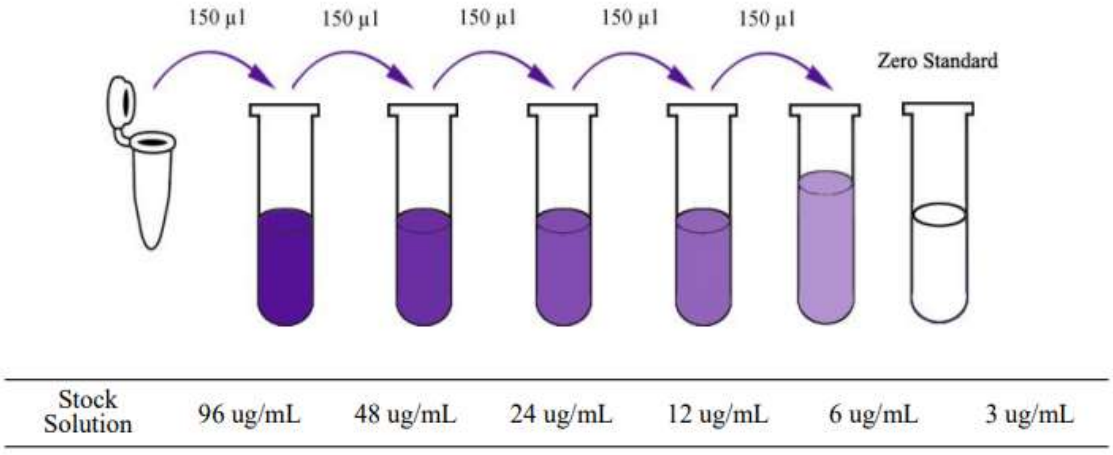


Figure (2-3) The Reagents Preparation for (LRG1) Concentrations

- Biotinylated Antigen Briefly centrifuged the biotinylated antigen vial then add 1ml Biotinylated Antigen Diluent to mixed well. And then pipetted all this solution back into the Biotinylated Antigen diluent vial to mixed well and generate a 6ml stock solution. Allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- Avidin-HRP Concentrate Briefly low- speed centrifuged the avidin-HRP Concentrates solution and then pipetted all avidin-HRP into the Avidin HRP Diluent vial. Mixed well to generate a 6ml stock solution. Allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- Washed Buffer Concentrate 25x Diluted 20ml of concentrated wash buffer with 480ml double distilled water to prepared 500 ml of wash buffer. If crystals have formed in the concentrate, warmed it in a 40°C water bath and mixed it gently until the crystals have completely dissolved.

Assay Procedure:

1. Prepared all reagents, standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay is performed at room temperature.

2. Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips will be stored at 2~8°C for up to one month.

3. Blank wells: Only added substrate solution A , substrate solution B and Stop solution as blank control.

4. Added 50 µl diluted standard to standard well, added 50 µl sample (Sample recommended dilution:2-5 times when necessary) to the sample well, and

added 50 µl biotinylated antigen to each well. Mixed well. Covered the plate with a sealer and incubated for 60 minutes at 37°C.

5. Removed the searler and the liquid in the well, washed five times with 300 µl wash buffer manually. Inverted the plate each time and decanted the contents hit 4-5 times on absorbent material to complete removed liquid. For automated washing, aspirated all wells and wash 5times with wash buffer. Blot the plate on absorbent material.

6. Added 50 µl avidin-HRP to the standard well and sample well, covered the plate with a sealer and incubated for 60 minutes at 37°C.

7. Removed the sealer and wash as described above.

8. Added 50 µl substrate solution A to each well and then added 50 µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

9. Added 50 µl Stop Solution to each well, the blue color will changed into yellow immediately.

10. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation Results

Averaged the duplicate readings for each standard, control, and sample. Created a standard curve by plotting the mean absorbance for each standard on the Y-axis against the target antigen concentration on the X-axis and drew a

best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the X axis versus the O.D. of the standards on the Y axis and the best fit line can be determined by regression analysis. The linear equation (X = Y+Calibration Value) can be used to calculate the standard curve where X is the log of the concentration of the standard and Y is the OD value of the standard. If samples have been diluted (2-5 times is recommended), the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.

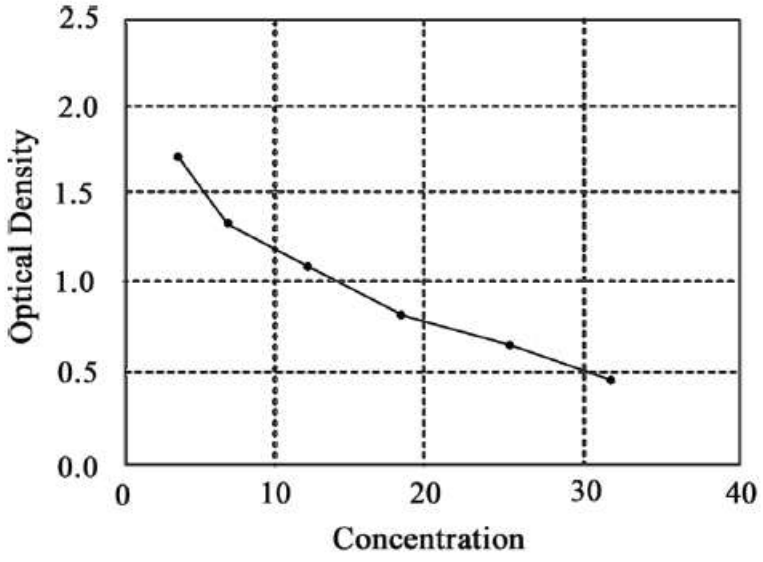


Figure (2-4) The Standard Curve for (LRG1) Concentrations

2.3.11.Measurement of Serum Insulin-Like Growth Factor – Binding Protein 7 (IGFBP7) Concentration.

Principle:

The kit an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IGFBP7 present in the sample is added and binds to antibodies coated on the wells and then biotinylated IGFBP7 antibody is added and binds to IGFBP7 in the sample. Then Streptavidin-HRP is added and bind to the Biotinylated IGFBP7 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IGFBP7. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The method (sandwitch immunoassay technique).

Reagents and Materials:

Component	**Quantity (96T)**	**Quantity (48T)**
Standard solution	0.5 ml x 1	0.5 ml x 1
Pre-coated ELISA plate	12 * 8 well strips x 1	12 * 4 well srips x 1
Standard diluent	3 ml x 1	3 ml x 1
Streptavidin-HRP	6 ml x 1	3 ml x 1
Stop solution	6 ml x 1	3 ml x 1
Substrate solution A	6 ml x 1	3 ml x 1
Substrate solution B	6 ml x 1	3 ml x 1
Wash buffer conc (25x)	20 ml x 1	20 ml x 1
Human IGFBP7 (AB)	1	1

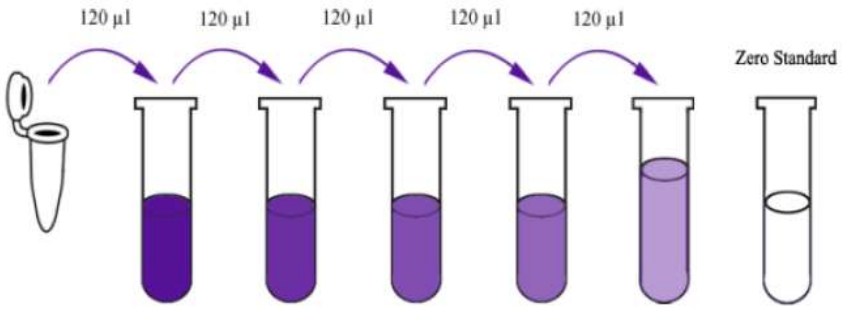
User instruction	2pcs	2pcs
Plate sealer	1	1

Preparation of Reagent:

• All reagents will be brought to room temperature before use.

• Standard Reconstitute the120 µl of the standard (24 ng/ml) with 120 µl of standard diluent to generate a 12 ng/ml standard stock solution. Allowed the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (12 ng/ml) 1:2 with standard diluent to produce 6ng/ml, 3ng/ml, 1.5ng/ml and 0.75 ng/ml solutions. Any remaining solution will be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

12 ng/ml	Standard NO.5	120 µl Original standard+120µl standard diluent
6 ng/ml	Standard NO.4	120 µl standard NO.5 + 120 µl standard diluent
3 ng/ml	Standard NO.3	120 µl standard NO.4 + 120UL standard diluent
1.5 ng/ml	Standard NO.2	120UL standard NO.3 + 120 µl standard diluent
0.75 ng/ml	Standard NO.1	120 µl standard NO.2 + 120 µl standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
24ng/ml	12ng/ml	6ng/ml	3ng/ml	1.5ng/ml	0.75ng/ml

Figure (2-5) The Reagents Preparation for (IGFBP7) Concentration

wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Assay Procedure:

1.Prepared all reagents, standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay is performed at room temperature.

2.Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C. 3.Add 50 µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4.Added 40 µl sample to sample wells and then add 10 µl Human IGFBP7 antibody to sample wells, then added 50 µl streptavidin-HRP to sample wells

and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.

5.Removed the sealer and wash the plate 5 times with wash buffer. Soaked wells with 300 µl wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated or decanted each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6.Added 50ul substrate solution A to each well and then added 50 µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

7.Added 50 µl Stop Solution to each well, the blue color will changed into yellow immediately.

8.Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result:

Constructed a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and drew a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Data:

The standard curve of E3857Hu is provided for demonstration only. A standard curve will be generated for each set of samples assayed.

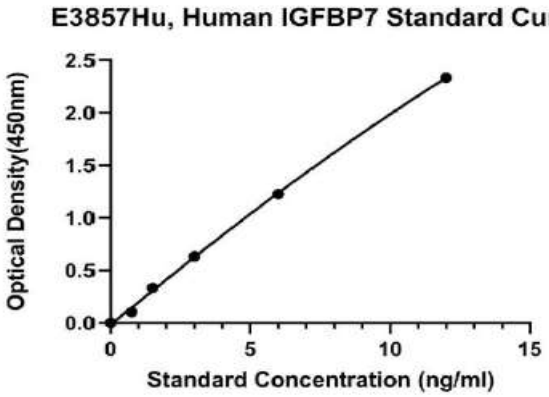


Figure (2-6) Standard Curve for (IGFBP7) Concentrations

2.4. Statistical Analysis

They were performed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were applied to summarize the data, with continuous variables expressed as mean ± standard deviation and categorical variables reported as frequencies (n) and percentages (%). The Kolmogorov-Smirnov test was used to assess data normality.

For inferential analysis, the two-independent-samples *t*-test was employed to compare continuous variables between groups. Correlations between biomarkers were evaluated using Pearson's correlation coefficient. Associations between variables were quantified using odds ratios (ORs) with 95% confidence intervals (CIs), derived from unconditional logistic regression. ANOVA test used to compare more than two groups.

Analytical tests identified significant differences in categorical variables across parameters, with a two-sided p-value < 0.05 considered statistically significant. Additionally, receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal threshold for critical Diabetics, balancing sensitivity and specificity.

Chapter Three

Results

3.Results

3.1. Demographic and Clinical Characteristics

The demographic and clinical characterstics of patients with diabetes mellitus type 2 (T2DM) and control groups were summarized in table (3-1). The table (3-1) presents the baseline characteristic of the study participants, comparing diabetic and non-diabetic groups. The diabetic group had the proportions of age as the following: from (20-30) year the proportion was 3 (5.0%), from (31-40) year, the proportion was15 (25.0%) , from (41-50) year, the proportion was 8 (13.3%),from (51-60) year, the proportion was 15 (25.0%) and (> 60) year, the proportion was 19 (31.7%). The non diabetic group showed the following age distribution from (20-30) year ,the proportion was 8 (26.7%), from (31- 40) year, the proportion was 8 (26.7%) , from (41-50) year , the proportion was (23.3%),from (51-60) year , the proportion was 5 (16.7%) and (<60) year, the proportion was 2 (6.7%). Both diabetic group and non diabetic group had equal sex distribution (50%) male and female (50%). The patients' BMI categories are distributed as following: 21 (35.0%) were classified having a normal BMI, 39 (65.0 %) were classified as having an overweight BMI , The control group consist of individuals the following proportions: 16 (53.3%) having a normal BMI, 14 (46.7%)

having an overweight BMI. The proportion of smoker patients in (diabetic group) was 28 (46.7), while the proportion of non smoker Patients in (diabetic group) was 32 (53.3%). The proportion of smoker individuals in (non diabetic group) was 12 (40 %),while the proportion of non smoker individuals in (non diabetic group) was 18 (60 %) in the current study. These are proportions found in the following table (3-1).

Table (3-1) Demographic and Clinical Characteristics

Characteristics		**Diabetic**	**Non-Diabetic**
Sex	Male	30 (50.0%)	15 (50.0%)
	Female	30 (50.0%)	15 (50.0%)
Age	20-30 years	3 (5.0%)	8 (26.7%)
	31-40 years	15 (25.0%)	8 (26.7%)
	41-50 years	8 (13.3%)	7 (23.3%)
	51-60 years	15 (25.0%)	5 (16.7%)
	>60 years	19 (31.7%)	2 (6.7%)
BMI	Normal weight	21 (35.0%)	16 (53.3%)
	Overweight	39 (65.0%)	14 (46.7%)
Smoking	No (%)	32 (53.3%)	18 (60%)
	Yes (%)	28 (46.7%)	12 (40%)

***Chi-square Test**

3.2. Distribution of Past Medical History

The current study highlights on the medical history differences between the study groups . The number of patients with family history of diabetes mellitus type 2 in diabetic group was 41(68.3 %) ,while non family history was 19 (31.7%). The number of individuals (non diabetic group) with family history was 13(43.3%), while non family history was 17 (56.7%), with p value (0.022), which was significant. The number of patients with hyperlipidemia of diabetes mellitus type 2 in diabetic group was 35 (58.3%), while non hyperlipidemia was 25 (41.7%). The number of individuals (non diabetic group) with hyperlipidemia was (16.7 %), while non hyperlipidemia was 25 (83.3 %) with p. value (< 0.001), which was significant. The number of Patients with hypertension of diabetes mellitus type 2 in diabetic group was 30 (50.0 %) , while non hypertension was 30 (50.0 %). The number of individuals (non diabetic group) with hypertension was 6 (20.0%) ,while non hypertension was 24 (80.0%) with p value (0.006), which was significant. The number of patients with physical activity of diabetes mellitus type 2 in diabetic group was 29 (48.3%), while non physical activity was 31(51.7%). The number of individuals (non diabetic group) with physical activity was 25 (83.3%), while non

physical activity was 5 (16.7%) with p value (0.001), which was significant. The proportions are found in the following table (3-2).

Table (3-2) Distribution of Past Medical History

Medical history		Diabetic	Non-Diabetic	P-value
Family history	No	19 (31.7%)	17 (56.7%)	0.022
	Yes	41 (68.3%)	13 (43.3%)	
Hyperlipidemia	No	25 (41.7%)	25 (83.3%)	<0.001
	Yes	35 (58.3%)	5 (16.7%)	
Hypertension	No	30 (50.0%)	24 (80.0 %)	0.006
	Yes	30 (50.0%)	6 (20.0%)	
Physical activity	No	31 (51.7%)	5 (16.7%)	0.001
	Yes	29 (48.3%)	25 (83.3%)	

***Chi-square Test, Differences are Significant at P-value< 0.05.**

The number of patients with treatment by (oral anti-diabetic drugs) of type 2 diabetes mellitus in diabetic group was 42 (70.0 %) while the number of patients with treatment by (insulin-injection) of type 2 diabetes mellitus in diabetic group was 18 (30.0%) .These are proportions can be represented in the following figure (3-1).

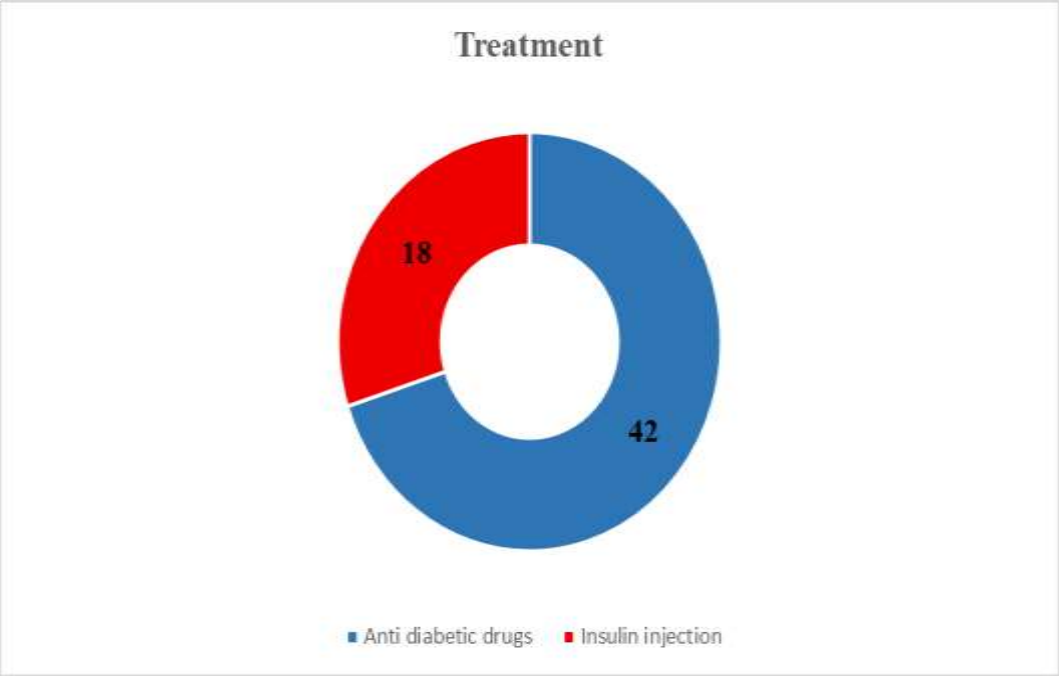


Figure (3.1) Distribution of Treatment Types

The number of patients with duration of type 2 diabetes mellitus (T2DM) from (1 - 5) year, the proportion was (22%), from (5 - 10) year, the proportion was (9%), from (10 - 15) year, the proportion was (14 %) and (> 15) year, the proportion was (15%). These are proportions can be represented in the following figure (3-2).

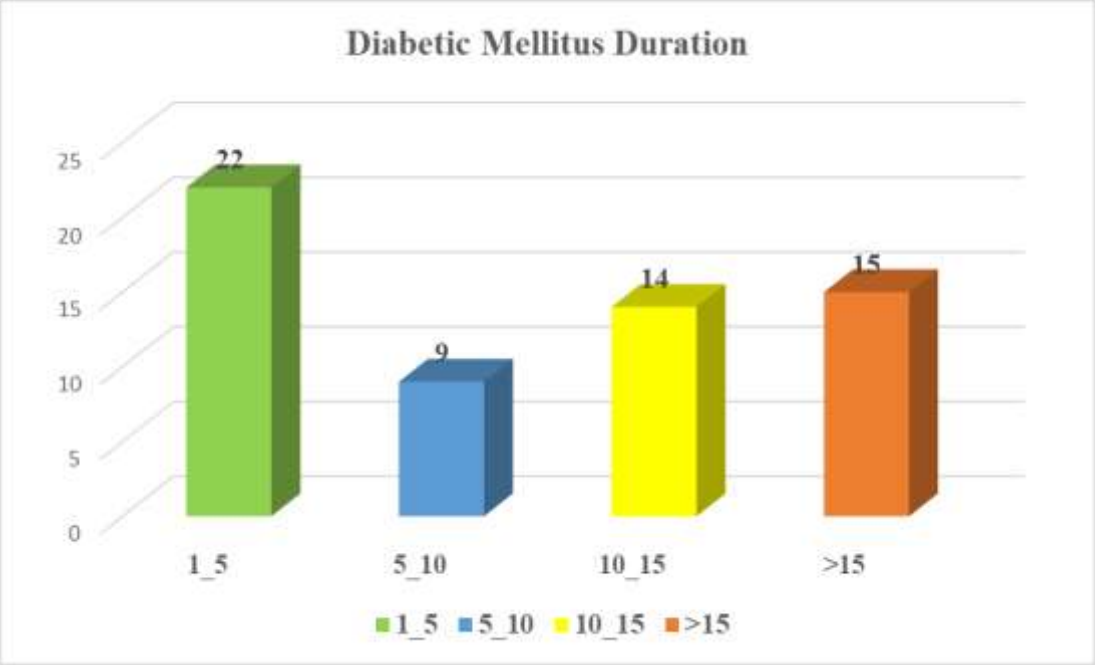


Figure (3-2) Distribution of Duration of Type 2 Diabetes Mellitus

3.3. Comparison of Routine Biomarkers in The study Groups

The current study routine biomarkers were compared between groups. Dabetic group had mean of fasting blood sugar (FBS) (208.51 ±93.61) mg/dl while non diabetic group had mean of fasting blood sugar (FBS) (88.28 ±7.54) mg/dl with p value (<0.001), which was significant. Diabetic group had mean of glycated hemoglobin (HbA1c) % (8.39±1.71) % , while non diabetic group had mean of glycated hemoglobin (HbA1c) % (5.01 ± 0.29) % with p value (<0.001),which was significant. Diabetic group had mean of insulin (14.76 ± 4.13)

µU/ml,while non diabetic group had mean of insulin (20.68±12.65)µU/ml with p value (0.001) , which was significant . Diabetic group had mean of cholesterol (207.80±49.28) mg/dl while non diabetic group had mean of cholesterol (140.87±11.19) mg/dl with p value (<0.001), which was significant . Diabetic group had mean of triglyceride (TG) (170.08±98.12) mg/dl, while non diabetic group had mean of triglyceride (TG) (125.20±7.26) mg/dl with p value (<0.014), which was significant. Diabetic group had mean of high density lipoprotein (HDL) (77.21±18.30) mg/dl, while non diabetic group had mean of high density lipoprotein (HDL) (95.02±10.89) mg/dl with p value (<0.001), the proportion was significan. Diabetic group had mean of low density lipoprotein (LDL) (88.79±23.76) mg/dl, while non diabetic group had mean of Low density lipoprotein (LDL) (69.05±10.14) mg/dl with value (<0.001), which was significant. Diabetic group had mean of very low density lipoprotein (VLDL) (33.95±19.65) mg/dl while non diabetic group had mean of very low density lipoprotein (VLDL) (25.04±1.45) mg/dl with p value (<0.015), which was significant. The proportions are found in the following table (3-3).

Table (3-3) Comparison of Routine Biomarkers in The Study Groups

Parameters	**Diabetic Mean ± SD(n=60)**	**Non-Diabetic Mean ± SD (n=30)**	**P-value**
FBS(mg/dl)	(208.51±93.61)	(88.28±7.54)	<0.001
HbA1C%	(8.39±1.71)	(5.01±0.29)	<0.001
Serum-insulin (µU/ml)	(14.76 ± 4.13)	(20.68 ± 12.65)	0.001
Cholesterol (mg/dl)	(207.80±49.28)	(140.87±11.19)	<0.001
TG (mg/dl)	(170.08±98.12)	(125.20±7.26)	<0.014
HDL (mg/dl)	(77.21±18.30)	(95.02±10.89)	<0.001
LDL(mg/dl)	(88.79±23.76)	(69.05±10.14)	<0.001
VLDL (mg/dl)	(33.95±19.65)	(25.04±1.45)	<0.015

*** n:Number of Patients, SD: Standard Deviation, Test: Two independent samples T.test, Differences are significant at P-value< 0.05.**

3.4. Comparison Mean Values of LRG1 and IGFBP7 between The Study Groups

The current study for two main biomarkers (LRG1) and (IGFBP7) in the comparison between two groups diabetic and non diabetic. The diabetic group had leucine rich alpha-2-glycoprotein-1 (LRG1) mean of (1.01±0.36) µg/ml, while non diabetic group had leucine rich alpha-2 glycoprotein-1 (LRG1) mean of (0.56±0.26) µg/ml with p.value (<0.001), which was significant. The diabetic group had insulin – like growth factor- Binding protein 7 (IGFBP7) mean of (1.09±0.33) ng/ml, while non diabetic group had insulin- like growth factor - binding protein 7 (IGFBP7) mean of (0.55±0.35) ng/ml with p value (<0.001) , which was significant. These are proportions found in the following table (3-4).

Table (3-4) Comparison Mean Values of LRG1 and IGFBP7 between The Study Groups

Biomarker	Diabetic Mean ± SD (n=60)	Non-Diabetic Mean ± SD (n=30)	P-value
LRG1(µg/ml)	(1.01±0.36)	(0.56±0.26)	<0.001
IGFBP7 (ng/ml)	(1.09±0.33)	(0.55±0.35)	<0.001

* **n: Number of Patients, SD: Standard Deviation, Test: Two independent samples T.test, Differences are significant at P-value< 0.05**

3.5. Comparison Mean Values of LRG1 and IGFBP7 According to Sex in Patients with Diabetes Mellitus

Table (3-5) showed that the mean of (LRG1) for male is (0.789±0.580) µg/ml and female is (0.896±0.551) µg/ml with p value (0.432), which was no significant. Table (3-5) showed also that the mean of (IGFBP7) for male is (0.878±0.472) ng/ml and female is (0.988±0.489) ng/ml with p value (0.325), which was non significant. The current study was (non significant) for both biomarkers. The proportions are found in the following table (5-3).

Table (3-5) Comparison Mean Values of LRG1 and IGFBP7 According to Sex in Patients with Diabetes Mellitus

Sex			
Biomarker	**Male Mean ± SD(n=30)**	**Female Mean ± SD(n=30)**	**P-value**
LRG1(µg/ml)	(0.789 ± 0.380)	(0.896 ± 0.351)	0.432
IGFBP7(ng/ml)	(0.878±0.372)	(0.988 ± 0.489)	0.425

*** n: Number of Patients, SD: Standard Deviation, Test: independent samples T.test, Differences are significant at P-value< 0.05**

3.6. Comparison Mean Values of LRG1 and IGFBP7 Accoding to Age in Patients with Diabetes Mellitus

Table (3-6) shown that the mean of (LRG1) for ages from (20- 40) year, the proportion was (0.323 ± 0.078) µg/ml, from (41-60) year, the proportion was (0.897 ± 0.286) µg/ml , and (> 60) year, the proportion was (1.272±0.324) µg/ml with p value (<0.001), which was significant. The table (3-6) shown also the mean of (IGFBP7) for ages from (20-40) year , the proportion was (0.419±0.139) ng/ml, from (41-60) year, the proportion was (0.977±0.288) ng/ml, and (> 60) year,

the proportion was (1.376±0.305) ng/m with p value (<0.001) which was significant. These are proportions elucidated that both LRG1 and IGFBP7 levels are elevated whenever the age is increase. The proportions are found in the following table (3-6).

Table (3.6). Comparison Mean Values of LRG1 and IGFBP7 According to Age in Patients with Diabetes Mellitus

Age				
Biomarker	**20-40 y N=18 Mean ± SD**	**41-60 y N=23 Mean ± SD**	**>60 y N=19 Mean ± SD**	**P-value**
LRG1 (µg/ml)	0.323±0.078	0.897±0.286	1.272±0.324	<0.001
IGFBP7 (ng/ml)	0.419±0.139	0.977±0.288	1.376±0.305	<0.001

*** n: Number of Patients , SD : Standard Deviation , Test : One way ANOVA , Differences are significant at P-value< 0.05**

3.7. Comparison Mean Values of LRG1 and IGFBP7 According to BMI in Patients with Diabetes Mellitus

The table (3-7) showed that the mean of both biomarkers LRG1 and IGFBP7.The normal weight for LRG1was (0.307 ± 0.129) µg/ml and the overweight for LRG1 was (1.132±0.407) µg/ml with p value (<0.001) which was significant. The normal weight for IGFBP7 was (0.403±0.202) ng/ml and the overweight for IGFBP7 was (1.219±0.212) ng/ml with p value (<0.001) , which was significant. The levels for both biomarkers are raised in overweight patients more than normal weight patients, and these differences are significant (p<0.001) for both biomarkers. The proportions are found in the following table (3-7).

Table (3-7) Comparison Mean Values of (LRG1) and(IGFBP7) According to BMI in Patients with Diabetes

BMI			
Biomarker	**Normal weight Mean ±SD (n=21)**	**Overweight Mean ±SD(n=39)**	**P-value**
LRG1 (µg/ml)	(0.307±0.129)	(1.132±0.407)	<0.001
IGFBP7 (ng/ml)	(0.403±0.202)	(1.219±0.212)	<0.001

***n: Number of Patients, SD: Standard Deviation,Test: Two independent samples T.test, Differences are significant at P value <0.05**

3.8. Comparison Mean Values of LRG1 and IGFBP7 According to Smoking in Patients with Diabetes Mellitus

The table (3-8) showed that the mean of **(**LRG1) for smoking was (1.266±0.323) µg/ml and non smoking was (0.473±0.147) µg/ml with p value (<0.001), which was significant. The table (3-8) also showed that the mean of (IGFBP7) for smoking was (1.219±0.316) ng/ml and non smoking was (0.567±0.180) ng/ml with p value (< 0.001), which was significant. The levels for both biomarkers are raised in smoker patients more than non smoker patients, and these differences are significant p value (<0.001) for both biomarkers. The proportions are found in the following table (3-8).

Table (3-8) Comparison Mean Values of LRG1 and IGFBP7 According to Smoking in Patients with Diabetes Mellitus

Smoking			
Biomarker	**Non-smoker Mean ± SD (n=32)**	**Smoker Mean ± SD (n=28)**	**P-value**
LRG1 (µg/ml)	0.473±0.147	1.266±0.323	<0.001
IGFBP7 (ng/ml)	0.567±0.180	1.219±0.316	<0.001

*** n: Number of Patients, SD: Standard Deviation, Test:To independent samples T.test, Differences are significant at P-value< 0.05**

3.9. Comparison Mean Values of LRG1 and IGFBP7 According to Physical activity in Patients with Diabetes Mellitus

The table (3-9) showed that the mean of (LRG1) for Physical activity was (0.464±0.415)µg/ml and non physical activity was (1.198±0.317) µg/ml with p value (<0.001), which was significant. Table (3-9) also showed that the mean of (IGFBP7) for physical activity was (1.219±0.324) ng/ml and non physical activity was (1.343±0.302) ng/ml with p value (<0.001), which was significant. The levels for both biomarkers are raised in non physical activity patients more than physical activity patients, and these difference are significant (p<0.001) for both biomarkers. The proportions are found in the following table (3-9).

Table (3-9) .Comparison Mean Values of LRG1 and IGFBP7 According to Physical Activity in Patients with Diabetes Mellitus

Physical activity			
Biomarker	**No Mean ± SD(n=32)**	**Yes Mean ± SD(n=28)**	**P-value**
LRG1 (µg/ml)	(1.198±0.317)	(0.464±0.415)	<0.001
IGFBP7 (ng/ml)	(1.343±0.302)	(1.219±0.324)	<0.001

*** n: Number of Patients , SD: Standard Deviation, Test: Two independent samples T. test, Differences are significant at P-value< 0.05**

3.10. Comparison Mean Values of LRG1 and IGFBP7 According to Family History in Patients with Diabetes Mellitus

The table (3-10) showed that the mean of (LRG1) for patients with family history was (0.889 ± 0.224) µg/ml and with non family history was (0.745±0.210) µg/ml with p value (0.324), which was non significant. The table (3-10) also shown that the mean of (IGFBP7) for patients with family history was (0.981±0.368) ng/ml and with non family history was (0.831 ± 0.323) ng/ml with p value (0.311), which was non significant. The levels for both biomarkers are not statistically significant. The proportions are found in the following table (3-10).

Table (3-10) Comparison Mean Values of LRG1 and IGFBP7 According to Family History in Patients with Diabetes Mellitus

Family History			
Biomarker	**No Mean ± SD (n=19)**	**Yes Mean ± SD (n=41)**	**P-value**
LRG1 (µg/ml)	0.745±0.210	0.889±0.224	0.324
IGFBP7 (ng/ml)	0.831±0.323	0.981±0.368	0.311

* **n: Number of Patients, SD: Standard Deviation, Test: Two independent samples T.test, Differences are significant at P-value< 0.05**

3.11. Comparison Mean Values of LRG1 and IGFBP7 According to Hypertension in Patients with Diabetes Mellitus

The table (3-11) showed that the mean of (LRG1) for patients with hypertension was (1.267±0.331) µg/ml and with non hypertension was (0.420±0.163) µg/ml with P value (<0.001), which was significant. The table (3-11) also showed that the mean of (IGFBP7) for Patients with hypertension was (1.344±0.314) ng/ml and with non hypertension was (0.522±0.144) ng/ml with p value (< 0.001), which was significant. The levels for both biomarkers are elevated in hypertension patients more than non hypertension patients, and these differences are significant (p < 0.001) for both biomarkers.The proportions are found in the following table(3-11).

Table (3-11) Comparison Mean Values of LRG1and IGFBP7 According to Hypertension in Patients with Diabetes Mellitus

Hypertension			
Biomarker	**No** **Mean ± SD (n=30)**	**Yes** **Mean ± SD (n=30)**	**P-value**
LRG1 µg/ml	0.420±0.163	1.267±0.331	<0.001
IGFBP7 ng/ml	0.522±0.144	1.344±0.314	<0.001

* **n: Number of Patients, SD: Standard Deviation, Test: Two independent samples T.test, Differences are significant at P-value< 0.05**

3.12. Comparison of LRG1 and IGFBP7 According to Hyperlipidemia in Patients with Diabetes Mellitus

The table (3-12) showed that the mean of (LRG1) for non hyperlipidemia was (0.328±0.126) µg/ml, hypercholesterolemia was (1.252±0.261) µg/ml hypertriglyceridemia was (0.353 ± 0.101) µg/ml , hypercholesterolemia and hypertriglyceridemia were (1.134±0.129)µg/ml with p value (<0.001), which was significant.The table (3-12) showed that the mean of (IGFBP7) for non hyperlipidemia was (0.407±0.118) ng/ml, hypercholesterolemia was (1.249±0.263) ng/ml, hypertriglyceridemia was (0.489±0.134) ng/ml, hypercholesterolemia and hypertriglycedemia were (1.269±0.265) ng/ml with p value (<0.001), which was significant, and these differences are significant ($p < 0.001$) for both biomarker. The proportions are found in the following table (3-12).

Table (3-12) Comparison of LRG1 and IGFBP7 According to Hyperlipidemia in Patients with Diabetes Mellitus

Hyperlipidemia					
Bio marker	**Non Hyperlipidemia Mean ± SD (n=14)**	**Hyper Cholesterol Mean ± SD (n=14)**	**Hyper Triglyceride Mean ± SD (n=10)**	**Hyper Cholesterol + Triglyceride Mean ± SD (n=22)**	**P-value**
LRG1 (µg/ml)	0.328 ± 0.126	1.252 ± 0.261	0.353 ± 0.101	1.134±0.129	<0.001
IGFBP7 (ng/ml)	0.407±0.118	1.249±0.263	0.489±0.134	1.269±0.265	<0.001

*** n: Number of Patients, SD: Standard Deviation,Test: One way ANOVA, Differences are significant at P-value< 0.05**

3.13. Comparison of LRG1 and IGFBP7 According Duration of T2DM in Patients with Diabetes Mellitus

The table (3-13) showed that duration of (T2DM) of the mean of (LRG1)for less than10 year was (0.426±0.155) ug/ml and greater than 10 years was (1.289±0.317) µg/ml with p value (<0.001), which was significant. The table also showed duration of (T2DM) of the mean of (IGFBP7) for less than 10 years was (0.518±0.131) ng/ml and greater than 10 years was (1.377±0.276) ng/ml with p value (< 0.001), which was significant. The levels for both biomarkers are elevated in

patients of duration of (T2DM) for greater than 10 years more than patients of duration of (T2DM) for less than 10 years and these differences are significant ($p<0.001$) for both biomarkers . The proportions are found in the following table (3-13).

Table (3-13) Comparison of LRG1 and IGFBP7 According to Duration of T2DM in Patients with Diabetes Mellitus

Duration of T2DM			
Biomarker	**<10 years Mean ± SD (n=31)**	**>10 years Mean ± SD (n=29)**	**P-value**
LRG1 (µg/ml)	0.426±0.155	1.289±0.317	<0.001
IGFBP7 (ng/ml)	0.518±0.131	1.377±0.276	<0.001

*** n: Number of Patients, SD: Standard Deviation, Test: T independent samples T.test, Differences are significant at P-value< 0.05.**

3.14. Comparison Mean Values of LRG1 and IGFBP7 According to Medications in Patients with Diabetes Mellitus

The table (3-14) showed that the mean of (LRG1) for medication by (Insulin-injection) was (1.216±0.266)µg/ml and medication by (Oral-anti Diabetic drugs) was (0.928±0.368) µg/ml with p value (<0.001),which was significant. The table (3-14) also showed that the mean of (IGFBP7)

, For medication by insulin injection was (1.402±0.318) ng/ml and Medication by anti-glycemic drug was (0.966±0.285) ng/ml with p value (< 0.001) , which was significant. The levels for both biomarkers are Elevated in patients medicated by (insulin-injection) more than patients medicated by (Oral anti-Diabetic durgs) , and these differences are significantly ($p<0.001$).These are proportions found in the following table (3-14).

Table (3-14) Comparison Mean Values of LRG1and IGFBP7 According to Medications in Patients with Diabetes Mellitus

Medications			
Biomarker	**Oral anti-Diabetic drugs Mean ± SD (n=42)**	**Insulin-injection Mean ± SD (n=18)**	**P-value**
LRG1 (µg/ml)	0.928±0.268	1.216±0.266	<0.001
IGFBP7 (ng/ml)	0.966±0.285	1.402±0.318	<0.001

*** n: Number of Patients, SD: Standard Deviation, Test: Two independent samples T.test, Differences are significant at P-value< 0.05**

3.15. Correlation Coefficients of LRG1 with IGFBP7 in Patients with Diabetes Mellitus

The table (3-15) shows positive relationship an significant correlation o f (LRG1) with (IGFBP7) in diabetic patients.The correlation coefficient was (0.887) and p value was (< 0.001), which was significant in the current study. The proportions are found in the following table (3-15).

Table (3-15) Correlation Coefficients of LRG1 with IGFBP7 in Patients with Diabetes Mellitus

Biomarker	LRG1 [P-value]	IGFBP7 [P-value]
LRG1 (µg/ml)	1	0.887 [<0.001]
IGFBP7 (ng/ml)	0.887 [<0.001]	1

* **R:Pearson Correlation Coefficient, Relation is significant at P- value<0.05.**

3.16. Correlation Coefficients of Diabetic Tests with LRG1 and IGFBP7 in Patients with Diabetes mellitus

The table (3-16) which explores relationship of LRG1 and IGFBP7 with diabetic tests, it is positive and moderate with FBS and HbA1c, which was significant ($p<0.05$). While correlation coefficient of insulin level with diabetic test are reverse weak and not significant. The proportions are elucidated in the following table (3-16).

Table (3-16) Correlation Coefficients of Diabetic Tests with LRG1 and IGFBP7 in Patients with Diabetes Mellitus.

Biomarker	LRG1	P-value	IGFBP7	P-value
FBS mg/dl	0.456	<0.001	0.370	0.004
HbA1C %	0.437	<0.001	0.335	0.009
Serum-insulin µU/ml	-0.07	0.572	-0.19	0.153

* **R: Pearson Correlation Coefficient, Relation is significant at P- value< 0.05**

3.17. Correlation Coefficients of Lipid Profile with LRG1 and IGFBP7 in Patients with Diabetes mellitus

The table (3-17) that shows the correlation of (LRG1) and (IGFBP7) with lipid profiles tests, it is positive and moderate with cholesterol ($p<0.001$), positive not significantly weak with triglyceride ($p>0.05$) for both the biomarkers. Negative correlation with HDL and significant ($p<0.001$), in LDL the correlation coefficient is positive moderate and significant ($p<0.001$), but VLDL level has not significant Correlation coefficient ($p>0.05$). The proportions are elucidated in the following table (3-17).

Table (3-17) Correlation Coefficients of Lipid Profile with LRG1 and IGFBP7 in Patients with Diabetes Mellitus

Lipid Profile	LRG1	P-value	IGFBP7	P-value
Cholesterol mg/dl	0.642	<0.001	0.642	0.001
TG mg/dl	0.092	0.482	0.170	0.193
HDL mg/dl	-0.726	<0.001	-0.694	<0.001
LDL mg/dl	0.543	<0.001	0.496	<0.001
VLDL mg/dl	0.094	0.477	0.171	0.191

* **R: Pearson Correlation Coefficient, Relation is significant at P- value< 0.05**

3.18. Association of LRG1 and IGFBP7 Levels in Patients with Control

The table (3-18) that shows the probability of LRG1 and IGFBP7 Levels raising with diabetic patients group more than control group, and it is statistically significant ($p<0.001$) for both biomarkers. The odd ratio for LRG 1 was (5.812) and for IGFBP7 was (5.962) respectively . The proportions are elucidated in the following table (3-18).

Table (3-18) Association of LRG1 and IGFBP7 Levels in Patients with Control

Biomarker	Odds ratio	CI 95%		P-value
		Lower	Upper	
LRG1 (µg/ml)	5.812	2.2659	6.6527	<0.001
IGFBP7 (ng/ml)	5.962	2.1360	5.2356	<0.001

*** Test: Odds Ratio, CI: Confidenc interval, association is significant at p-value < 0.05.**

3.19. Roc Test of LRG1 and IGFBP7 in Diabetic Patients Group

ROC analysis demonstrated strong diagnostic performance for both biomarkers. LRG1 had an AUC of 0.853 ($p<0.001$), with 78% sensitivity and 70% specificity at a cut off of 0.76 µg/ml, IGFBP7 had an AUC of 0.856 ($p <0.001$), with 70% sensitivity and 70% specificity at a cut off of 0.86 ng/ml . Figures 1 and 2 visually support these findings. The proportions are elucidated in the following table (3-19).

Table (3-19) ROC Test of LRG1 and IGFBP7 in Diabetic Patients Group

Biomarker	AUC	P-value	Cut off	Sensitivity	Specificity
LRG1 (µg/ml)	0.853	<0.001	0.76	0.78	0.70
IGFBP7 (ng/ml)	0.856	<0.001	0.86	0.70	0.70

*Roc: Receiver operating characteristic , significant at $p \leq 0.05$; Area Under curve

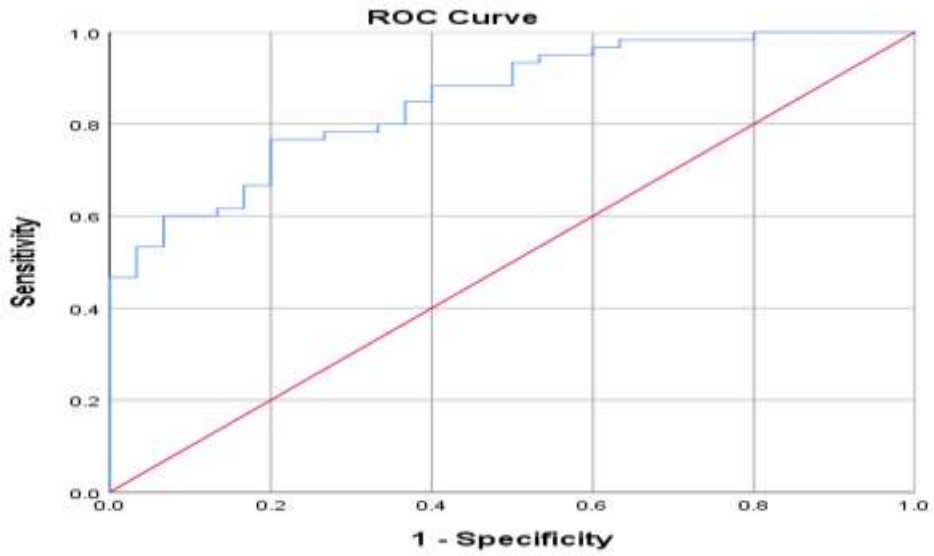


Figure (3-3) Receiver Operating Characteristic (ROC) Curve of LRG1 in Studied Groups

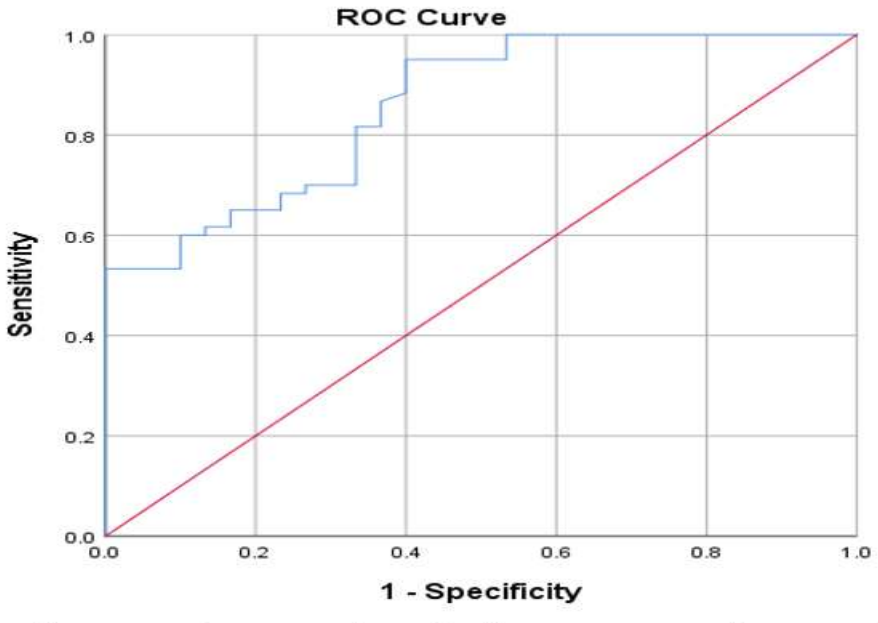


Figure (3-4) Receiver Operating characteristic (ROC) curve of IGFBP7 in Studied Groups

Chapter Four
Discussion

4. Discussion

4.1. Demographic and Clinical Characteristics

In the current study, the percentage of male is equal to that of Female as shown in the table (3-1). In previous study which is showed the prevalence of type 2 diabetes mellitus is increasing in both sexes, but men are usually diagnosed at a younger age and lower body fat mass than women. Worldwide, an estimated 17.7 million more men than women have diabetes mellitus. Women appear to bear a greater risk factor burden at the time of their type 2 diabetes diagnosis, especially obesity .Moreover , psychosocial stress might play a more prominent role in diabetes risk in women. Across their lifespan, women experience greater hormone fluctuations and body change due to reproductive factors than men. **[Kautzky et al ., 2023].** The current study observed that majority of patients fell into the age range of > 60years (31.7%), while the age groups from (31-40) years and from (51-60) years are accounted for (25.0%). In previous study found elderly.. adults have a much greater occurrence of diabetes type 2 disorders compared to other demographic Groups. The age is risk factor for Type 2 diabetes mellitus which is increased with advanced of the age due to increased insulin resistance and reduced insulin secretion**[Huang , 2022]**.

The current study observed that a significant proportion of patients were classified as overweight (65.0%) and normal weight (35.0%). In the previous study observed that increased body fat is likely to promote T2D by impairing insulin sensitivity, and possibly insulin secretion, particularly when there is ectopic fat in the liver, muscle, and pancreas. However, the main organ for glucose disposal, and fat oxidation, is skeletal muscle, so decreased muscle mass might also be expected to promote T2D, and conditions with muscle loss or atrophy do exhibit impaired glucose tolerance **[Han et al.,2019]**.The current study found that less than half of diabetic patients were smokers. In previous studies also found that less than half of diabetic patients were smokers **[Kar et al.,2019]**. The high levels of nicotine from smoking cigarettes can make the cells in the body less responsive to insulin which makes the blood sugar levels higher people with type 2 diabetes mellitus who are expose to high amounts of nicotine may need to regulate their blood glucose levels **[US. Department of health and human services ,2021]**.

4.2. Distribution of Past Medical History

In the current study, it was found that most of T2DM patients had family history of T2DM, compared to less than half of the control group had family history of T2DM as indicated in the table (3-2). In previous study observed that a family history of type 2 diabetes mellitus in a first-degree relative including a parent or sibling is a strong independent risk factor for T2DM, with greater than two-fold excess risk having an affected parent or sibling compared to non family history. This risk factor is increased with influenced by both genetic and shared environmental factors within family **[Kral et al ., 2019]**. The current study found that T2DM patients had hyperlipidemia more than half compared to control group had less than half of hyperlipidemia. In previous study found that diabetic patients with hyperlipidemia often manifest marked elevation of low- density lipoprotein (LDL), triglycerides (TRG), and omega-6 free fatty acids. Elevation of omega-6 polyunsaturated acids in turn contribute to formation of LDL/TRG. Hyperglycemia is often accompanied by hyperlipidemia in both type 1 and type 2 diabetes with increased total cholesterol and decreased high density lipoprotein (HDL) **[Zhou et al ., 2015]** .The current study observed that T2DM patients had half of hypertension compared to

control group had less than half of hypertension. In previous study which is showed high blood pressure in patients with type 2 diabetes mellitus leads to an increase in blood glucose levels because it makes the blood vessels less flexible, which effects the regulation of blood glucose levels. It also makes the body's cells less responsive to insulin, which leads to high blood glucose levels in patients with type 2 diabetes mellitus (T2D) **[Pavlou et al., 2018]**. In the current study found that T2DM patients had less than half of physical activity compared to control group had more than half of physical activity.In previous study which is showed the use of physical activities in patients with Type 2 diabetes mellitus (T2DM) leads to a decrease and regulation of blood glucose and fat levels, as well as reducing the body's cells resistance for insulin and improving the sensitivity of the insulin in the body **[Tian et al.,2023]**.The present study, found the number of (T2DM) patients with treatment by (Oral anti-Diabetic drugs) was (70.0 %) while the number of (T2DM) patients with treatment by (insulin- injection) was (30.0%) as indicated in Figure (3-1).The number of (T2DM) patients with duration of (T2DM) from (1 - 5) year, the proportion was (22%), from (5 - 10) year, the proportion was (9%),from (10 - 15) year, the proportion was (14 %) and (> 15) year, the proportion was (15%) as shown in Figure (3-2). These results are

consistent with the prior study **[Mishra et al ., 2021]**.

4.3. Biomarkers

In current study, it was observed that the mean of (FBS) was significantly higher in T2DM patients more than control group as indicated in Table (3-3). In previous study which is showed high levels of fasting blood sugar in patients with type 2 diabetes mellitus (T2DM) are due to several factors, including decreased insulin production by the pancreas (β - cell) , insulin resistance, eating large amounts of carbohydrates , lack of physical activity and taking some medications that increase blood glucose levels **[Raymond et al., 2023]**. In the current study, it was observed that the mean of (HbA1c) was significantly higher in T2DM patients more than control group as stated in Table (3-3). In previous study which is showed glycated hemoglobin increases due to high blood glucose levels over a long period of time which usually occurs in patients with diabetes or pre-diabetes. This mean that the blood glucose levels is frequently high, which leads to glucose binding to the hemoglobin in the red blood cells permanently, increasing the percentage of glycated hemoglobin **[ALzahrani et al.,2019].** The current study, it was observed that the mean of insulin was significantly lower in T2DM patients compared to control group as indicated in Table

(3-3). In previous study which is showed that the insulin level in patients with Type 2 diabetes mellitus (T2DM) is usually low due to presence defect in β cell of the pancreas which becomes unable to produce a sufficient amount of insulin, which leads to decrease in the insulin level, or the body's cells becomes resistant to insulin and this leads to excess of blood glucose levels **[Vazquez Arreola et al., 2022]**.

4.4. Lipid profile

In the present study observed that the mean of serum total cholesterol correlation was significantly higher in T2DM patients more than control group as indicated in Table (3-3). In previous study which is showed that the increase in total cholesterol levels in T2DM patients can be explained by insulin resistance which disrupts lipid metabolism. Insulin resistance leads to increased VLDL (very low-density lipoprotein) production in the liver and decreased lipoprotein lipase activity, which contributes to the accumulation of LDL and total cholesterol in the blood **[Trisna et al ., 2024]**. In the current study observed that the mean of serum (triglyceride, LDL, VLDL) correlation was significantly higher in T2DM patients more than Control group as elucidated in Table (3-3).In previous study which is showed that hyperglycaemia is associated with increased levels of

lipids in the blood. Hyperglycaemia can affect lipid metabolism through several mechanisms, including increased lipolysis, increased triglyceride synthesis, and inhibition of lipoprotein lipase activity. Additionally, the underlying insulin resistance of T2DM contributes to dyslipidaemia by increasing VLDL production in the liver and reducing LDL removal from the circulation**[Trisan et al.,2024]**. The present study observed that the mean of serum (HDL) correlation was significantly lower in T2DM patients compared to control group as indicated in Table (3-3). In previous study which is showed that low level of HDL-C is frequently encountered in T2DM, along with hypertriglyceridemia. This decrease comes under mechanisms that affect both the absolute number of particles (HDL-P) and their distribution into subclasses of distinct size or functionality .The causes responsible for lowering HDL-C interact at multiple levels, from impaired synthesis to abnormal maturation and increased catabolism of HDLs **[Hermans et al.,2017]**.

4.5. Leucine Rich-Alpha 2 Glycoprotein 1 (LRG1)

In the current study, there was statistically significant difference in the mean of (LRG1) between T2DM patients and the control group as indicated in Table (3-4) where observed elevated (LRG1) level in diabetic group compared to control group. LRG1 is a protein that carries a

negative charge, so it represents an ideal binding site for the positively charged transforming growth factor beta (TGFβ), this binding results in a defect in function of (TGFβ) which is responsible for the growth, regulation and specialization of (β cells) of the pancreas, which are responsible for insulin secretion. This leads to a decrease in insulin secretion and an increase in blood glucose levels **[Camilli et al., 2022]**. In the present study observed there was non statistically significant difference in the mean of LRG1 between male and female T2DM patients as shown in Table (3-5). In previous study, it was found that gender had non significant influence on (LRG1) level because the gender does not affect on (T2DM) disease, this leads to the level of LRG1 not being affected by this factor **[Gurung et al., 2021].** In the current study, there was statistically significant difference in the mean of LRG1between T2DM patients for the factors (age, BMI and smoking) as indicated in Tables (3-6), (3-7) and (3-8). In previous study, it was found that (age, BMI and Smoking) had significant influence on LRG1 level because the increase of these factors will an increase the risk of (T2DM) disease, which leads to an increase in the concentration of LRG1 **[Liu et al ., 2021]**. The present study observed there was statistically significant difference in the mean of LRG1 between T2DM patients for

physical activity where observed that serum (LRG1) level in T2DM patients with absence physical activity more than those T2DM patients with presence physical activity as stated in Table (3-9). The prior study , it was found that (physical activity) had significant influence on LRG1 concentration because the presence this factor will a decrease of risk (T2DM) disease , which leads to a decrease in the level of LRG1**[Organ et al., 2022]**. The present study observed there was non statistically significant difference in the mean of LRG1 between T2DM patients for family history as indicated in Table (3-10). The prior study, it was found that (family history) had non significant influence on LRG1 level because family history does not affect on (T2DM) severity, this leads to the level of LRG1 not being affected by this factor **[Gur et al.,2021].** The current study observed there was statistically significant difference in the mean of LRG1 between T2DM patients for (hypertension, hyperlipidemia and duration of T2DM) as shown in Tables (3-11), (3-12) and (3-13). In previous study , it was found that (hypertension, hyperlipidemia and duration of T2DM) had significant influence on LRG1 level because the increase of these factors will an increase of risk (T2DM) disease, which leads to an increase in the level of LRG1 **[Liu et al.,2021]**. The present study, there was statistically significant difference

in the mean of LRG1 between T2DM patients for (medication) where observed that serum (LRG1) level was in T2DM patients treated with insulin more than those treated with (Oral anti-diabetic drugs) as indicated in Table (3-14). This due to the glucose levels in T2DM patients treated with (insulin) more than those treated with (Oral-anti diabetic drug). This leads to elevated serum (LRG1) level in T2DM patients treated with insulin more than those treated with (Oral-anti diabetic drugs)**[Liu et al., 2017]**. In the present study, there was positive relation and significant correlation between biomarkers (LRG1) and (IGFBP7) in diabetic patients as indicated in Table (3-15) because both biomarkers are significant positive correlation with (T2DM) disease. The current study, observed that (LRG1) level has significantly correlated with (fasting blood sugar and glycated heamoglobin) but it was non significant correlated with (serum-insulin) as indicated in Table (3-16). In previous study found that serum (LRG1) level was significant positive correlation with (fasting blood sugar and glycated heamoglobin) while it was non significant correlation with (serum-insulin) **[Morales et al., 2024]**.The current study, observed that (LRG1) level was significant positive correlation with (total-cholesterol and LDL-C) while it was significant negative correlation with (HDL-C) in addition to, it was non significant correlation with (triglyceride and

VLDL-C) as stated in Table (3-17). In prior research, found that increased of fats causes insulin resistance which disrupts lipid metabolism which leads to increased VLDL producion in the liver and decreased lipoprotein lipase activity which contributed to the accumulation of LDL and total cholesterol. This leads to excess blood glucose levels and (LRG1) level **[Trisna et al.,2024]**. In the current study, there was statistically significant association between (LRG1) and (T2DM) where observed raised of LRG1 level in diabetic patients compared to control group. Odd ratio (OR) was (5.812) with p value (<0.001) as shown in Table (3-18). In research prior observed there was statistically significant association between (LRG1) level and (T2DM). Odd ratio indicated to the strength association between (LRG1) level and (T2DM) **[Low et al.,2024]**.The present study observed that (Roc analysis) of LRG1demonstrated strong diagnostic performance that had an AUC of (0.853), p value (<0.001) with sensitivity was (0.78) and specificity was (0.70) at a cut off of (0.76) as elucidated in Table (3-19) and figure (3-3). The prior researches, found that the results are indicated to the strong diagnostic performance for (LRG1) in (T2DM) patients **[Chen et al ., 2019]**.

4.6. Insulin-Like Growth Factor Binding Protein 7(IGFBP7)

In the current study, it was observed that the mean of diabetic group was significantly higher in (T2DM) patients more than control group as shown in table (3-4). In previous study which is showed increased (IGFBP7) level have been found in the plasma of (T2DM) patients and described as statistically significant predictors of type 2 diabetes mellitus (T2DM) due to elevated of (IGFBP7) gene in islets of the pancreas for diabetic group more than control group as well as (IGFBP7) reduced of insulin secretion through impaired P21-activated kinase1(PAK1)function which participated in insulin secretion from pancreas cells. Serum (IGFBP7) levels are increases with increase of insulin resistance for T2DM patient **[Westholm et al.,2024]**. In the current study, observed there was non statistically significant difference in the mean of (IGFBP7) between male and female (T2DM) patients as indicated in Table (3-5). In previous study unlike with present study, it was found elevated serum (IGFBP7) levels in men more than women due to (IGFBP7) DNA methylation levels were associated with (T2DM) in men but not in women **[Gu et al.,2023].** In the current study, there was statistically significant difference in the mean of IGFBP7 between T2DM patients for the factors (age, BMI and smoking) as indicated in Tables (3-6), (3-7) and (3-8). In previous study, it was

found that (age, BMI and Smoking) had significant influence on IGFBP7 level because the increase of these factors will an increase the risk of T2DM disease, which leads to an increase in the concentration of IGFBP7 **[Godina et al.,2021]**.The present study observed there was statistically significant difference in the mean of IGFBP7 betweenT2DM patients for physical activity where observed that serum IGFBP7 level in T2DM patients with absence physical activity more than those T2DM patients with presence physical activity as stated in Table (3-9). The prior study, it was found that (physical activity) had significant influence on IGFBP7 concentration because the presence this factor will a decrease of risk (T2DM) disease, which leads to a decrease in the level of IGFBP7. The exercise has protective effects by inducing the pancreatic beta cells to increase insulin secretion, thereby activating the insulin signaling path-way, upregulating GLUT-4 expression, and enhancing intracellular energy metabolism where it facilitates glucose uptake and utilization by the body's cells **[Seo et al ., 2019]**. The present study observed there was no statistically significant difference in the mean of IGFBP7 between T2DM patients for family history as indicated in Table (3-10). The prior study, it was found that (family history) had non significant influence on IGFBP7 level because family history does not affect on (T2DM) severity,

This leads to the level of IGFBP7 not being affected by this factor **[Lisowska et al.,2019]**. The current study observed there was statistically significant difference in the mean of IGFBP7 between T2DM patients for (hypertension, hyperlipidemia and duration of T2DM) as shown in Tables (3-11), (3-12) and (3-13). In previous study , it was found that (hypertension, hyperlipidemia and duration of T2DM) had significant influence on IGFBP7 level because the increase of these factors will an increase of risk (T2DM) disease, which leads to an increase in the level of IGFBP7 **[Zhu et al.,2024]**. In the present study, there was statistically significant difference in the mean of IGFPBP7 between T2DM patients for (medication) where observed that serum (IGFBP7) level was in T2DM patients treated with (insulin) more than those treated with (anti-glycemic drug) as indicated in Table (3-14). This due to the glucose levels in T2DM patients treated with insulin more than those treated with (Oral anti-diabetic drugs) . This leads to elevated serum IGFBP7 level in T2DM patients treated with (insulin) more than those treated with (Oral anti-diabetic drugs) **[Al-Qerem et al.,2021]**.In the present study , there was positive relation and significant correlation between biomarkers (LRG1) and (IGFBP7) in diabetic patients as indicated in Table (3-15) because both biomarkers are significant positive correlation with (T2DM) disease.

The current study, observed that serum IGFBP7 level has significantly correlated with (fasting blood sugar and glycated heamoglobin) but it was non significant correlated with (serum- insulin) as indicated in Table (3-16). In previous study found that serum (IGFBP7)level was significant positive correlation with (fasting blood sugar and glycated heamoglobin) but it was non significant correlation with (serum-insulin) [**AL-fartosy et al.,2017]**.The present study, observed that (IGFBP7) was significant Positive correlation with (total - cholesterol and LDL-C) while it was significant negative correlation with (HDL-C) in addition to, it was non significant correlation with (triglyceride and VLDL-C) as stated in Table (3-17). In prior research, found that increased of fats causes insulin resistance which disrupts lipid metabolism which leads to increased VLDL production in the liver and decrease lipoprotein lipase activity which contributed to the accumulation of LDL and total cholesterol. This leads to excess blood glucose levels and (IGFBP7) level **[Trisna et al., 2024]**. In the current study, there was statistically significant association between IGFBP7 and (T2DM) where observed raised of IGFP7 Level in diabetic patients compared to control group. Odd ratio (OR) was (5.962) with p value (<0.001) as shown in Table (3-18). In research prior observed there was statistically significant association

between (IGFBP7) level and (T2DM). Odd ratio indicated to the strength association between (IGFBP7) level and (T2DM) **[Li et al ., 2024]**. The present study observed that (ROC analysis) of (IGFBP7) demonstrated strong diagnostic performance that had an AUC of (0.856), p value (<0.001) with sensitivity was (0.70) and specificity was (0.70) at a cutoff of (0.86) as elucidated in Table (3-19) and Figure (3-4).The prior researches, found that the results are indicated to the strong diagnostic performance for (IGFBP7) in (T2DM) patients **[Zhu et al ., 2024]**.

Chapter Five

Conclusions and Recommendations

5. Conclusions and Recommendations

5.1. Conclusions

From all data and correlations of different variables in the present study, it could be concluded that

1. Both (LRG1) and (IGFBP7) are valuable biomarkers in predictive and development of (T2DM) patients as well as they were considered as risk factors for development of (T2DM) patients.
2. Significantly elevated serum levels of both biomarkers (LRG1) and (IGFBP7) were observed in (T2DM) patients compared to control group , suggesting that their potential role in identifying the progression and and development of type 2 diabetes mellitus (T2DM).
3. There was statistically significant differences in biomarkers Level which are found across demographic variables (age, BMI and smoking) while there was non statistically significant differences in biomarkers levels with (sex).
4. ROC analysis demonstrated strong diagnostic performance for both (LRG1) and (IGFBP7) in distinguishing (T2DM) patients from Control group.

5.2. Recommendations

This study recommends that it is necessary to:

1. Increase the sample size which is used in measurement of both biomarkers (LRG1) and (IGFBP7) and other parameters to avoid occurrence the problems in the tests.
2. Study strong association between both biomarkers (LRG1) and (IGFBP7) in other diseases such as retinitis.
3. It is recommended to use of both biomarkers (LRG1) and (IGFBP7) in predictive and progression of patients with type 2 diabetes mellitus (T2DM).

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Appendices

Sample no
Patient data:
Age:
Sex : male female
Smoking : yes no
History :
BP : lower high Physical activity : yes no Body mass index (BMI)
Hypertension yes no
Hyperlipidemia yes no
Family history; yes no
T2DM yes no
DM findings;
Type-2
Treatment of T2DM:
1-Drug
2-Insulin
Duration of T2DM:
Complication of T2DM:
1-Retinopathy
2-Neuropathy
3-Nephropathy
4-Diabetic foot
5-History ischemic heart disease
Drug:
1-Lowering sugar agent
2-Hyperlipidemia drug

الخلاصة

مرض السكري هو مجموعة من الاضطرابات الايضية تتميز بارتفاع مستويات السكر في الدم نتيجة خلل في افراز الانسولين , مقاومة الانسولين او الاثنين معا . الشكلين الرئيسيين لداء السكري كانا يعرفان بالنوع الأول والنوع الثاني. الشكل الأكثر سيادة هو داء السكري النوع الثاني وتعد نسبته لاكثرمن (90 %) من حالات السكري النوع الثاني الذي عادة يبدا بمقاومة الانسولين, اضطراب الانسولين في عدم استخدام الانسولين من قبل الخلايا بشكل صحيح او نقص في افراز الانسولين من خلايا البنكرياس.

هذه الدراسة تهدف لايجاد العلاقة بين (LRG1) و(IGFBP7) بحدوث وتطور مرض السكري النوع الثاني .الدراسة الحالية من نوع (case control study) تضمنت (90) من الافراد تتراوح اعمارهم من (70-20) سنة حيث اجريت على (60) مريض مصاب بداء السكري النوع الثاني من مركز الامام الحسن للغدد الصم والسكري في محافظة كربلاء المقدسة وكذلك على (30) من مجموعة الاصحاء (اصحاء ظاهريا) خلال الفترة من تشرين الثاني (2024) الى اذار (2025) وجمعت نماذج الدم من حالة الصيام لجميع المشاركين في هذه الدراسة.

اخذ التاريخ المرضي لجميع المشاركين , قياس ضغط الدم , مؤشر كتلة الجسم , سكرالدم في حالة الصيام ,النسبة المئوية لتركيزالسكر في الهيموكلوبين , الانسولين وملف الدهون بالإضافة الى قياس (LRG1) بواسطة التقنية التنافسية المناعية باستخدام جهاز ELISA بينما (IGFBP7) قد تم قياسة بواسطة تقنية السندويش المناعية باستخدام جهاز ELISA.

الدراسة الحالية أظهرت زيادة مستويات كل من سكرالدم في حالة الصيام والنسبة المئوية لتركيز السكر في الهيموكلوبين في مجموعة المرضى اكثر من مجموعة الاصحاء بينما قيم الانسولين كانت اقل مقارنة بمجموعة الاصحاء التي كانت ضمن المستوى الطبيعي. الدراسة الحالية أظهرت زيادة مستويات

الكوليسترول, الدهون الثلاثية ,اليبوبروتين ذو الكثافة المنخفضة واليبوبروتين ذو الكثافة المنخفضة جدا في مجموعة المرضى اكثر من مجموعة الاصحاء بينما مستويات اليبوبروتين ذو الكثافة العالية ازدادت بمجموعة الاصحاء اكثر من مجموعة المرضى. في الدراسة الحالية لوحظ ان مستويات (LRG1) و(IGFBP7) قد ازدادت في مجموعة المرضى اكثر من مجموعة الاصحاء بقيمة احتمالية (>0.001) ومن ثم استنتجت ان كل من الماركرين (LRG1)و(IGFBP7) هي عوامل خطورة وتنبا لتطور مرض السكري النوع الثاني.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء - كلية الطب
فرع الكيمياء والكيمياء الحياتية

التشخيص باستخدام المؤثرات الحيوية الكلايكوبروتين1الفا2 الغني بالانسولين و البروتين7 الرابط لعامل النمو المشابه للانسولين في مصل مرضى السكري العراقيين من النوع الثاني

رسالة الماجستير

مقدمة الى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية / جامعة كربلاء كجز من متطلبات نيل شهادة الماجستير في (الكيمياء السريرية)

من قبل

خالد محسن ناصح

بكالوريوس علوم كيمياء / جامعة كربلاء/ 2012

باشراف كل من

ا.م .د ماهر عبود مخيف
جامعة كربلاء/ كلية الطب

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جامعة كربلاء/ كلية الطب

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