Republic of Iraq

Ministry of Higher Education and

Scientific Research

University of Kerbala - College of Medicine

Department of Chemistry and Biochemistry

Role of heat shock protein 70 and vitamin D3-binding protein and its relation to vitamin D3 receptor genes in obese women with type 2 diabetes

A thesis

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

By

Baraa Abdul Kareem Mutar Hussein

B.Sc. in Department of Pathological Analyses- AlSafwa University College (2017)

Supervised By

Professor
Dr. Fadhil Jawad Al-Tu'ma
Department of Chemistry and Biochemistry
College of Medicine
University of Kerbala

Professor
Dr. Thikra Ali Allwsh
Department of Chemistry
College of Science
University of Mosul

2023 A. D. **1445 A. H.**

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿٢﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿٥﴾)

صدق الله العلي العظيم

(سورة العلق – الآية 1-5)

Dedication

For those who are more generous than all of us,

My brother the martyr Ahmed Abdul-Kareem

Thanks to his great sacrifice, I stand before you today.

To the patients who helped me to complete my mission

To my supervisors of this thesis,

Professor Dr. Fadhil Jawad Al-Tu'ma (University of Kerbala)

Professor Dr. Thikra Ali Allwsh (University of Mosul)

Dr. Ammar Gany Yassin, who help me

To my father, the generous person who built kingdoms of trust in my heart, who painted the features of happiness on my face free of charge

For my mother, the great human being, the true treasure is a paradise that God created for me.

I thank my dear brother for supporting me (Ali)

To my husband, I offer you all the bouquets of gratitude in this universe.

To my child, thank you for being in my life (Ahmed)

To all of them, I offer my sincere thanks and appreciation

Baraa

Acknowledgement

First and foremost, I would want to express my gratitude and thanks to the patients and healthy volunteers who helped me collect blood samples and participated in the completion of this study project.

I would like to express my heartfelt gratitude to the Dean-ship of the College of Medicine, University of Kerbala for accepting me to study for a master's degree in clinical chemistry.

I would like to express my deep and sincere gratitude to my supervisors, Prof. Dr. Fadhil Jawad Al-Tu'ma (University of Kerbala) and Prof. Dr. Thikra Ali Allwsh (University of Mosul), for their inspiring guidance, help, and encouragement, which were the essential motivation to continue this work. I wish them a long life with my best wishes and to thank the staff of Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala for providing various requirements and equipment to accomplish this project.

I would like to thank the laboratory team of Al-Hussein Teaching Hospital and Al-Hassan canter of diabetes and endocrinology unit / Kerbala health directorate – Iraq for their assistance in conducting laboratory testing and their moral support.

Finally, I would like to express my thanks, gratitude, and love to my friends for their support and assistance.

Baraa

Supervisors Certification

We certify that this M.Sc. thesis entitled: -

(Role of Heat Shock Protein-70 and Vitamin D3 Binding Protein with its Relationship to Vitamin D3 Receptor Genes Polymorphism in Obese Women with Type 2 Diabetes)

was prepared by (**Baraa Abdul Kareem Mutar Hussein**) 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**).

Professor

Dr. Fadhil Jawad Al-Tu'ma

Department of Chemistry and Biochemistry

College of Medicine

University of Kerbala

Professor

Dr. Thikra Ali Allwsh

Department of Chemistry

College of Science

University of Mosul

In the view of the available recommendation, I forward this M.Sc. thesis for debate by the examining committee

Assist. Prof. Dr. Atheer Hameed Al-Ghanmi

Head of Department of Chemistry and Biochemistry

College of Medicine / University of Kerbala

Examining Committee Certification

We, the examining committee, certify that we have read this M.Sc. thesis entitled: -

(Role of Heat Shock Protein-70 and Vitamin D3 Binding Protein with its Relationship to Vitamin D3 Receptor Genes Polymorphism in Obese Women with Type 2 Diabetes)

We have examined the postgraduate student **(Baraa Abdul-Kareem Mutar1)** in the content of this thesis and, in our opinion; it meets the standard for the degree of master in **(Clinical Chemistry)** and it is adequate with **(Excellent)** degree.

Signature:

Assist. Prof. Dr. Rehab Jassim Mohammed
College of Veterinary Medicine
University of Kerbala
Date: 17/7/ 2023

(Member)

Signature:

Assist. Prof. Dr. Maher Abbood Mukheef
College of Pharmacy
University of Kerbala
Date: 21/ 8/ 2023

(Member)

Signature:

Prof. Dr. Fadhil Jawad Al-Tu'ma
College of Medicine
University of Kerbala
Date: 18/7/2023

(Member / Supervisor)

Signature:

Prof. Dr. Thikra Ali Allwsh
College of Science
University of Mosul
Date: 15/7/2023

(Member / Supervisor)

Signature:

Prof. Dr. Muna Hussein Ali
College of Science - University of Mosul
Date: 15/7/ 2023
(Chairman)

Approved by the College of Medicine - University of Kerbala

Signature:

Date: / /2023
Prof. Dr. Riyadh Dayhood Al-Zubaidi
Dean of the College of Medicine

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Complete Name
(25, OH) D3	25 Hydroxy Cholecalciferol
2-h PG	2-Hour Plasma Glucose
A	Adenine
AA	Amino Acids
AB	Anti-Body
ADA	American Diabetes Association
AT	Adipose Tissue
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Blood Pressure
C	Cytosine
Ca^{+2}	Calcium
CAD	Coronary Artery Disease
CLD	Cell Lysis Buffer
CNCD	Chronic Non-Communicable Diseases
CVDs	Coronary Vascular Disorders
DDH_2O	Deionized Distal Water
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FGF23	Fibroblast Growth Factor 23
FPG	Fasting Plasma Glucose
G	Guanine
GDM	Gestational Diabetes Mellitus
GDIP	Glucose-Dependent Insulinotropic Polypeptide

GLP-1	Glucagon-Like Peptide-1
GOD	Glucose Oxidase
GWAS	Genome-Wide Association Studies
HbA1C	Glycated Hemoglobin
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
HRP	Horseradish Peroxidase
HSP-70	Heat Shock Proteins-70
HSPs	Heat Shock Protein
IBD	Inflammatory Bowel Disease
IDDM	Insulin-Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IR	Insulin Resistance
IRSs	Insulin Resistance Substrates
K	Potassium
MENA	Middle East and North Africa
mRNA	Messenger Ribose Nucleotide Acid
NAFLD	Non-Alcoholic Fatty Liver Disease
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
OR	Odd Ratio
PASA	PCR Amplification of Specific Alleles
PCR	polymerase Chain Reaction
POD	Peroxidase
R	Reagent
RBS	Random Blood Sugar
RNA	Ribose Nucleotide Acid
ROC	Receiver Operating Curve
RXR	Retinoic acid X Receptor

SD	Standard Deviation
SNPs	Single Nucleotide Polymorphisms
SPS	Stiff Person Syndrome
SPSS	Statistical Package for the Social Sciences Software
T	Thymine
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TLR-2	Toll-Like Receptor-2
TLR-4	Toll-Like Receptor-4
TAGs	Triacylglycerols
TMP	Trimethethylopropane
TNF	Tumor Necrosis Factor
NF-b	Necrosis Factor b
TPR	Tetratricopeptide repeats
TTAB	Tetradecyl Tri Methyl Ammonium Bromide
VDBP	Vitamin D Binding Protein
VDR	Vitamin D Receptors
VDRE	Vitamin D Response Elements
WAT	White Adipose Tissue
WHO	World Health Organization
WHR	Waste Hip Ratio

Summary

The current study included 45 woman with type 2 diabetes mellitus attend to Al_Hussein Teaching Hospital and Al_Hassan center of diabetes and endocrinology unit / kerbala health Directorate_lraq,their ages ranged between (30_67) year, and they were divided into two groups. Depending on the body mass index (BMI), it included the group of obese women with an average body mass of more than 30 kg/m2, and the group of normal weight women with average body mass of less than 30 kg/m2, the sampling period lasted from the beginning of April (2022) to March (2023). As well as 45 healthy women with out any health problem and at the same ages as a control group proteins.

The results observed indicated that HSP-70 and VDBP levels shown a non-significant decrease in all of type 2 diabetic groups as compared with apparently healthy control group, while the C- peptide were significantly decreased in type 2 diabetic patients when compared with apparently control group. In addition, the random blood glucose and HbA1c% were shown significant elevation in type 2 diabetic patients as compared with apparently healthy control groups. The observed data indicated that HSP70 , VDBP and C-peptide levels when comparing between type 2 diabetic patients and control in obese groups shown a risk factors depending upon the odd ratio observed (OR = 1.156(1.649-1.148), 0.917(0.733-1.148)and 1.049(1.011-1.088)) respectively, but only C-peptide was shown to be significant. In BMI the levels of HSP- 70, VDBP and C-peptide, as shown the HSP70, VDBP and C- peptide was non-significant in

obese groups, while the HSP-70VDBP and C-peptide was shown significant in normal weight groups. The optimal diagnostic points for HSP70 was (sensitivity =90%, specificity = 87%) at a level (Cut-off points) = 29.086, while C-Peptide levels: (sensitivity = 93%, specificity = 88%) at a level (Cut-off points) = 134.76, and VDBP (sensitivity = 96%, specificity
= 78%) at a level (Cut-off points) = 56.423 all markers have p- values of the AUC were <0.001 and statistically significant.

Accordingly, it was concluded that a significant relationship between circulating VDBP levels and T2DM. VDBP appears to able to contribute to the treatment of obesity and diabetes because of its anorexigenic and antihyperglycemic effects. In addition, C- peptide is a known biomarker of insulin resistance and beta-cell function. High specificity and sensitivity analyzed results were obtained by ROC analysis for both markers in T2DM.And the result of genotype, In addition, When compared VDR with polymerase gene in patients showed increased frequencies of HSP-70: CT + TT and TT genotypes in (rs1544410) for CT (OR = 0.972(0.901 - 1.048), p = 0.464; TT (OR = 0.988(0.921 - 1.059), p = 0.734, respectively), VDBP: CT + TT and TT genotypes in (rs1544410) for CT (OR = 0.998(0.969 - 1.027), p = 0.875; TT (OR = 0.988(0.961 - 1.015), p = 0.384,. C-Peptide : CT + TT and TT genotypes in (rs1544410) for CT (OR = 1.003(0.997 - 1.009), p = 0.338; TT (OR 0.998(0.990 - 1.006), p = 0.593, Just significant differences in 25(OH)D3

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disordercharacterized by increased blood glucose levels due to insulin deficiency or insulin-resistant (IR) cells **(Zou *et al.*, 2018)**. Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounting for 90–95% of all diagnosed cases of diabetes mellitus (DM). T2DM is prevalent among adults and the elderly **(Zheng *et al.*, 2018)**. The pancreatic gland produces the essential hormone insulin. It facilitates the passage of glucose from the circulation to the body's cells, where it is converted into energy. Hyperglycemia is the defining characteristic of diabetes, caused by a cell's inability to respond to insulin or an insulin deficiency. DM's chronic hyperglycemia affects and damages various organs, particularly the kidneys, nerves, blood vessels, and eyes **(Obied *et al.*, 2019 ; Tan, 2015)**. Obesity and inactivity are the two most important risk factors for T2DM **(Glovaci *et al*., 2019)**. In 2019, there were 4.2 million fatalities from DM, making it the ninth leading cause of death worldwide, affecting approximately 462 million people or 6.2% of the global population ***(*Khan *et al.*, 2020)**.

Heat shock proteins (HSPs), highly stable polypeptides, are practically present in every body cell. One of their essential functions is comparable to that of molecular chaperones, which actively contribute tomaintaining protein homeostasis and cell survival ***(*Sharma *et al.*, 2009 *;* Wang *et al.*, 2021)**. Heat shock proteins-70 (HSP-70), a family chaperone, has been implicated in the pathophysiology of insulin resistance, which leads to (T2DM), according to multiple studies conducted in the last ten years.

Can also restore insulin sensitivity in the resistant tissue **(Krause, Bock, *et al.*, 2015; Krause, Heck, *et al.*, 2015)**. However, even with significant weight loss and long-term maintenance of lifestyle changes, there is always the possibility of recurrence. Therefore, it complements lifestyle changes and reduces the risk of T2DM in individuals with prediabetes.

In the past decade, low blood levels of 25-hydroxy cholecalciferol 25(OH) D3 have been identified as a risk factor for T2DM. Vitamin D supplementation has been proposed as a prospective strategy for reducing diabetes risk **(Lu *et al.*, 2018 ; Pittas *et al.*, 2007)**. Vitamin D isessential for bone mineralization, growth, and strength via its direct effect on bone cells (osteoblasts and osteoclasts), regulation of bone remodelling, and interaction with non-skeletal systems such as the intestine and pancreas **(Hou *et al.*, 2018)**. Due to the widespread distribution of vitamin D receptors (VDR) in numerous tissues, the diversity of vitamin D's effects on the body, and the correlation between vitamin D and the number of diseases, vitamin D has attracted increasing interest in several scientific fields. Insulin receptors in its responsive tissues, such as muscle and adipose tissue, are changed as a result of VDR in pancreatic cells. **(Grammatiki *et al.*, 2017)**. The nuclear membrane-expressed VDR is essential for vitamin D's biological effects **(Sun, 2018)**. Vitamin D binding protein (VDBP) is the primary carrier for transporting vitamin D from the production site to the target tissue. It binds to approximately 85% of vitamin D metabolites **(Bikle and Schwartz, 2019)**.

1.1. Diabetes mellitus

DM is a collection of diverse metabolic disorders caused by insulin deficiency, insulin resistance, or both, and it typically manifests as hyperglycemia and glucose intolerance. resulting in abnormalities in the metabolism of carbohydrates, lipids, and proteins and disruption of the regulatory systems that regulate the storage and mobilization of metabolic fuel ***(*Padhi *et al.*, 2020)**. According to the International Diabetes Federation (IDF), in 2019, 4.2 million persons died of diabetes. By 2045, it is anticipated that 700 million people between the ages of (20-79) years will have diabetes, an increase from the current number of 463 million. Diabetes was the primary factor contributingto at least 720 billion US dollars in health expenditures in 2019 as shown in (Fig.1-2) **(Galicia-Garcia *et al.*, 2020)**.

Globally and in the United States, the prevalence of T2DM, which accounts for (90–95%) of all cases of diagnosed diabetes, continues to rise rapidly **(Kirtland *et al*., 2015 ; Mayer-Davis *et al*., 2017)**. Increasing numbers of seniors, socioeconomic progress, urbanization, highly processed foods, and adecline in physical activity are just some factors contributing to the exponential growth. Because there are few symptoms or indicators in theearly years of the disease, approximately half of those with T2DM are unaware they have the disease. Symptoms go undiagnosed and contribute to diabetes complications well before the diagnosis is confirmed **(ATLAS, 2019)**.

Despite the alarming prevalence of DM, approximately 193 million individuals worldwide lack a diagnosis. Most of these individuals, more than 120 million, reside in Southeast Asia and the Western Pacific. They are oblivious that they have the disease due to factors such as access to healthcare and the lack of symptoms and signs **(Saeedi *et al*., 2019)**. **As show in (Fig 1-1)**.

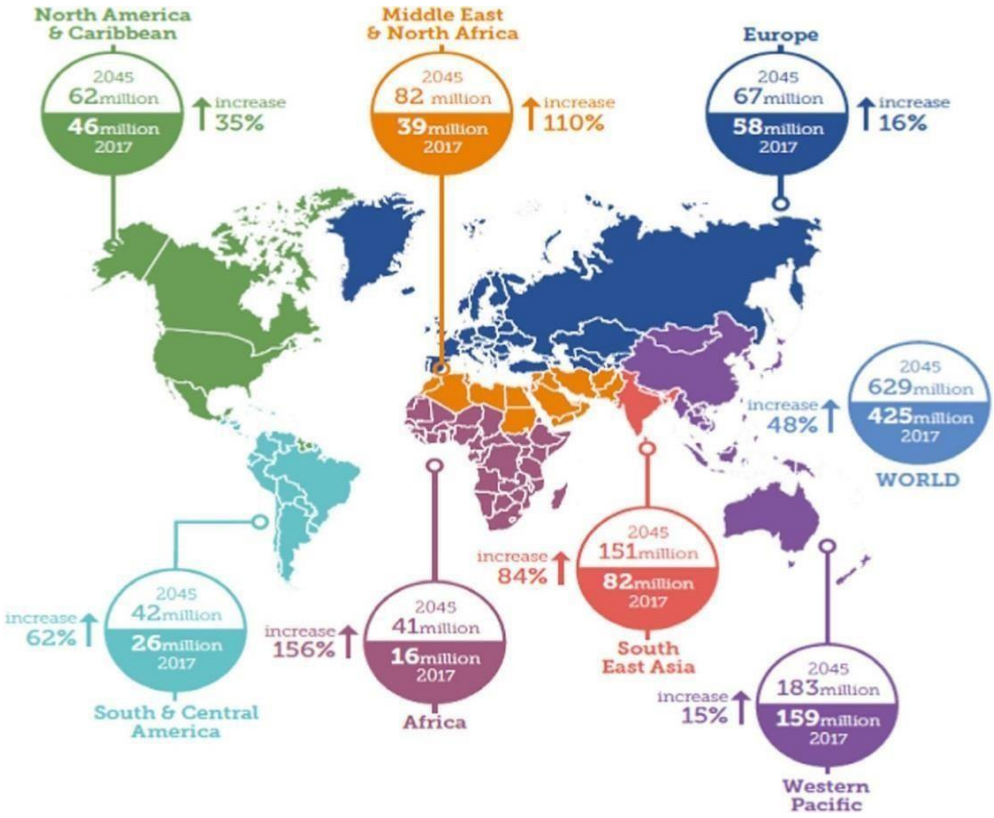


Fig. 1-1: Number of people with diabetes worldwide and per region in 2017 and 2045. (with permission from International Diabetes Federation. IDF Diabetes Atlas, 8th ed. Brussels, Belgium: International Diabetes Federation, 2017. (Diabetes Atlas, 2019) (Saeedi *et al.*, 2019)

1.1.1. Types of diabetes mellitus

The American Diabetes Association (ADA) classifies diabetes mellitus according to its etiology as follows:

1. Autoimmune idiopathic diabetes Mellitus of type 1 (T1DM).

2. Type 2 Diabetes Mellitus (T2DM).

3. Gestational diabetes Mellitus (GDM) and other specific varieties of diabetes mellitus **(Souza *et al*., 2016)**.

1.1.2. Epidemiology of diabetes mellitus

DM is a condition resulting from an imbalance in glucose levels. (90% - 95%) of diabetic patients have T2DM and GDM, whereas only (5% - 10%) have T1DM **(Benjamin *et al*., 2019)**.

1.1.3. Complication of diabetes mellitus

Dm is a known risk factor for Cardiovascular Disease and the early advent of CVD **(Health *et al*., 2011)**. Diabetes-related abnormalities in metabolic energy metabolism cause diabetic dyslipidemia **(Chehade *et al*., 2013)**.

1.1.4. Diagnosis of diabetes mellitus

Diabetes can be diagnosed using plasma glucose criteria, such as the Fasting Plasma Glucose (FPG) value or the 2-hour plasma glucose (2-h PG) value during a 75-gram oral glucose tolerance test (OGTT), as well as Glycated Hemoglobin (HbA1c) criteria **(Gillett, 2009)** as shown in **(Table 1.1)**.

Table (1-1): Diagnosis of diabetes (Punthakee *et al*., 2018).

FBG ≥ (7.0) mmol/L **Fast = No caloric income for at least 8 hours**
HbA1C ≥ (6.5) % (in adults) **By using standard and validate assays in the absence of factors that may influencethe accuracy of the test (A.1C.) and not for the suspect. T1DM**
2 h Blood glucose in a (75) g oral glucose tolerance test **O.G.TT ≥ (11.1) mmol/L**
In Random blood glucose ≥ (11.1) mmol/L **Random = not fasting for 12 hours or less**

1.1.5. Abnormalities in metabolism of diabetes mellitus

Diabetes mellitus is a condition of macromolecule metabolism characterized by a decreased capacity of the body to produce or respond to insulin, which helps to maintain healthy levels of blood glucose **(Kumar *et al*., 2020)**. When the pancreatic gland is no longer able to produce insulin or when the body is unable to use the insulin it produces, a chronic illness may develop. Insulin is a hormone produced by the duct that functions as a kind of lock to allow aldohexose from the food eaten to pass from the bloodstream into the body's cells where it may be used as an energy source. All foods with macromolecules countermine into aldohexose in the blood. Endocrine facilitates aldohexose uptake by

cells **(Kumar *et al*., 2020)**. The endocrine system transports blood sugar into cells so they can store it or use it as fuel. With polygenic disease, body either won't produce enough endocrine or won't use the endocrine it does produce well. kidneys, eyes, nerves and otherorgans can be harmed in polygenic disease is untreated high blood glucose levels **(Ahmad *et al*., 2021)**. If create little to no endocrine, or if are endocrine-resistant, a lot of sugar stays in blood. The glucose levels of those with polygenic disease are greater than usual **(Kähm *et al*., 2018)**.

1.1.6. Diabetes mellitus and genetic predispositions

T2DM is currently viewed as a condition having a significant genetic component. As a result, (35–50%) of patients have relatives who have diabetes, compared to 15% of persons without this illness. Compared to (1-6%) of healthy individuals, (10–30%) of the patient's parents who are tested for T2DM have the condition. According to the results of the Framingham Offspring Study, if one parent had diabetes, the relative risk for the child was 3.6; if both parents had, the risk was
6.0. According to this study, whether the mother or the father has the condition, the risk for diabetes in sons or daughters of diabetic parents is the same. **(Siderova, 2019)**.

1.2. Type 2 diabetes mellitus

The majority of instances of diabetes (90–95%) are of T2DM. It is characterized by hyperglycemia in the context of hyperinsulinemia, which is caused by insulin resistance and leads to metabolic dysfunction and the inability to create enough insulin to meet the body's needs **(Cancienne *et al*., 2018)**. To combat insulin resistance, one must secrete

more insulin due to diminished insulin-mediated glucose absorption in the peripheral tissues (by muscle and fat), insufficient reduction of hepatic glucose output, and inefficient triglyceride uptake by fat **(Zierath, 2019)**. The main signs of this illness include polyuria, polydipsia, polyphagia, weight loss, weariness, and eye distance. Diabetic patients who have persistent hyperglycemia may suffer dramatic complications if they are not treated **(Kumar *et al.*, 2013)**.

1.2.1. Epidemiology of type 2 diabetes mellitus

The Middle East and North Africa (MENA) region of the IDF has the second-highest prevalence of diabetes. Among the MENA Region (21 nations and territories, including Iraq), diabetes is to blame for 373557 fatalities. Diabetes accounts for 51.8% of mortality in patients under the age of 60, placing the region second among IDF regions **(Abusaib *et al.*, 2020)**. The prevalence of T2DM in Iraq reached epidemic levels in 2007, impacting an estimated 2 million people, or 7.43% of the nation's total population **(Abbas *et al.*, 2020)**. Between 8.5% and 13.9% of Iraqis have T2DM, which affects about 1.4 million people. 19.7% of people aged 19 to 94 in Basrah, southern Iraq, were found to have diabetes, according to a study of 5400 participants **(Abusaib *et al.*, 2020)**.

1.2.2. Causes of type 2 diabetes mellitus

The single most significant factor in uncovering the genetic causes of T2DM over the past ten years has been hypothesis-free genome-wide association studies (GWAS), which have found about 100 linked genomic regions or loci **(Morris, 2018)**. Type 2 diabetes is the most common form

of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise **(Baynes, 2015)**. T2D is believed to arise due to complex interactions between genetic information, developmental exposures and environmental factors such as diet, physical activity, and pollution **(Nowlin *et al.*, 2012 and Ma *et al.*, 2014)**. The characteristic of overeating and inactivity is excess fat, which has been a major area of genetic research. Genes that are strongly expressed in the central nervous systemhave been associated with general obesity in people of diverse ancestries in extensive investigations of BMI and related metrics **(Heitkamp *et al.*, 2021)**.

1.2.3. Hormonal effect of type 2 diabetes mellitus

Our comprehension of diabetes has been fundamentally altered by the identification of insulin and glucagon as glucose metabolism regulators. The pancreatic islet of Langerhans -cells converts proinsulin into the 51-amino-acid peptide insulin **(Ojha *et al.*, 2019)**.

1.2.4. Risk factors of type 2 diabetes mellitus

Several risk factors could increase a person's likelihood of developing T2DM. The following categories apply to T2DM risk factors:

1- Risk factors that can be changed include being overweight or obese, not exercising regularly, having high blood pressure (hypertension), and having abnormal cholesterol (lipid) levels.

2- Non-modifiable risk variables include things like socioeconomic status, demographics, genetics, age, mental health, and a family history of gestational diabetes **(Almubarak, 2016)**.

1.2.5. Type 2 diabetes mellitus and molecular changes

Adipose tissue, skeletal muscle, and the liver all undergo molecular and phenotypic alterations that lead to insulin resistance and ultimately T2D **(Lin and Sun, 2010)**. Since the majority of the body's insulin- stimulated glucose absorption occurs in the skeletal muscle, glucosehomeostasis in T2DM patients is significantly impacted by tissue abnormalities **(Lin and Sun, 2010)**. Impaired mitochondrial biogenesis contributes to the development of insulin resistance **(Martins *et al*., 2012)**. Myokines are a component of a complex network that facilitates communication between muscles, the liver, adipose tissue, the brain, and other organs. They also play a role in the growth (myogenesis) and regeneration of muscles within the muscle itself **(Oh *et al.*, 2016)**.

1.3. Obesity

Obesity is typically measured with the body mass index (BMI) table (1-2), but anthropometric classification systems do not account for the presence or severity of comorbidities ***(*Pischon *et al.*, 2008*)***. According to the most recent data, in 2019, obesity ascended to the fifth position on the list of global risk factors for premature death. Approximately 3 million people worldwide die each year due to obesity ***(*Murray *et al.*, 2020*)***. Obesity is a significant risk factor for developing chronic non-communicable diseases (CNCD) in humans. The most prevalent non-communicable diseases are cardiovascular, cerebrovascular, oncological, diabetic, respiratory, and musculoskeletal disorders. In addition, obesity significantly affects the development and prognosis of several infectious diseases, including a recent coronavirus infection. All of the factors above have the potential to increase the strain on global healthcare systems, and

it will require substantial investments to halt and reverse these effects **(Kim and Drapkina, 2022 ; Murray *et al.*, 2020)**.

Table (1-2): Obesity classification according to World Health Organization(WHO) 2012 (Nasser *et al.*, 2019)

Classification	Body mass index (kg/m^2)
Underweight	<18 .50
Normal range	18.50 - 24.99
Overweight	≥25-29.5
Obese	≥30.00
Obese class I	30.00 - 34.99
Obese class II	35.00 - 39.99
Obese class III	≥40.00

1.3.1. Causes of obesity

Our general comprehension of the causes of obesity is that an increase in caloric intake through overeating and a decrease in physical activity are the most significant contributors. Increasing wages, urbanization, genetic predisposition, pharmaceuticals, mental illness, economic policies, endocrine abnormalities, and exposure to hormone- disrupting substances are some of the most common causes of obesity **(Mohajan and Mohajan, 2023)**. In the 21st century, there has been a transition from a monogenetic to a multigenetic perspective on obesity ***(*Lee *et al.*, 2022)**. Prior research

has identified causal associations between higher BMI and waist/hip WHR adjusted for BMI, T2DM, and coronary artery disease (CAD) using a small number of obesity- associated single nucleotide polymorphisms (SNPs) **(Emdin *et al.*, 2017;Lotta *et al.*, 2018)**.

1.3.2. Risk factors of obesity

Changing lifestyles in the last century and sedentary workplaces and genetic factors are the main known risk factors influencing the development of obesity **(Mangemba and San Sebastian, 2020 ; Yang *et al.*, 2018)**. In addition, indicators of metabolic risk include insulin resistance and hyperglycemia **(Health *et al.*, 2011)**. Diet quality can be measured to better comprehend common dietary patterns **(Index, 201**

1.3.3. Complications in metabolism

As show in (Fig 1-2).

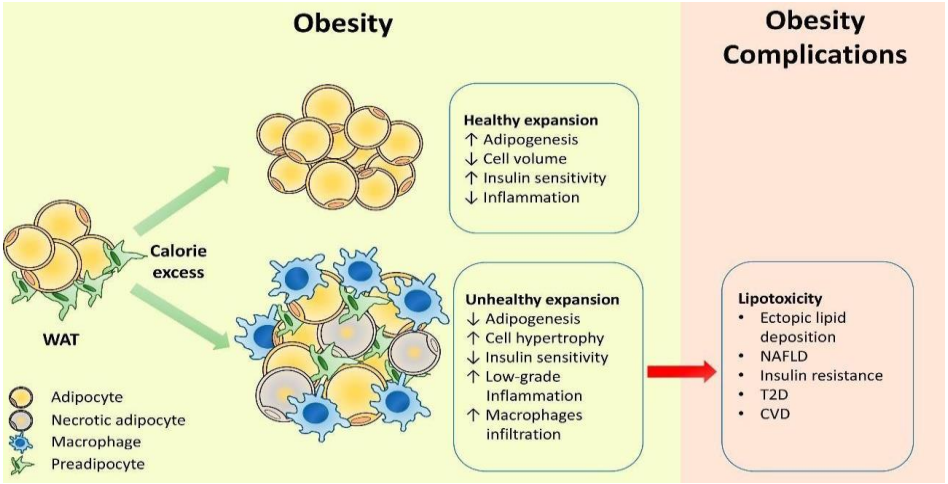


Fig. 1.2: White adipose tissue in obesity. White adipose tissue responds to caloric excess through a healthy or unhealthy expansion. Healthy expansion through adipocyte hyperplasia protects against the metabolic complications of obesity. Unhealthy expansion through adipocyte hypertrophy promotes obesity-associated metabolic complications. WAT, white adipose tissue; T2D, type 2 diabetes; NAFLD, non-alcoholic fatty liver disease; CVD, cardiovascular disease (Gaborit *et al.*, 2015 ; Singh *et al.*, 2017)

1.3.4. Obesity and diseases

Obesity is a complex, chronic disease that is characterized by a marked increase in body adipose tissue mass. It is also associated with abnormalities in the metabolism of lipids and sugars, chronic inflammation, and oxidative stress, as well as an increased risk of a number of diseases, most notably cancer, diabetes, and cardiovascular disease, and a shortened life span ***(*Leung *et al*., 2015; Włodarczyk and Nowicka, 2019).** The accumulation of DNA damage in obese individuals has been described, and it has been hypothesized that this damage may contribute to the development of obesity-related diseases **(Zaki *et al.*, 2018)**.

1.4. Biochemical markers

1.4.1. Hormone insulin

The B-cells of the pancreas, which are cell clusters found in the endocrine section of the pancreas, produce the peptide hormone insulin. The mainhormone regulating how tissues use nourishment is insulin. Its anabolic metabolic effects promote the production of protein, triacylglycerols (TAGs), and glycogen. The 51 amino acids that makeup insulin are organized into two polypeptide chains, A (21 amino acids) and B (30 amino acids), which are connected by two disulfide bridges as well as intramolecular disulfide bridges between the amino acid residues of theA chain **(Newsholme et al., 2017)**.

1.4.1.1. Biosynthesis of insulin

The creation of pre-pro-insulin, a bigger precursor polypeptide chain with 109 amino acids, is the first step in the biosynthesis of insulin. The endoplasmic reticulum quickly converts it into pro-insulin by removing 23 amino acid residues. This results in the formation of pro-insulin with 86 amino acids, which is subsequently delivered to the Golgi apparatus where it is cleaved by the pro-hormone convertase enzyme **(Tokarz *et a*l., 2018)**. Thus, 51 amino acid insulin is created after the separation of the linking peptide, or C-peptide, which has 31 amino acids. Although the quantities of insulin and C-peptide are equal, the latter has a longer half-life than the former, making it a useful indicator for estimating the production and secretion of the former **(Akai *et al.*, 2015)**.

1.4.1.2. Regulation of insulin secretion

The B-cells of the pancreas react to many stimuli, such as glucose, arginine, and hormones derived from gastrointestinal peptides, by secreting insulin:

1. The initial step in glucose-stimulated insulin release is glucose entry into the B cell via GLUT-2, followed by phosphorylation by the glucokinase enzyme and the production of ATP. The ensuing rise in the ATP/ADP ratio causes the ATP-sensitive potassium (K+-ATP) channel to close, the cell membrane to become depolarized, and calcium to enter through voltage-dependent calcium channels, leading to the exocytosis of insulin granules **(Thorens, 2015)**.

2. The initial step in glucose-stimulated insulin release is glucose entry into the B cell via GLUT-2, followed by phosphorylation by the glucokinase enzyme and the production of ATP. The ensuing rise in the ATP/ADP ratio causes the ATP-sensitive potassium (K+-ATP) channel to close, the cell membrane to become depolarized, and calcium to enter through voltage-dependent calcium channels, leading to the exocytosis of insulin granules **(Kahn *et al.*, 2006)**.

3. The hormones in the gut play an important function as another regulator of beta-cells. They note that after consuming glucose, insulin output increases. This finding implies that after ingesting glucose, some substances promote the release of insulin. These variables have been demonstrated to be gut "massagers" (termed incretins) GLP-1, a glucagon-like peptide-1, and GIP, a gastric inhibitory polypeptide, are two incretins (GIP) The release of these polypeptide hormones are reduced in T2DM and obesity **(Zaccardi *et al.*, 2016)** as shown in (**Fig. 1-3)**.

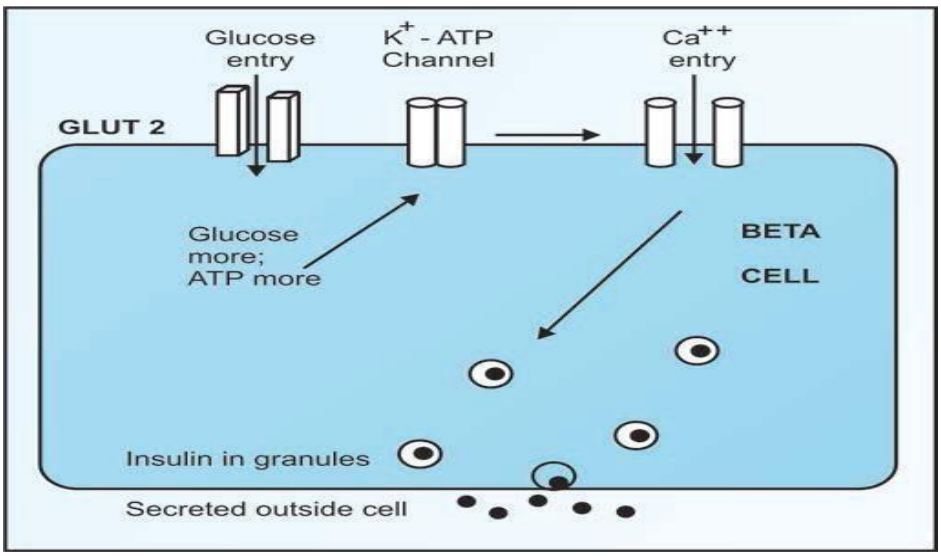


Fig. 1-3: Mechanism of insulin secretion (Vasudevan *et al.*, 2013).

1.4.1.3. Physiological roles of insulin

Insulin primarily controls the body's energy supply by maintaining a balance of micronutrient levels during the fed state **(Sakurai *et al.*, 2021)**. Transporting intracellular glucose to tissues and cells that depend on insulin, such as the liver, muscle, and adipose tissue, requiresthe hormone insulin. Any imbalance in external energy sources causes the lipids stored in adipose tissue to break down, which ultimately speeds up insulin release **(Alves-Bezerra and Cohen, 2017)**.

1.4.1.4. Mechanism of insulin signaling

1. **Insulin receptor:** Linking to a particular plasma membrane receptor on the target cells causes insulin to start working. These receptors are less plentiful and less responsive to insulin in target tissue in obesity (diabetes mellitus Type 2). A glycoprotein with 2 alpha and 2 beta subunits makes up the insulin receptor. The insulin-binding site is present in the subunits on the extracellular side. The subunits are visible on the cytoplasmic side of the cell after passing through thecell membrane. Tyrosine kinase activity is present in the beta subunit. The insulin receptor is therefore classified as a tyrosine-kinase receptor **(Vasudevan *et al.*, 2013)** as shown in **(Fig. 1-4)**.
2. **Signal transduction:** Initially, insulin binds to the alpha subunit; this activation of the tyrosine kinase activity of the beta subunit causes the beta subunit to become autophosphorylated. A collection of proteins known as "insulin receptor substrates (IRSs)" are one of several cell-signaling reactions that are facilitated by autophosphorylation. At least four IRSs with similar structural features but different tissue

distributions have been identified. Via the activation of many pathways that affect gene expression, cell metabolism, and growth, phosphorylated IRS proteins are linked with other signaling moleculesthrough certain domains **(Graham *et al.*, 2004)**.

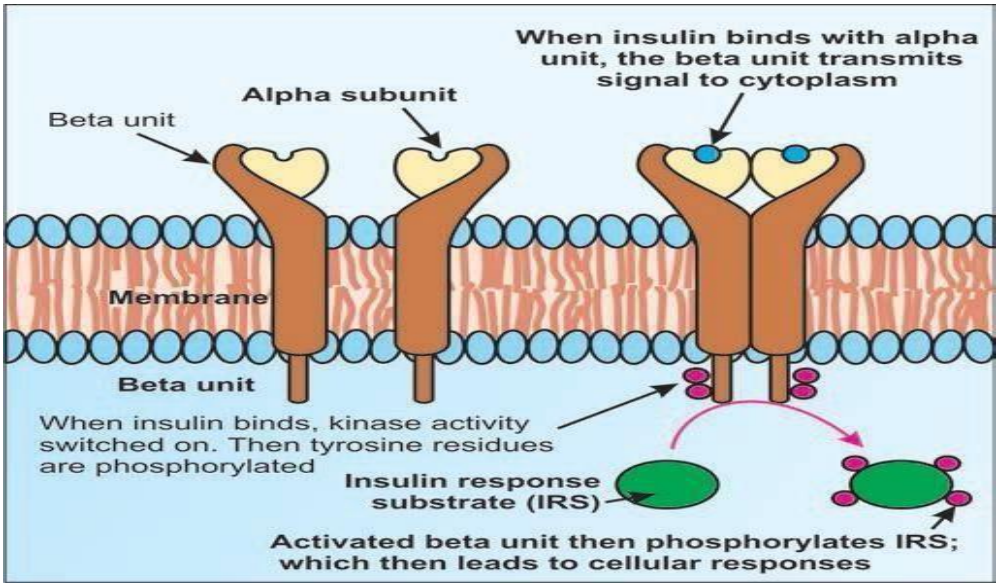


Fig. 1-4: Insulin receptor

1.4.1.5. Insulin resistance

Insulin resistance (IR) is a pathological condition of the body in which cells are unable to sense insulin hormones and cells lose insulin sensitivity **(Himanshu *et al.*, 2020)**. IR is commonly linked with obesity, which is a pathophysiologic factor of T2DM **(Becheva and Kirkova-Bogdanova, 2022)**. The etiology of T2DM is accelerated by obesity by promoting insulin resistance. Because of limited understanding of insulin resistance, T2DM therapeutic options are limited.

Nonetheless, several research has discussed the connection between insulin resistance and lipotoxicity, hyperinsulinemia, inflammation, and mitochondrial dysfunction **(Ota, 2015)**. Via the establishment of insulin resistance, endoplasmic reticulum stress, oxidative stress, genetic background, aging, hypoxia, and lipodystrophy are also implicated in the pathogenesis of T2DM. However, none of those theories have resulted in the development of T2DM treatment options. Lack of consensus regarding cross-linked mechanisms of insulin resistance in T2DM may be the cause **(Ota, 2015)**.

1.4.1.6. Effect of vitamin D_3 on insulin

According to the latest scientific research, a deficiency in vitamin D reduces insulin synthesis and secretion. Observational studies on humans demonstrate a negative association between vitamin D and insulin sensitivity, prediabetes, and dysglycemia **(Jain and Micinski, 2013)**. Numerous studies conducted over the past decade have shown that vitamin D plays an important role in reducing the risk of T2DM **(Al- Shoumer and Al-Essa, 2015)**. The current recommendation for adequate bone health from the Institute of Medicine is (600-800) IU of vitamin D per day **(Apidechkul *et al.*, 2022)**.

1.4.2. Vitamin D_3

Vitamin D3 ($25(OH)D_3$ is a steroid hormone that, in addition to its well-known role in Ca^{+2} homeostasis and bone metabolism, controls a number of other biological functions in the body **(Chang and Lee, 2019)**. Vitamin D_3 is the only vitamin and it acts as a steroid hormone. Ithas two different chemical structures and is fat-soluble vitamin **(Rhodes *et al.*, 2021)**.

1- Food sources of vitamin D2 (ergocalciferol) are limited to plants.

2- When ultraviolet light strikes 7-dehydrocholesterol in the epidermis, vitamin D3 (cholecalciferol) is synthesized. Vitamin D3 is a fat-soluble vitamin that is abundant in animal organs, especially the liver as shown in **(Fig 1-5) (Kumar *et al.*, 2013).**

Vitamin D3 is essential for the absorption of minerals like calcium and phosphorus, and it is also connected to an expanding list of non-skeletal disorders, such as diabetes and its complications **(Dilaveris *et al.*, 2019)**.

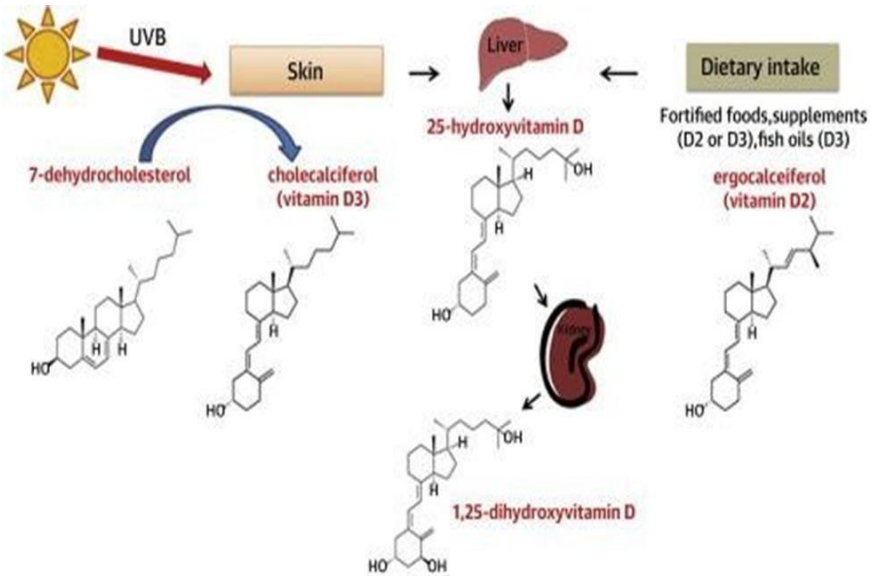


Fig. 1-5: Forms of vitamin D, photobiosynthesis, and activation (Al Mheid and Quyyumi, 2017)

1.4.2.1. Sources of vitamin D_3

Fish oil is a vital component of a healthy diet. There is some vitamin D in the liver and eggs, but only around 5% of daily needs. Despite this, mushrooms can provide a trace amount of vitamin D2, despite their dependence on UV light for growth. The majority of humans get their vitamin D from exposure to UV-B photons produced by cholesterol in the skin **(Hyppönen and Power, 2007)**.

1.4.2.2. Metabolism of vitamin D_3

Vitamin D is primarily obtained by most individuals through the skin's production in response to exposure to UV-B radiation. 7-dehydrocholesterol is converted to cholecalciferol when exposed to UVB light in the skin's upper epidermis, which triggers photolysis of the B-ring structure and subsequent isomerization. These modifications to the molecule's structure make it too large to pass through the plasma membrane, so it must enter the extracellular region by another means. Next, it is attracted to the capillary bed, where it interacts with vitamin D binding protein (DBP) to the liver. In the summer, maximum production is attained after (10–15) minutes of sun exposure, depending on skin pigmentation **(Hanel and Carlberg, 2020)**. Then, 25- hydroxylase transforms vitamin D_3 into 25-hydroxycholecalciferol $25(OH)D_3$. Although not only in the liver, this process primarily takes place there. The enzyme 25-hydroxyvitamin D1-hydroxylase, which is present in proximal tubular cells in kidneys, mediates the final stage of the synthesis of active 1,25-dihydroxycholecalciferol ($1,25(OH)_2$ D). To meet their own needs, non-kidney cells can also create $1,25(OH)_2$ D **(Bikle and Christakos, 2020)**. As seen in **(Fig1-6).** Only free vitamin D is thought to be able to penetrate most cells. Megalin and

Kubilin, two cell surface receptors, must be expressed for protein-bound vitamin D to enter cells **(Wongdee and Charoenphandhu, 2015)**. The interaction between the vitamin D/VDR complex and the vitamin D response element in the genome occurs when free vitamin D diffuses across the plasma membrane and binds to the vitamin D receptor (VDR) in the nucleus **(Carlberg and Muñoz, 2022)**.

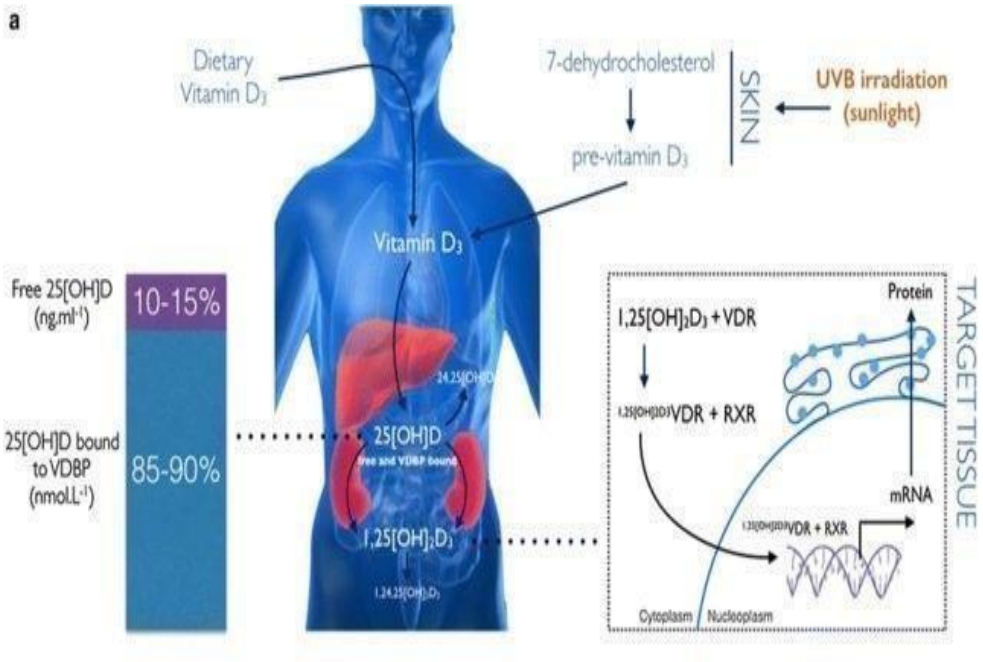


Fig. 1-6: Metabolic pathways of vitamin D_3 *(Owens et al., 2018)*.

1.4.2.3. Chemistry of vitamin D3

Vitamin D_3 is a fat-soluble vitamin consisting of the plant form known as ergocalciferol (vitamin D2) and the animal form known as cholecalciferol (vitamin D3) **(Giustina *et al.*, 2019)**. Vitamin D has long been associated with bone health because it promotes calcium absorption in the small intestine, maintains serum calcium and phosphate levels, and affects bone production and remodeling by acting on osteoblasts and

osteoclasts cells. In addition to its well-known effects on mineral homeostasis, vitamin D also has unique impacts on cell proliferation and differentiation, regulation of the innate and adaptive immune systems, protection of cardiovascular and neurological diseases, and even anti-aging benefits **(Gil *et al*., 2018)**. Vitamin D_3 can be found in fatty fish, fortified foods, and nutritional supplements. depicts how 7-dehydrocholesterol in the skin absorbs ultraviolet B rays from sunlight, causing the body to generate vitamin D_3 in addition to getting it from food sources. The body starts producing vitamin D as a result of this process **(Herrick *et al*., 2019)**. Although many cellular activities require vitamin D, its activity can be impeded by several causes, including decreased vitamin D production, a deficiency in vitamin D hydroxylases in cells, and lower VDR concentration. Since vitamin D activity is reduced, numerous chronic diseases, including cancer, osteoporosis, diabetes, erectile dysfunction, and atherosclerosis, may be brought on **(Kim *et al.*, 2020)**. When vitamin D_3 is hydroxylated in the liver and kidneys, it transforms into $1,25(OH)_2D_3$, or 1,25-dihydroxy vitamin D_3. Liver cells, immune cells, and bone cells are among the target locations where it acts. **(Pilz *et al.*, 2019)**. Vitamin D_3 has a well-known role in calcium and phosphate balance, which impacts bone growth and resorption. Low vitamin D_3 status is also associated with several municable diseases as well as an elevated risk of infectious disorders, most notably upper respiratorytract infections. But the debate over whether low vitamin D_3 levels are a factor in or a consequence of illness has persisted **(Mitchell, 2020)**.

Rickets is brought on by inadequate vitamin D_3 during growth. In adults, however, it results in osteomalacia and various degrees of osteoporomalacia. Low vitamin D_3 levels raise bone turnover, reduce bone density, and are linked to an increased risk of fractures **(Sassi *et al.*, 2018)**. Many variables affect vitamin D_3 status, including those that affect skin production, vitamin D_3 bioavailability and metabolism, and acquired and hereditary diseases of vitamin D_3 responsiveness and metabolism **(Charoenngam *et al*., 2019)**.

1.4.2.4. Vitamin D_3 and diabetes mellitus

The correlation between normal vitamin D_3 levels and appropriate insulin sensitivity and glucose homeostasis is supported by a large body of research **(Szymczak-Pajor and Śliwińska, 2019)**. For instance, recent clinical research in T2DM patients found that vitamin D_3 treatment for 6 months dramatically improved metabolic decline and glucose homeostasis **(Lemieux *et al.*, 2019)**. DM is more common in people with low levels of vitamin D3 **(L. Wang *et al.*, 2020)**. In addition, it has been proven that the polymorphism of genes involved in calcitriol production raises the risk of insulin resistance and diabetes **(Al-Daghri *et al.*, 2017)**. This data indicates that vitamin D3 may have a role in the pathogenesis of insulin resistance and diabetes **(Al-Shoumer and Al-Essa, 2015)**.

1.4.2.5. Vitamin D3 and type 2 diabetes mellitus

African Americans, Asians, and Latinos, rising obesity, advanced age, and inactivity (which may result in less time spent outdoors or exposure to sunshine) are risk factors for both vitamin D_3 deficiency and type 2

diabetes, which suggests that these associations may not be random

(Saintonge *et al.*, 2009). Recent studies on the various functions of vitamin D_3 have demonstrated the presence of specialized vitamin D receptors (VDR) in pancreatic B-cells as well as the production of 1 - hydroxylase, which is responsible for converting 25(OH)D to ($1,25(OH)_2D$) in addition to being activated in the kidney. Human insulin gene promoter has vitamin D_3 response elements, and skeletal muscle contains VDR. Additionally, $1,25(OH)_2D$ directly activates the transcription of the human insulin receptor gene, the peroxisome activator of receptor proliferators, raises the expression of the insulin receptor, and improves insulin-mediated glucose transport **(Alvarez and Ashraf, 2010)**. Human skeletal muscle and adipose tissue both express vitamin D_3 receptors, making them the primary determinants of peripheral insulin sensitivity. It is important to note that both VDRexpression and insulin sensitivity decline with aging in skeletal muscle **(Bischoff-Ferrari *et al.*, 2004)**. Insufficient vitamin D_3 affects intracellular calcium, which in turn affects insulin output and sensitivity **(Pittas *et al.*, 2007)**. Since it has been demonstrated that low 25- hydroxyvitamin D_3 levels impact pancreatic cell function and IR, low 25- hydroxyvitamin D_3 levels have become a potential risk factor for type 2 diabetes in recent years **(Pittas *et al.*, 2019)**. Therefore, there are several

observational studies on the association between the prevalence of diabetes and serum 25 (OH) D concentration, and practically all of this research supports this relationship **(Jorde, 2019)**.

1.4.2.6. Vitamin D3 and diabetes mellitus complications

Based on vitamin D_3 known effects (antiproliferative, immunomodulatory, angiogenic, inhibition of the renin-angiotensin-aldosterone system, and inhibition of the expression of neurotrophic factor), which probably interact with the pathogenesis of diabetes complications, hypovitaminosis D has been linked to long-term microvascular and macrovascular complications of diabetes ***(*Alam *et al.*, 2016)**.

1.4.2.7. Effect of vitamin D3 on bone

Vitamin D_3 helps prevent osteoporosis and fractures while promoting bone health. Inadequate dietary vitamin D_3 and vitamin D_3 deficiency are common in older adults and are associated with a higher risk of fractures **(Anderson, 2017)**. Osteoclasts are cells found in bone that can break down bone tissue, while osteoblasts are cells that create new bone cells. The presence of vitamin D_3 receptors (VDR) in osteoblasts, osteoclasts, and osteocytes contributes to the preservation of bone health by controlling the remodeling of bone **(Hou *et al.*, 2018)**. The $1,25(OH)_2D_3$ metabolites of vitamin D_3 regulate the osteoblast genesis of bone marrow stromal cells via autocrine and paracrine processes **(Posa *et al.*, 2016)**. By inducing the production of hormones such as fibroblast growth facto 23 (FGF23) and osteogenic hormones, vitamin D_3 controls the remodeling of bones(prostaglandin E2, nitric oxide, and ATP) **(St. John *et al.*, 2014)**.

1.4.2.8. Vitamin D receptor (VDR)

The majority of vitamin D_3 effects depend on its receptor, which is mostly liganded by $1,25(OH)_2D_3$. The vitamin D receptor is thought to function as a transcription factor. VDR is expressed by intestinal epithelial cells, osteoblasts, parathyroid cells, and distal renal tubules, and it plays a crucial role in the equilibrium of calcium and phosphate. The vitamin D receptor is a member of the steroid hormone receptor family. The small intestine was where VDR was first identified, but it has now been found in practically all tissues. It should come as no surprise that vitamin D affects several biological processes through VDR **(Bikle, 2020)**.

The vitamin D_3 receptor acts as a heterodimer in the nucleus and regulates vitamin D_3 target genes together with the retinoic acid X receptor (RXR). The heterodimer compound interacts with the vitamin D_3respond elements (VDRE) in DNA, thereby regulating the transcription of many vitamin D_3 target genes, including bone-related genes, osteocalcin, and osteopontin **(Bikle, 2020)**.

1.4.3. Vitamin D binding protein (VDBP)

Transport of vitamin D_3 to different tissues and cell types inside the body is facilitated by vitamin D_3 attached to VDBP, which also controls the total quantity of vitamin D_3 available for the body **(Chun, 2012)**. The 25 hydroxyvitamin D ($25(OH)D_3$) and 1, 25 hydroxyvitamin D are transported by the vitamin DBP, a highly polymorphic protein (1,25OHD) **(Bouillon *et al.*, 2020).** DBP is a multifunctional protein that has been

well-preserved throughout vertebrate evolution and is mostly made in the liver. It has a molecular weight of (52–59) kDa. In human plasma, DBP has a half-life of about 1.7 days. The mature human DBP structure is 458 amino acids (AA) long **(Bouillon *et al.*, 2020)**.

Approximately (85-90%) of the circulating 25(OH)D is bound by the DBP, which is in charge of conveying vitamin D3 and its metabolites from the point of manufacture to the target tissues **(Bikle and Christakos, 2020)**. In recent years, attention has been drawn to the biological significance of DBP, which extends beyond its function in transporting vitamin D and may even play a part in inflammation and immunomodulation **(Chun *et al.*, 2019)**.

1.4.4. Heat shock protein-70 (HSP-70)

Heat shock protein 70 is a highly conserved family of stress-responsive proteins **(Radons, 2016)**. It has been revealed that HSP70 functions in modifying circumstances associated with chronic diseases like diabetes **(Nakhjavani *et al.*, 2010)**. Heat shock proteins (HSPs) have molecular weights that range from (15 -110) kDa. They are widely distributed and well-known proteins **(Jee, 2016)**. They are believed to be defensive molecules that can express themselves in response to a variety of cellular stressors and play a variety of tasks **(Heise and Fort, 2011)**. such as oxidative, thermal, and ischemia stress **(Morteza et al., 2013)**. Heat shock proteins function as chaperone proteins and can aid in the refolding of denatured proteins **(Al-Zuhaeri *et al.*, 2022)**. Their capabilities have recently been expanded to encompass cell signaling

control **(Calderwood *et al*., 2007)**. Its participation in long-term health issues such as diabetes, obesity, and insulin resistance **(Chung *et al.*, 2008)**. In mammalian cells, both enzymatic and non-enzymatic processes can produce reactive oxygen species (ROS), Diabetes-related inflammatory diseases and vascular problems, which are brought on by the production and action of reactive oxygen species are known to be influenced by hyperglycemia **(Wei et al., 2009)**.

1.4.4.1. Heat shock protein-70 with type 2 diabetes mellitus

Heat shock proteins play important roles in offering defense mechanisms against disease, particularly diabetes. Heat shock proteins (HSPs) are a group of conserved proteins due to their crucial role in providing cellular protection. To improve cellular protection in the case of diabetes mellitus, the expression of HSP has been examined from pharmacological and genetic modulation points of view. Taking into consideration that HSPs are released in the extracellular space has led researchers to conduct clinical studies to explore the potential of using HSPs and anti-HSP antibodies as serum biomarkers of DMT2 complications **(Bellini *et al*., 2017)**.

1.4.4.2. Classification and functional role of heat shock protein-70

HSPs are a member of a limited family of elevated proteins that have been referred to as stress proteins. The molecular mass of HSPs determines how they are categorized. Constitutive HSPs, such as HSP90,HSP40, and HSP70, have cellular housekeeping roles **(Saibil, 2013)**. Two ATP-dependent HSPs that control unfolded proteins are HSP70 and HSP90, which are both highly conserved. HSP90 is a protein homeostasis-related

ATP-dependent chaperone **(Schopf *et al.*, 2017)**.HSPs are also involved in a wide range of pathological disorders,including cancer, neurological illnesses, and infectious diseases. It should be noted that HSP90 helps many other chaperones, such as HSP70 and HSP40, as well as cochaperones, such as those with tetratricopeptide repeats (TPR), refold numerous denatured proteins **(Howard *et al.*, 2010)**. Many types of HSP70 are either constitutive or inducible. Heat stress induces a significant expression of induced HSP70 family chaperones and acts as an HSP70 catalyst for brain cells **(Brown, 2007)**. It is possible to increase the stability of denatured proteins by combining the chaperone activities of HSP70 and HSP90 **(Sherman andGoldberg, 2001)**.

1.4.5. C-Peptide

C-peptide testing is a helpful and popular method of assessing pancreatic beta cell activity. Its production is equivalent to that of endogenous insulin, but its secretion is more consistent over a longer period. Samples of serum and urine are used in estimate techniques. After proinsulin is broken down, equal amounts of insulin and the "C- peptide," a 31 AA peptide, are produced. Compared to insulin, c- peptide degenerates more slowly (half-life of 20–30 min, while the half-life of insulin is 3–5 min). C-peptide levels in healthy people's fasting plasma range from (0.3-0.6) nmol/l, rising to (1-3) nmol/l after meals. First-pass metabolism of insulin occurs in the liver, but c-peptide has a low rate of hepatic clearance **(Leighton *et al.*, 2017)**.

1.4.6. Genetic studies

Each individual is distinct because only around 0.1% of the variability in any two genomes can be accounted for. The fundamental units of human DNA are made up of four distinct nucleotides. (Adenine; A, Cytosine; C,

Guanine; G, Thymine; T). These nitrogen bases can be modified in a variety of ways, including by rearranging, exchanging, duplicating, or deleting them **(Le Marchand *and* Wilkens, 2008)**. Thesemodifications increased somatically at a high pace, especially in cancer cells where they were enriched. Moreover, these alterations might take place in germ cells and be passed on to future generations as constitutional variants. Sequence changes can occur anywhere in the genome's DNA, and the bulk of them happened in non-coding areas, which have no or little impact on the operation of the cell. Different DNA repair systems often act to keep high genome integrity, but never with total fidelity **(Olden *et al.*, 2001)**.

1.4.6.1. Single nucleotide gene polymorphism

A single nucleotide (A, T, C, or G) in the genome sequence can change to cause an SNP (pronounce "snips"), which alters the DNA sequence. AAGGCTAA in the DNA sequence, for instance, might become ATGGCTAA due to an SNP.AnA SNP must occur in at least 1% of the population to be recognized. Throughout the 3-billion-base human genome, SNPs which account for nearly 90% of all genetic modifications occur every (100 – 300) bases. Both the genome's coding and noncoding sections are susceptible to SNPs **(Medvedev *et al.*, 2017)**.

1.4.6.2. Detection of single-nucleotide polymorphism

The polymerase chain reaction (PCR) is an amplification technique for the revelation of SNPs. The general procedure involves raising the temperature of the reaction to 95˚C to separate the two strands of DNA then lowering to the annealing temperature which is specific for the primers to hybridize, and then elevating to the optimal DNA polymerase temperature of 72˚C for primer elongation. This process is frequent

cyclically, generating billions of copies of the target sequence **Figure (1-7) (Lui *et al.*, 2009)**. There are various types of PCR and {amplification-refractory mutation systems (ARMS)-PCR is a main sort. It is a simple technique used for detecting SNPs based on the use of sequence-specific PCR primers to promote amplification of extracted DNA only when the targ*et al*lele is contained within the sample **(Little *et al*., 2001).**

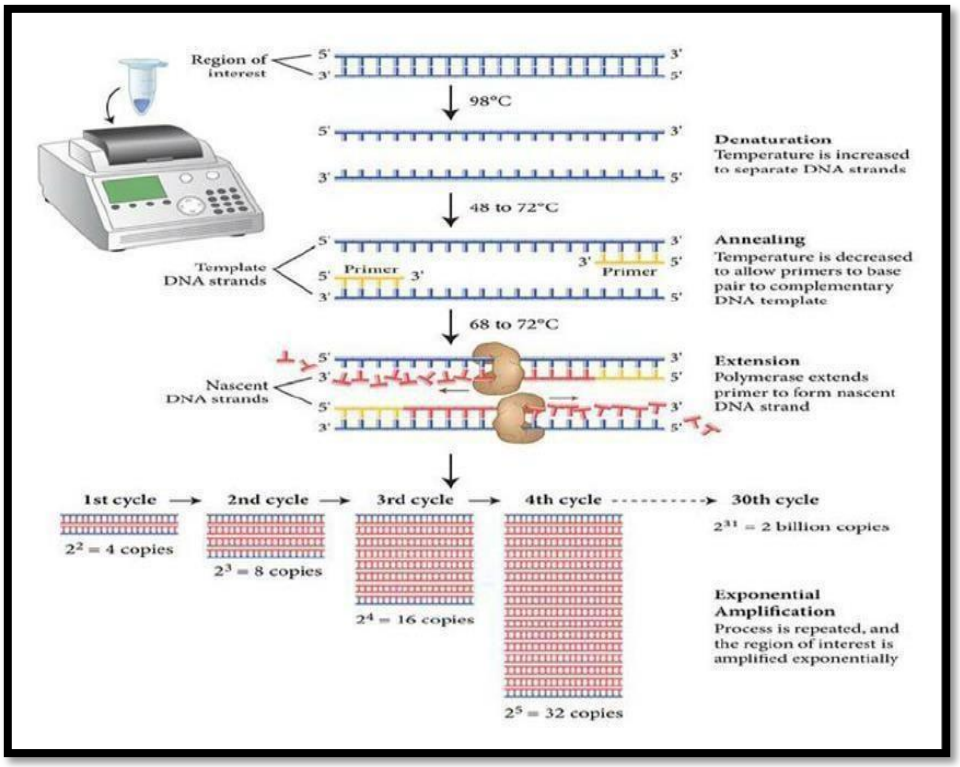


Fig. 1-7: PCR steps (Mridha, 2015).

1.4.6.3. Vitamin D3 receptor gene

Factor nuclear transcription by the mediation of 1,25(OH)2D3, VDR influences calcium absorption, bone remodeling, and the rate of mineralization. There are 11 exons in the 3q11 region on the long arm of chromosome 12, and (2-9) of them are actively transcribed **(Horst-Sikorska *et al*., 2013)**. Numerous investigations have revealed that the nuclear receptor superfamily's VDR gene, which is found on

chromosome 12's long arm (12q13.11), plays a significant role in the etiology of osteoporosis **(Ali and Jebor, 2021)**.

1.4.6.4. Vitamin D receptor gene polymorphism

It has been investigated if the polymorphism of the vitamin D receptor gene correlates with various human disorders. Hyperparathyroidism, infectious illnesses, inflammatory bowel disease (IBD), and prostatic tumors are all associated with VDR gene polymorphism **(Mahdi, 2021)**. The majority of research on VDR polymorphisms has been done in Caucasian populations and has concentrated on six SNPs: (1) rs10735810 or FokI in exon 2; (2) rs1544410 or BsmI in intron 8; (3) rs731236 or TaqI in exon 9; (4) rs7975232 or ApaI in intron 8; (5) rs757343 or Tru91 in intron 8; and (UTR) **(Mosaad *et al.*, 2014)**. There have been described over ten distinct sequence variants in the 3/UTR, including a poly(A) repeat polymorphism **(Uitterlinden** et al., **2004)**. The VDR gene has four polymorphisms (ApaI, BsmI, TaqI, and FokI), each of which is biallelic and often investigated about BMD and osteoporosis: FokI (rs228570 T/C), ApaI (rs7975232 A/C), BsmI (rs1544410 A/G), and TaqI (rs731236, T/C) **(Kow *et al.*, 2019)**.

1.4.6.5. Vitamin D receptor gene polymorphism of BsmI

SNP in the VDR gene modulates glucose intolerance, insulin secretion, and sensitivity **(Manchanda and Bid, 2012)**. The VDR gene's genetic variation can alter insulin secretion, leading to insulin resistance, as well as vitamin D synthesis, transportation, and action **(Sung *et al.*, 2012)**. The gene product that mediates the effect of vitamin D is called VDBP. When cholecalciferol binds to VDBP, it enters the bloodstream. Intron 8's BsmI polymorphism in the VDR gene is linked to the

development of type 2 diabetes mellitus **(Nosratabadi *et al.*, 2010)**. reported that T2DM patients have a considerably higher prevalence of the BsmI SNP. Several studies showed a similar connection between BsmI polymorphism and T2DM in other groups **(Ortlepp *et al.*, 2003)**. BsmI SNP and the risk of T2DM in various ethnic groups are thus not conclusively linked. SNP is connected to therapeutic responsiveness and illness susceptibility **(Anuradha, 2013)**.

1.4.6.6. Vitamin D3 receptor gene with type 2 diabetes mellitus

T2DM and VDR polymorphisms are still not linked. Many genetic VDR polymorphisms have been identified in studies carried out in different places with varying populations of people. Just one research has examined the relationship between the VDR BsmI (rs1544410) polymorphism and vitamin D insufficiency, obesity, and insulin resistance among non-diabetic subjects across various age groups to date, and it was conducted in the central area of Malaysia **(Rahmadhani *et al.*, 2017)**. Accordingly, the aforementioned study found that the BsmI(rs1544410) polymorphism was associated with an increased risk for vitamin D deficiency and insulin resistance among the Malaysian population **(Rahmadhani *et al.*, 2017)**. The adjustment of glycemiccontrol variables (i.e., vitamin D, calcium, magnesium, and phosphate levels) in Malaysian patients with T2DM has not, however, been sufficiently researched. In T2DM patients, hypomagnesemia is the electrolyte imbalance that most usually correlates with glycemic control **(Rajagambeeram *et al.*, 2020)**.

1.4.6.7. VDR - BsmI gene polymorphism in obese women

The risk of metabolic syndrome elements including abdominal obesity has also been linked to vitamin D_3 insufficiency **(Karonova *et al.*,2018)**. effect of vitamin D_3 on adipogenesis; the nuclear receptor VDR is encoded by the VDR gene. **(Zhu *et al.*, 2018)**. The control of hormone-sensitive genes and the modulation of vitamin D pathways are both important functions of the VDR gene. It's interesting to note that the VDR gene is expressed in both pancreatic beta cells and adipocytes. As a result, it may affect body composition either directly by controlling adipocyte development and metabolism or indirectly by modulating insulin levels **(Pike *et al.*, 2016)**. The presence of the polymorphisms FokI, BsmI, ApaI, and TaqI has been linked to the importance of VDR in the genesis of obesity **(Wysoczańska-Klaczyńska *et al.*, 2018)**. SNPs like BsmI thatresult in reduced VDR mRNA and protein expression may enhance one'ssusceptibility to obesity **(Zhu *et al.*, 2018)**. Research like the one by Al- Hazmi et al. showed that polymorphisms in the VDR gene's BsmI locus were strongly related to the susceptibility to obesity **(Al-Hazmi *et al.*, 2017)**.

*****The loss of beta-cell mass and the subsequent changes in clinical parameters, such as decreased C-peptide levels, elevated HbA1c, and fasting and 2 h postprandial hyperglycemia, are now caused by the lack of a non-invasive biomarker for early identification of islet stress and malfunction. It would be easy and non-invasive to test indicators of islet cell stress and damage during annual physicals. Diagnostic biomarkers for

diabetes should ideally be non-invasive, highly selective, neutral to normal physiological and metabolic alterations, and easily detectable in circulation. The most crucial need is that a biomarker to be detectable in the bloodstream before the start of irreversible beta-cell mass loss **(Bigagli and Lodovici, 2019 ; Zhao *et al*., 2019)**.

1.4.7.Aims of Study:

The aims of the presented study are to:

1. Investigation of sera levels of 25(OH)D_3 among Iraqi women with type 2 diabetes mellitus and compared with the control group.
2. Investigation of sera levels of vitamin D_3 binding protein among Iraqi women with type 2 diabetes mellitus and compared with the control group.
3. Identification of vitamin D_3 receptors single nucleotide gene polymorphism in obese women with type 2 diabetes mellitus by gene sequencing.
4. Determination of sera levels of each of insulin, glucose, and blood HbA1c% in patients and control group.
5. Investigation of sera levels of heat shock protein-70 in female patients with T2DM.
6. Study the correlations between various phenotypes and genotypes' resultant data to see the association between the genetic variants and the obesity of type 2 diabetic patients.
7. Study of gene sequencing of VDR gene.

2. Materials and Methods

2.1. Subjects and study design

The present work included a case-control study; An overall of 90 samples (females) were studied, 45 female patients with T2DM (23 of them were obese, 22 of them were not obese. While 45 cases of obese and non-obese apparently healthy individuals were dealt with in a control group. The ethical research committee, College of Medicine / University of Kerbala, approved the study protocol. All blood specimens (patients and control) were collected from individuals admitted to Al-Hussein Teaching Hospital and Al-Hassan Center of Diabetes and endocrinology unit, Kerbala Health Directorates, Kerbala – Iraq. The study was managed over the period from April 2022 to March 2023. The specimen collected were carried by ice box to the molecular laboratory of the Biochemistry Department in the College of Medicine, University of Kerbela, to be analyzed. The age of all subjects ranged from 30 to 67 years.

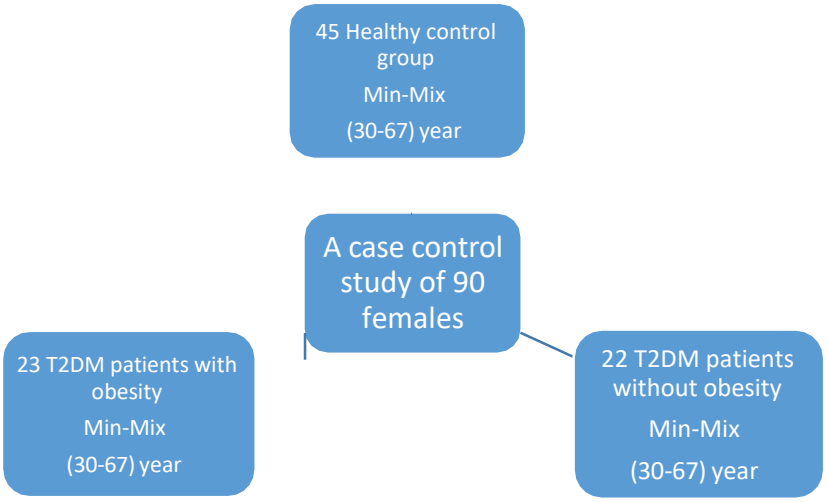


Fig. (2-1): Scheme of subject groups

2.1.1. Patient group

Patients from Al-Hussein Teaching Hospital and Al-Hassan Centre for Diabetes and Endocrinology facility in Kerbala were randomly selected.

2.1.1.1. Inclusion criteria of patients group

Obese and non-obese type 2 diabetic women were included in this study.

2.1.1.2. The exclusion criteria

1. Renal diseases
2. Heart diseases
3. Liver diseases
4. Thyroid diseases

2.1.2. Control group

Subjects were selected from the general population and exhibited a robust appearance control individuals.

2.1.2.1. Inclusion criteria of control group

a. FBS is less than 110 (mg/dl).
b. HbA1c less than 5.7 %.
c. Body mass index (BMI) 18.5-25

2.1.3. Approval of the ethical committee

The study protocol has been approved by the Research Ethics Committee of the College of Medicine at the University of Kerbala, as well as a committee from Al-Hussein Teaching Hospital and Al-Hassan Centre of Diabetes and Endocrinology Unit, under the Kerbala Health

Directorates in Iraq. This ensures that the research is conducted with the highest ethical standards and guidelines.

2.1.4. Study protocol

The medical history, height, and weight of the patient were recorded. Blood was then extracted from the patients veins. A portion of the blood was then transferred to a gel tube, centrifuged to obtain serum, and stored until the measurement time. The concentrations of numerous parameters included random blood glucose, HbA1c%, C-Peptide, HSP-70, VDBP, 25-(OH) D3, insulin resistance, and insulin . The remained blood was deposited in an EDTA tube for genomic DNA extraction used for molecular analysis of VDR and its sequencing.

2.1.5. Blood sampling

Seven ml of blood was drawn from the vein of all subjects by using a disposable syringe and then divided into two parts:

The first part (3 ml) was placed in a gel tube and left at room temperature for about (30 min) for clotting, then put in the centrifuge at 4000 x g to obtain serum which was used for the determination of biochemical parameters levels, including blood glucose by using the enzymatic colorimetric method and Heat shock protein70 (HSP 70) level by using ELISA kit, Vitamin D3 Binding Protein (VDBP) level by using ELISA kit, $25(OH)D_3$ level by using ELISA kit, insulin level by using ELISA kit, and C-peptide level by using ELISA kit. The remaining blood (4 ml) was put in tow (EDTA) containing. The first EDTA tube containing (2 ml) of blood used to determine the HbA1c% level and the second EDTA tube was stored by freezing at (- 20) ˚C until using for DNA extraction, and then performingvarious molecular analyses.

2.2. Materials

2.2.1. Chemicals and kits

All chemicals and ready kits analysis which were used in this study are listed in Table (2-1)below.

Table 2.1: The Chemicals and ready kits analysis used in the estimation of some biochemical and molecular parameters

No.	Chemicals and Kits	Company	Country
1	Genomic DNA Extraction kit	Promega	USA
2	Agarose	Promega	USA
3	Ethidium Bromide	Promega	USA
4	Master Mix Kit	Promega	USA
5	Nuclease Free Water	Promega	USA
6	Primers	Macrogen	Korea
7	Tris Borate Edta(TBE) Buffer X10	Promega	USA
8	PCR Premix	Promega	USA
9	DNA leader	Promega	USA
10	COBAS HbA1c kit	Roche	Germany
11	Ethanol 95%	Promega	USA
12	Heat shock protein 70 (HSP-70) kit	Pars biochem	China
13	Human C-Peptide Elisa kit	Pars biochem	China
15	COBAS Blood glucose kit	Roche	Germany
16	Vitamin D3 binding protein (VDBP) kit	Pars biochem	China
17	Human Vitamin D_3 kit	Roche	Germany
18	Hormone Insulin kit	Roche	Germany

2.2.2. Instruments and apparatus

The Instruments and apparatus, and tools used in this study are shown in the table (2-2) below.

Table 2.2: Instruments and apparatus used in the work methods of this study

No.	Instruments and Apparatus	Company	Country
1	PCR-thermocycler	Thermo Fisher Scientific	USA
2	Vortex-Mixture	Quality Lab System	England
3	Deep Freeze	Nikai	Japan
4	Refrigerated Centrifuge	Bio base	China
5	Sensitive Balance	Sartorius	Germany
6	UV Transilluminator	Bio base	China
7	Microwave Oven	Samsung	Korea
8	Mini spin centrifuge	Bio base	China
10	Nanodrop UV Spectrophotometer	Quawell Q5000	China
12	Incubator	Bio base	China
13	Electrophoresis apparatus	Drawell	China
14	Photo documentation	Bio base	China
15	ElLISA reader	Bio Tek	ELX800-USA
16	ELISA washer	Bio Tek	ELX800-USA
17	Water bath	LabTech	Korea
18	Cobas c311Analyzer	Roche	Germany
19	Cobas Integra 400 Plus	Roche	Germany

2.2.3. Tools

The Tools with their supplier which were used in this study are listed in Table (2.3) below.

Table 2.3: Tools used in the study

No.	Tools	Suppliers
1	Pipette(100-1000µl)	DRAGON MED/ USA
2	Micropipette (10-100 µl)	DRAGON LAB/ USA
3	Gilson Tips,1000µl (blue)	China
4	Gilson Micro-tips, 100µl	China
5	Eppendorf Tubes	China
5	Gel tubes	China
6	EDTA tubes	China
7	Gloves	China
8	Syringe	China

2.3. Methods

2.3.1. Assessment of body mass index BMI

The body mass index (BMI) was estimated by the following equation:

$$\text{BMI (kg/m}^2\text{)} = \text{Wight in kg} / (\text{Height in meter})^2$$

For both patients and control groups, weight was classified according to their BMI as shown below **(Organization, 2016)**.

a. Underweight < 18.5

b. Normal weight 18.5-24.9

c. Overweight 25.0 – 29.9

d. Obese 30.0 -34.9

e. Extremely Obese > 35

2.3.2. Assessment of waist circumference and waist to hip ratio

The west hip ratio (WHR) was estimated by the following equation in which the calculate WHR by dividing waist circumference by hip circumference.

WHR=Waist in cm/ Hip in cm

According to a study in (2021) by World Health Organization

(WHO)a moderate WHR is:

- 0.9 or less in men
- 0.85 or less in women.

Waist-to-hip ratio chart

Health risk	Women
Low	0.80 or lower
Moderate	0.81-0.85
High	0.86 or higher

2.3.3. Estimation of human vitamin D-binding protein (VDBP) concentration in serum

The VDBP concentration in the serum was estimated using the ready assay kit of Chinese origin, and by using the Enzyme – Linked Immunosorbent Assay (ELISA)

Principle

Using Purified Human DBP antibody to coat microtiter plate wells, make solid-phase antibody, then add DBP to wells, Combined DBP antibody which With HRP labelled becomes antibody-antigen-enzyme-antibody complex, after washing completely, adding TMB substrate solution, TMB substrate turns blue. The reaction is terminated by adding a sulphuric acid solution, and the Colour change is measured spectrophotometrically at 450 nm. The concentration of DBP in the samples is then calculated by comparing their optical density (OD) with the standard curve.

Table (2-4): Materials provided with the kit

Materials providedwith the kit	96 determinations	Storage
User manual	1	
Closure plate membrane	2	
Sealed bags	1	
Microelisa strip plate	1	2-8 ℃
Standard：72 ng/ml	0.5 ml × 1 bottle	2-8 ℃
Standard diluent	1.5 ml × 1 bottle	2-8 ℃
HRP-Conjugate reagent	6 ml × 1 bottle	2-8 ℃
Sample diluent	6 ml×1 bottle	2-8 ℃
Chromogen Solution A	6 ml × 1 bottle	2-8 ℃
Chromogen Solution B	6 ml × 1 bottle	2-8 ℃
Stop Solution	6 ml × 1 bottle	2-8 ℃
wash solution	（20 ml × 20 fold） × 1bottle	2-8 ℃

Procedure

1. Sample diluted and added to standard: prepared 10 standard wells on coated ELISA plates, 100 L of the standard were added to wells Iand II, followed by 50 L of standard dilution to wells I and II, and mixed; 100 liters were removed from the first and second wells and added to the third and fourth wells separately. then 50 L of the standard dilution were added to the third and fourth wells and mix; 50 liters

50 liters were added to the fifth and sixth wells; 50 L of standard dilution was added to wells five and six; Then 50 liters were removed from the fifth and sixth wells and the seventh and eighth wells were added; Then 50 liters of standard dilution was added to wells 7 and 8. The removed group should be equilibrated for 15 to 30 minutes at room temperature, and the ELISA plates should be coated if not to be used after opening. The plate should be stored in an airtight container. Purging buffer separated crystallization; Heating water can help melt when it mixeswell; 50 liters were removed from the seventh and eighth wells and added to the ninth and tenth wells, and 50 liters of standard dilution was added to the ninth and tenth wells; and mixed. Was removed 50 L from the ninth and tenth wells and discarded them (50 L of the sample was added to each well). Dilution (density: 48 ng/mL， 32 ng/mL, 16 ng/mL， 8 ng/mL， 4 ng/mL) Incubation: After the plate was sealed with the sealing plate membrane, it was incubated for 30 minutes at 37℃.

2. Configuration of liquid: 30-fold wash solution diluted 30- fold (or 20-fold) with distilled water and reserve.

3. Washing uncover the closure plate membrane, discard the liquid, dry byswing, washing buffer was added washing buffer to every well, leave for the 30s then drain, repeat 5 times, and drying by pat.

4. A volume of 50 μL of HRP-Conjugate reagent was added to each well except for theempty control well.

5. Incubation is described in Step 3.

6. Washing was done as described in step 3

7. Coloring: A volume of 50 µl of chromogen solution added 50 µl chromogen solution B to each well, and was mixed with gently shaking and incubated at 37℃ for 15 minutes. avoid light during coloring.
8. Termination: 50 µL of stop solution was added to each well to terminate the reaction. The color of the well should change from blue toyellow.
9. The O.D. absorbance was read. at 450 nm using a microtiter plate reader. The OD value of the blank control well was set as zero. The assay should be performed within 15 minutes after the addition of the stop solution.

Calculation

Referring to the standard curve of DBP concentration estimation in serum in unit (ng/ml) as shown in the (fig 2-2)

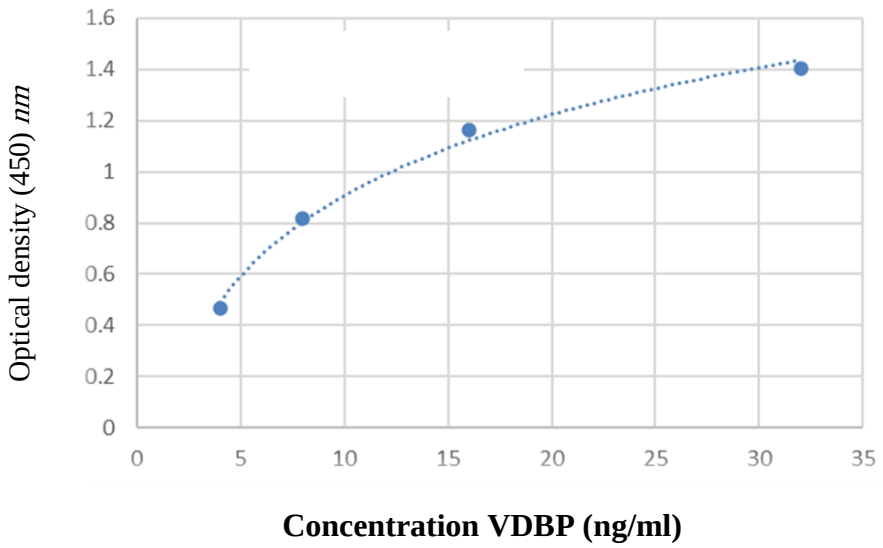


Fig. (2-2): standard curve of VDBP concentration estimation in serum

2.3.4. Estimation of human heat shock protein - 70 (HSP_70) in serum concentration

The HSP-70 concentration in the serum was estimated using the ready assay kit of Chinese origin, and by using the enzyme- Linked Immeunosorbent Assay (ELISA) technique.

Principle

The reagent assay human HSP-70 concentration in the sample purified Human HSP-70antibody to coat microtiter plate wells, make solid-phase antibody, and then add HSP-70 to wells; combined HSP-70antibody that binds to both HSP-70 and solid-phase antibody. After a thorough rinsing, antibody-antigen-enzyme-antibody complexes containing HRP-labeled HRP become antibody-antigen-enzyme-antibody complexes. When the TMB substrate solution is added, the substrate turns blue. The addition of a sulphuric acid solution terminates an HRP-catalyzed reaction. The change in hue is measured spectrophotometrically at 450 nm wavelength. The HSP-70 concentration in the samples is then determined by comparing the samples' optical density (OD) to the standard curve.

Table 2.5: Materials provided with the kit

Materials provided withthe kit	96 determinations	Storage
User manual	1	
Closure plate membrane	2	
Sealed bags	1	
Microelisa strip plate	1	2-8 ℃
Standard：72 ng/ml	0.5 ml × 1 bottle	2-8 ℃
Standard diluent	1.5 ml × 1 bottle	2-8 ℃
HRP-Conjugate reagent	6 ml × 1 bottle	2-8 ℃
Sample diluent	6 ml × 1 bottle	2-8 ℃
Chromogen Solution A	6 ml × 1 bottle	2-8 ℃
Chromogen Solution B	6 ml ×1 bottle	2-8 ℃
Stop Solution	6 ml × 1 bottle	2-8 ℃
wash solution	（20 ml × 20 fold） × 1bottle	2-8 ℃

Procedure

1. Sample diluted and added to standard: prepared 10 standard wells on coated ELISA plates, 100 L of the standard was added to wells 1 and2, followed by 50 L of standard dilution to wells 1 and 2, then mixed; Remove 100 liters from the first and second wells and add it separately to the third and fourth wells. Then the standard 50 L dilution was added to the third and fourth wells and mixed; Then remove 50 liters from the third and fourth wells, then dump and add 50 liters to the fifth and sixth

wells.; Then a 50 L standard dilution was added to the fifth and sixth wells and mixed; 50 liters was removed from the fifth and sixth wells and added to the seventh and eighth wells; Then adding the standard dilution of 50 liters to the seventh and eighth wells. The kit equilibrated for 15 to 30 minutes at room temperature after being removed from the refrigerator. If the coated ELISA plates are not to be used after opening, they stored in a sealed bag. Wash buffer will separate crystallization, can be heated to dissolve well, and mix; 50L were removed from the 7^{th} and 8th wells and added to the 9th and 10th wells, the standard dilution added 50 L to the 9th and 10th wells, mixed, and then 50 L were removed from the 9th and 10th wellsdiscarded (a 50 L sample was added to each well after dilution, (density: 48 ng/ml, 32 ng/ml

2. Sample added: set the empty wells separately (empty comparison wells add sample and HRP-Conjugate reagent, the other process is the same). Test sample well. 40 μL sample dilution was added to the test sample well, then the 10 μL test sample (final 5-fold dilution sample) was added, and then the sample was added to the wells, the wall of the well be touched as much as possible, and gently mixed.

3. Incubate: After the plate was sealed with the plate sealing film, it was incubated for 30 minutes at 37 ℃.

4. Composition fluid: washing solution 30-fold diluted 30- fold (or 20-fold) with distilled water and reserve.

5. Closing plate membrane washed, discarded liquid, then swing dried, wash solution added to each well, still for 30 seconds, then blotted, repeated 5 times, pat dry.
6. A volume of 50 µL of HRP-Conjugate reagent was added to each well except for theempty control well.
7. Incubation is described in Step 3.
8. Washing was done as described in step 3
9. Coloring: Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes.
10. Termination: 50 µL of stop solution was added to each well to terminate the reaction. The color of the well should change from blue to yellow.
11. The O.D. absorbance was read. at 450 nm using a microtiter plate reader. The OD value of the blank control well was set as zero. The assay performed within 15 minutes after the addition of the stop solution.

Calculation

Referring to the standard curve of HSP-70 concentration estimation serum in unit (ng/ml) as shown in the (fig 2-4)

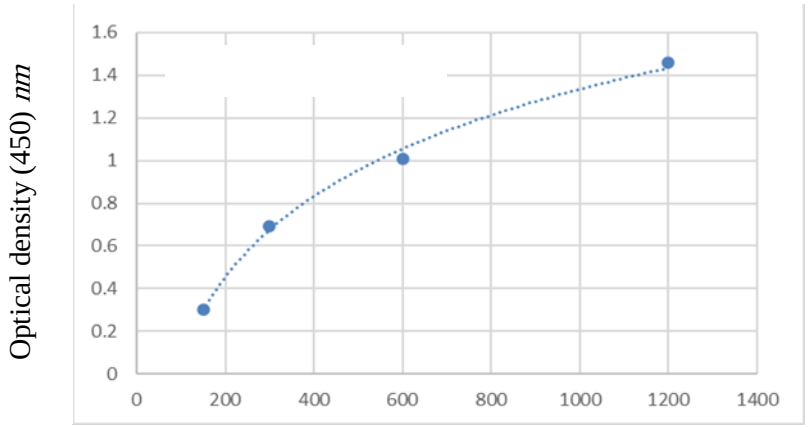


Fig. (2-3): standard curve of HSP-70 concentration estimation in serum

2.3.5. Estimation of human insulin concentration in serum using ELISA Kit

The Insulin concentration in the serum was estimated using the ready assay kit of German origin, and by using the enzyme- Linked Immunosorbent Assay (ELISA) technique.

Principle

The DEMEDITEC Insulin ELISA Kit is a solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule. An aliquot of patient samples containing endogenous Insulin is incubated in the coated well with enzyme conjugate, an anti-Insulin antibody conjugated with Biotin. After incubation, the unbound conjugate is washed off. During the second incubation step, Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti- Insulin antibody. The amount of bound HRP complex is proportional to the concentration of Insulin in the sample. Having added the

substrate solution, the intensity of colour developed is proportional to the concentration of Insulin in the patient sample.

Reagent Preparation

Bring all reagents and the required number of strips to room temperature before use.

Procedure

Each run must include a standard curve.

1. The required number of Microtiter wells is secured in the frame holder.

2. 25 µl of each standard, control and sample were dispensed with new disposable tips into the appropriate wells.

3. 25 µL of enzyme conjugate was dispensed into each well. Then blend well for 10 seconds. It is important to have complete mixing at this step.

4. Incubate for 30 minutes at room temperature.

5. The contents of the wells were quickly agitated. Rinse wells 3 times with 400 µL of diluted wash solution per well if a dishwasher was used—or rinse wells 3 times with 300 µL of diluted wash solution per well for manual washing. Wells were hit sharply on absorbent paper to remove remaining droplets.

6. A volume of 50 µL of the enzyme complex was added to each well.

7. Incubate for 30 minutes at room temperature

8. The contents of the wells were quickly agitated. Wells were rinsed 3 times with 400 µL of diluted wash solution per well. If using a dishwasher, wells were rinsed 3 times with 300 µL of diluted washing solution per well for hand washing. Wells were hit sharply on absorbent paper to remove remaining droplets.

9. A volume of 50 µL of the substrate solution was added to each well.

10. Incubate for 15 minutes at room temperature.

11. The enzymatic reaction was stopped by adding 50 µL of the stop solution to each well.

12. Determine the absorbance (OD) of the solution in each well at 450 nm (red) and at 620–630 nm using a microtiter plate reader.

Example of typical standard curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 µIU/mL)	0.03
Standard 1 (6.25 µIU/mL)	0.07
Standard 2 (12.5 µIU/mL)	0.14
Standard 3 (25 µIU/mL)	0.35
Standard 4 (50 µIU/mL)	0.88
Standard 5 (100 µIU/mL)	2.05

2.3.6. Estimation of human vitamin D_3 of 25OH-VD_3 concentration in serum

The Vitamin D_3 concentration in the serum was estimated using the ready assay kit of German origin, and by using the enzyme- Linked Immunosorbent Assay (ELISA) technique.

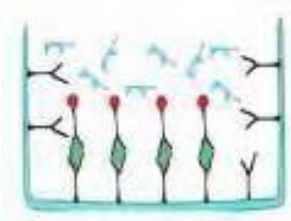
Principle

This kit utilizes the Double Antibody Sandwich ELISA technique. The pre-coated antibody is an anti-Human VD monoclonal antibody,while the detection antibody is a biotinylated polyclonal antibody. Samples and biotinylated antibodies are added into ELISA plate wells andwashed out with PBS or TBS after their respective additions to the wells. Then Avidin-peroxidase conjugates are added to the wells in after. TMB substrate is used for coloration after the enzyme conjugate has already been thoroughly washed out of the wells by PBS or TBS. TMB reacts to form a blue product from the peroxidase activity and finally turns yellow after adding the stop solution (Color Reagent C). The color intensity and quantity of target analyte in the sample are positively correlated.

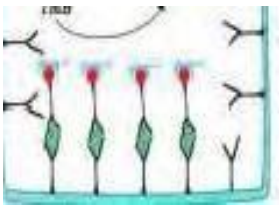
General Schematic of the Double-Antibody Sandwich Principle:

The first step

The second step



The third step



The fourth step

Test Preparation

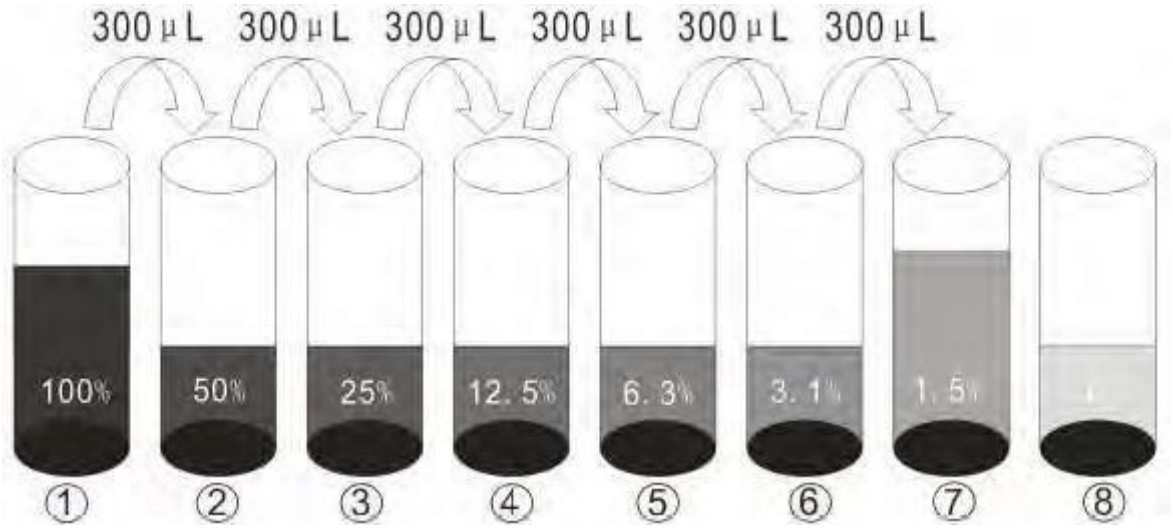
1. The ELISA kit taken out of the refrigerator 20 minutes in advance, and we start testing as soon as it is brought to room temperature.

2. dilute the laundry concentrate packet with double distilled water (1:25). We will return the unused amount to the bin.

3. Standard: added 1.0 mL of standard diluent to the lyophilized standard vial and left it for 30 minutes. After the standard is completely dissolved, then I mix a little and mark it with a sticker on the tube. The following concentration values are recommended for the standard curve: 400, 200, 100, 50, 25, 12.5, and 6.25ng/mL. It must be absolutely ensured that the freeze-dried standard is completelydissolved and mixed thoroughly.

4. Legend of the standard sample dilution method: 7 clean tubes were taken and labelled with their expected concentrations (200- 100-50, 25, 12.5, 6.25, 0 ng/ml). added 300 µl standard dilution into each tube.

Suck out the diluted 300 µL of the reconstituted standard and add it to the tube labelled 200 ng/mL and mix well. Pipette 300 µL diluent from a 200 ng/mL tube, then add to 100 ng/mL diluent and mix well. These steps were repeated with a standard of 6.25ng/mL. The standard diluent in the 0ng/mL tube is the negative control. The reconstituted standard solution（400 ng/mL) should be discarded after running the assay



Note: Reconstituted standard stock solution cannot be reused.

5. Biotin-treated antibody: Appropriate volume of Biotinylated antibody solution was removed for the number of wells intend to examine, and diluted with antibody diluted 1:100. This should be prepared 30 minutes in advance,

6. Enzyme conjugate: Appropriate amount of enzyme conjugate solution was removed for the number of wells to be examined, and diluted with enzyme diluent at a ratio of 1:100. This prepared 30 minutes in advance, and strongly recommend not to reuse for additional assays.

7. Reagent: A solution of reagent was prepared 30 minutes in advance by adding reagent A and reagent B in a ratio of 9:1.

Reference curve

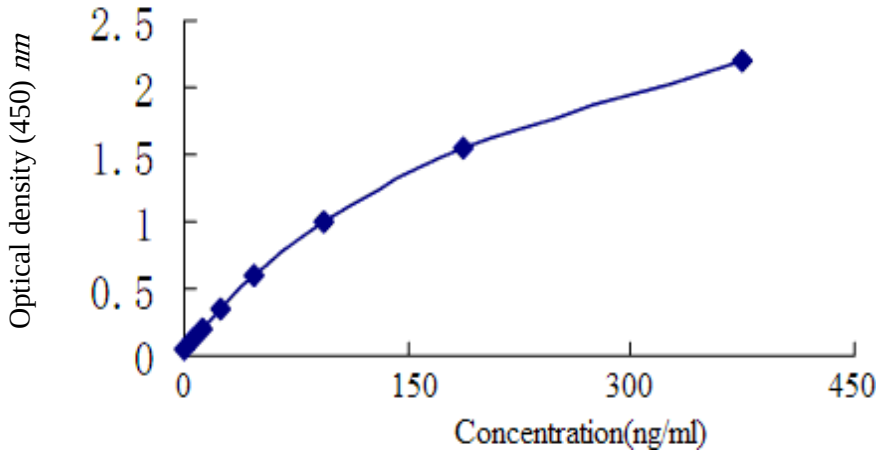


Fig. (2-4): Standard curve of vitamin D3 estimation in serum

2.3.7. Estimation of human C-Peptide concentration in serum

The C-Peptide concentration in the serum was estimated using the ready assay kit of German origin, and by using the enzyme- Linked Immunosorbent Assay (ELISA) technique.

Principle:

The reagent measures the level of Human C-Peptide in the sample used. Coat microtiter plate wells with purified Human C-Peptide antibody to make solid-phase antibody; add C-Peptide to wells; combine C-Peptide antibody with HRP-labeled antibody to form an antibody-antigen- enzyme-antibody complex; after washing, add TMB substrate solution; TMB substrate turns blue. The reaction is terminated by adding a sulphuric acid solution, and the colour change is measured spectrophotometrically at 450 nm. Afterwards, the concentration of C-

Peptide in the samples is determined by comparing the samples' optical density (OD) to the standard curve.

Table 2.6: Materials provided with the kit C-Peptide concentration

Materials provided withthe kit	96determinations	Storage
User manual	1	
Closure plate membrane	2	
Sealed bags	1	
Micro Elisa strip plate	1	2-8 ℃
Standard：135pg/ml	0.5 ml × 1 bottle	2-8 ℃
Standard diluent	1.5 ml × 1 bottle	2-8 ℃
HRP-Conjugate reagent	6 ml × 1 bottle	2-8 ℃
Sample diluent	6 ml ×1 bottle	2-8 ℃
Chromogen Solution A	6 ml × 1 bottle	2-8 ℃
Chromogen Solution B	6 ml × 1 bottle	2-8 ℃
Stop Solution	6 ml × 1 bottle	2-8 ℃
wash solution	（20 ml ×30 fold) ×1bottle	2-8 ℃

Preparation of reagents:

The stock solutions were produced according to the kit's instructions. Before use, all reagents were freshly prepared at ambient temperature.

Dilution of Standards:

Dilute the standard in small tubes, then pipette 50ul from each tube into the well of the microplate; each tube contains two wells.

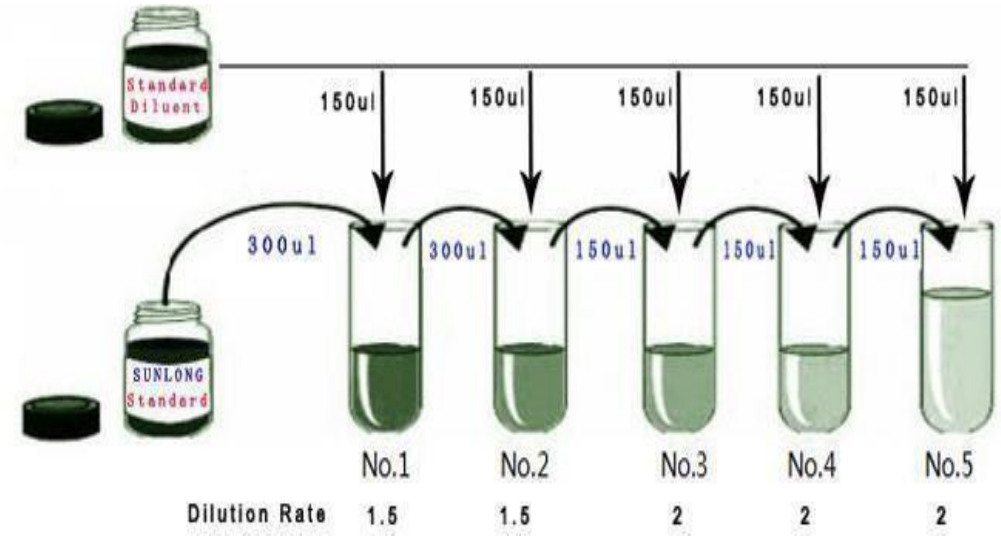


Table 2.7: Dilution of Standards

60 U/ml	**Standard No.1**	**300µl Original Standard + 150µl Standard diluents**
40 U/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
20 U/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
10 U/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
5 U/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

Procedures:

1. Sample dilution and standard addition: 10 standard wells were placed on coated ELISA plates, 100 L standard was added to the first and second wells, then the 50 L standard dilution was added to the first and second wells, and mixed; Remove 100 liters from the first and second wells and add it separately to the third and fourth wells. then add the standard 50-litre dilution to the third and fourth wells and mix; then removed 50 liters from the third and fourth wells, then dumped and added 50 liters to the fifth and sixth wells; Then add a 50 L standard dilution to the fifth and sixth wells and mix; 50 liters were removed from the fifth and sixth wells and the seventh and eighth wells were added; Then a 50 L standard dilution was added to the seventh and eighth wells and then the sample was added: set the empty wells separately (empty comparison wells add sample and HRP- Conjugate reagent; the other each process step is the same). Testsample well. 40 μL sample dilution was added to the test sample well, then the 10 μL test sample (final 5-fold dilution sample) was added, andthen the sample was added to the wells, the wall of the well be touched as much as possible, and gently mixed.
2. Incubate: After closing the plate with a plate-closing membrane, incubate for 30 minutes at 37℃.
3. Composition fluid: washing solution 30-fold diluted 30-fold with distilled water and reserve.
4. The exposed closure plate membrane was washed, liquid discarded, swing dried, wash solution added to each well, still for 30 seconds, filtered, repeated 5 times, and patted dry.

5. A volume of 50 µL of HRP-Conjugate reagent was added to each well except for theempty control well.
6. Incubation is described in Step 3.
7. Washing was done as described in step 3
8. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes.
9. Termination: 50 µL of stop solution was added to each well to terminate the reaction. The color of the well should change from blue toyellow.
10. The O.D. absorbance was read. at 450 nm using a microtiter plate reader. The OD value of the blank control well was set as zero. The assay should be performed within 15 minutes after the addition of the stop solution.

Calculation of result

Took the standard density as horizontal and the OD value as vertical, drew the standard curve on graph paper and then determined the corresponding density according to the sample OD value by the sample curve, multiplied by the dilution multiple, or calculated the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, and then determined the sample density, multiplied by the dilution factor.

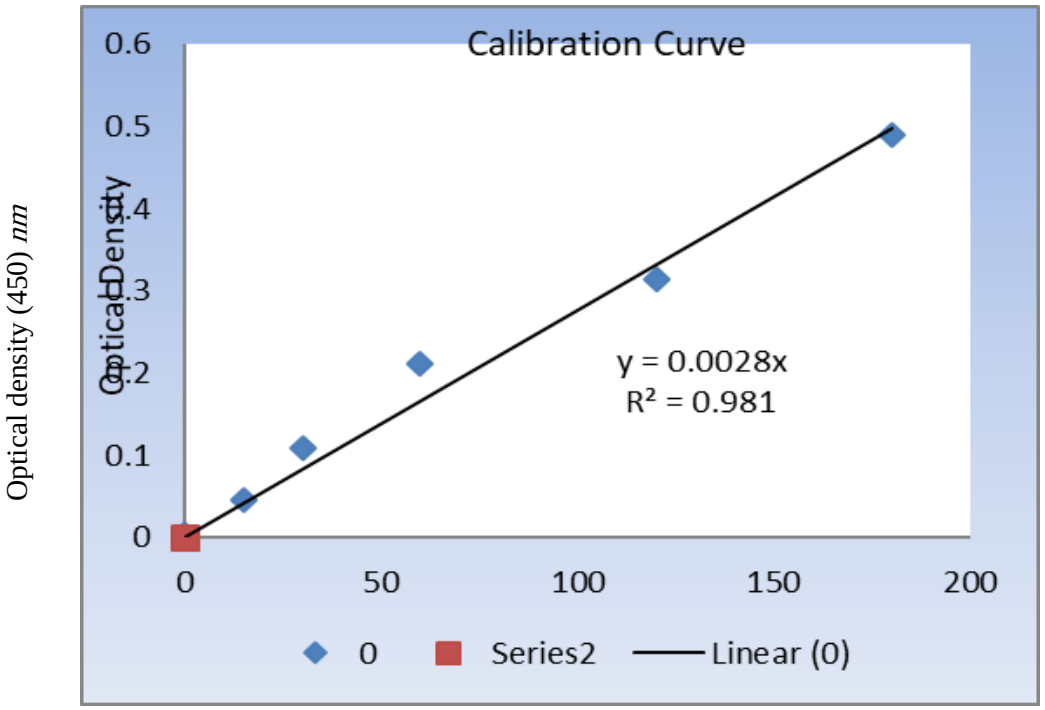


Fig. (2.5): Standard curve of C-Peptide concentration estimation in serum

2.3.8. Estimation of blood glucose concentration

Principle:

Estimation of blood glucose concentration in serum using the "Enzymatic colorimetric method" involves the oxidation of glucose to D-gluconate (gluconic acid) by glucose oxidase (GOD) with the production of hydrogen peroxide, followed by the oxidation of phenol and 4-amino antipyrine (4-AA) by peroxidase (POD) to form a red colour complex whose absorbance (measured at 500 nm) is proportional **(Green *et al.*, 1987),(Trinder, 1969)**.

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-Gluconate} + H_2O_2$$

$$\text{4-Aminoantuipyrine} + \text{4-Phenol} + 2H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents

Table 2-8: Reagents for determination of Glucose Concentration

	Content	Concentration
Reagent 1	Phosphate buffer	100 mmol/L, pH 7.5
	glucose oxidase (GOD)	10 KU/L
	Peroxidase (POD)	2 KU/L
	Amino antipyrine (4-AA)	0.5 mmol/L
	Phenol	5 mmol/L

Calculation:

Automatic calculation by using the Roche COBAS c311.

2.3.9. Determination of HbA1c%

Estimation of glycated hemoglobin (HbA1c) using "COBAS INTEGRA 400 plus" and "COBAS HbA1c kit," the normal concentration being less than 7% and the risk concentraton being equal to or greater than 7%.

Principle

This method is based on using " Immune turbidimetric assay ", which involves the first addition of Tetradecyl Tri methyl Ammonium Bromide (TTAB) on the whole blood as the hemolytic reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes), the second step involved the addition of R1 (Antibody reagent) glycol hemoglobin (HbA1c) which glycated at N-terminal of B-chain in the sample react with (anti-HbA1c AB) and cause agglutination. The agglutination rate is measured photometrically at 550 nm and is proportional to the HbA1c concentration. The final result is promptly calculated and reported as a percentage of HbA1c. **(Metus *et al.*, 1999)**.

2.4. Molecular study

2.4.1. DNA extraction

Patient and control blood samples were collected in EDTA containers, and DNA was extracted from whole-blood samples using the ReliaPrepTM Blood DNA Miniprep System (Promega).

Principle:

Proteinase K was utilized by the Principle DNA extraction reagent to lyse cells and degrade protein, allowing DNA to bind to the matrix of the spin column. Using the inundated buffer, contaminants were eliminated, and the elution buffer was used to elute genomic DNA that had been purified. The DNA was appropriate for PCR and agarose gel analysis after purification.

Protocol of DNA extraction from f blood:

1. The blood sample was mixed for at least 10 minutes in a rotisserie shaker at room temperature. If the blood was frozen, then the sample was left to thaw completely before mixing for 10 minutes.

2. A volume of 20 µL of proteinase K (PK) solution was poured into a 1.5 mL microcentrifuge tube.

3. Blood (200 µl) was added and mixed briefly into the tube containing proteinase K (PK) solution.

4. A volume of 200 µL of cell lysis solution (CLD) was added to the tube, then capped and taxed for at least 10 seconds to mix.

5. The tube was incubated for 10 minutes at 56 °C.

6. A ReliaPrepTM ligation column was inserted into an empty collection tube while the blood sample was incubated.

7. The tube was taken out from the heating block, 250 µL of the binding buffer solution was added, the tube was sealed, and it was rotated with a vortex mixer for 10 seconds to mix. At this point, the analyzer is dark green

8. The contents of the tube were placed into a ReliaPrepTM linking column, capped, and placed in a microcentrifuge.

9. Centrifuge for 1 minute at full speed (14,000 rpm). Check the binding column to ensure that the solution has passed completely through the membrane.

10. The collection tube containing the flow-through was removed, and the liquid was disposed of as hazardous waste.

11. The ligation column was placed in a brand new collection tube, 500 µl of column wash solution was poured into the column, and the centrifuge was set to its highest speed for 3 min. Then the flux was discarded.

12. Step 11 was repeated twice for a total of three washes

13. The column was placed in a clean 1.5 mL microcentrifuge tube.

14. Nuclease-Free Water (50–200µl) was added to the column, and centrifuged for 1 minute at maximum speed (14000 x g).**Note:** Eluting in 50 µl considerably boosts DNA concentration but results in a 25–30% reduction in yield.

15. The ReliaPrep™ Binding Column was discarded, and elute was saved at -23oC **(Chacon Cortes & Griffiths, 2014).**

2.4.2. Estimation of DNA concentration and purity

Principle:

Maximum absorbance for nucleic acids and proteins occurs at 260 and 280 nm, respectively. The ratio of absorbances at these wavelengths has been used as a measure of purity in both nucleic acid and protein extractions. When the ratio is between (1.8 - 2.0) DNA is commonly considered to be pure. The ratio A260/A230 is routinely calculated because absorbance at 230 nm is also considered to be the consequence of additional contamination. Concerning purified nucleic acid, the 260/230 values are frequently higher than the expected 260/280 values. 260/230 readings typically range from 2.0 to 2.2. (Analysis based on the wavelength of DNA or RNA. 260/230 Ratios)

"Some contaminants, such as phenol, have unique properties. However, many pollutants share similar characteristics. Below 230 nm, there is absorption. When interpreting anomalous 260/230 readings, it is essential to consider both the sample and the extraction procedure (Assessment of Nucleic Acid Purity, nanodrop.com). 260/280 Ratios "Abnormal 260/280 ratios typically indicate that the sample is contaminated with protein or a reagent such as phenol, or that the measurement was flawed.

Procedure

1. The unit was switched on and allowed to finish its startup calibration.

2. The sample blanks were prepared (reference) in the same solution used to dissolve the sample.

3. The sample solutions were prepared.

4. The cuvette was cleaned with a wipe.

5. Blank solution (1 µl) was added and analyzed as a reference.

6. Then, 1 µl of the sample was added and the concentration value was displayed on the screen of the Bio drop device.

7. The cuvette was cleaned with a wipe.

2.4.3. Polymerase chain reaction

In a few hours, PCR can amplify a small volume of template DNA OR RNA into large quantities. This was accomplished by combining the DNAwith primers on the DNA's side (forward and reverse), free nucleotides (dNTPs for DNA, NTPs for RNA), BSMI polymerase (of the thermophile species Thermus aquatics, whose polymerase can tolerate exceptionally high temperatures), and buffer. Alternating between hot and frigid

temperatures denatured and reannealed the DNA, with the polymerase continually adding new complementary strands. **(Butt *et al.*, 2018)** .

There were three steps to any PCR that are cycled about 25-35 times as shown in figure (2-3) which include:

- **Denaturation:** This phase involves separating the double DNA strands into two single strands by heating at approximately 94-98 °C.

- **Annealing:** At (55-65 °C), DNA primers (short single-strand DNA fragments) bind to the extremities of each DNA strand and initiate the reaction.

- **Extension:** This phase takes place at 72-74°C and involves the extension of primers to form a complementary strand to the template strand.

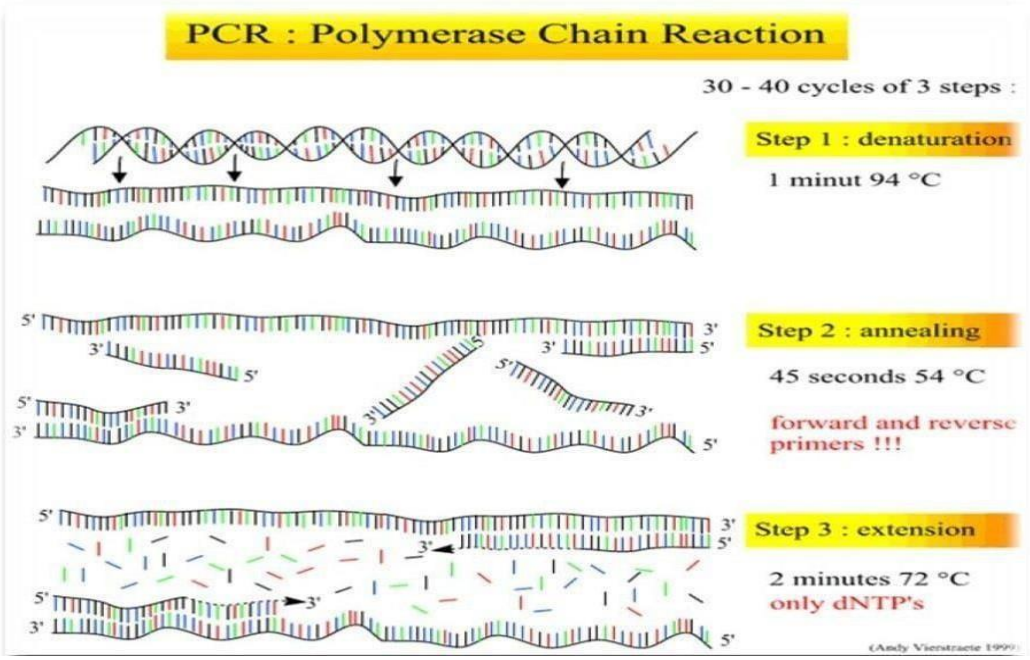


Fig. (2-6): PCR mechanism and experimental steps (Theis *et al.*, 2007)

2.4.4. Amplification of DNA

The target gene (VDR gene) was amplified using ALLEL SPECIFIC - PCR with a specific primer. The ALLEL SPECIFIC -PCR reactions were conducted in 25 *l* volumes in PCR tubes under sterile conditions; the total volume of the reaction mixture was brought to 25 *l* using DDH2O, and the master mix containing optimal concentrations of reaction requirements (MgCl2 1.5 mM, each dNTPs 200 M) was used, table (2-9).

Table 2-9: Components of master mix for detection of VDR gene

No.	Material	Volume(µl)
1	Master Mix	10
2	Forward	1.5
3	Reverse	1.5
4	Template DNA	3
5	DDH2O	7
Total		23µl

2.4.5. Optimization of PCR conditions

Different volumes of primer (0.5 µl,1 µl, 1.5 µl) with different volumes of template DNA (1 µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl) and different temperatures of the reaction conditions were trailed to optimize the conditions of the reaction. PCR tube was centrifuged for 30 seconds at 2000 xg in a micro-centrifuge to mix solutions well at room temperature then tubes were placed in the thermocycler to start the reaction. Programs

of the PCR protocol reaction for VDR gene polymorphism for BSMI (rs1544410) were illustrated in tables (2-4) in rs1544410 (200) bp band indicated the presence of the allele; if no amplification product, this means the absence of an allele in the gel. See table (2-10).

Table 2-10: Allele-specific –PCR program for detection of VDR gene polymorphism rs1544410 SNP

Type of Cycle	Temperature, ^{o}C	Time	No. of Cycles
Initial denaturation	95 ^{o}C	3 min	1
Denaturation	95 ^{o}C	30 Sec	30
Annealing	60 ^{o}C	35 Sec	
Extension	72 ^{o}C	55 Sec	
Final extension	72 ^{o}C	5 min	1
Total time: 1 hour and 50 min			

Table 2-11: Bands obtained from the amplification product of VDR, (rs1544410) SNPs

No of bands	(rs1544410)	Size of bands, (bp)
Wild Type	CC	200 C
Heterozygous	CT	200 C 200 T
Homozygous	TT	

2.4.6. Primers preparation

A primer is a short, single-strand DNA fragment consisting of oligonucleotides (18–22 bases in length) that are complementary to the target DNA region. In the absence of this chemical, the amplification process cannot be initiated on a single DNA molecule as it was in the past. Therefore, it must be annealed to the single DNA strands that result from denaturing double-stranded DNA.

A pair of primers designed for the VDR gene was used to conduct the polymerase chain reaction. Using Genius software, all gene information and SNPs details were compiled based on the NCBI database.

Preparation of the primers in the following steps:

Materials: Lyophilized primers, Sterile dH20

1. The tube was rolled up before the cap was opened.
2. A master stock was prepared, pmoles/µl, and the required amount of sterile dH_20 was added according to the manufacturer to obtain 100pmoles/µl (Master Stock).
3. The tube has been properly mixed to evenly resuspend the primers.
4. To prepare 10 µL of working stock, ten µL of master stock was transferred into a 0.5 mL Eppendorf tube containing 90 µL of sterile dH20 (working stock).
5. The master stock was stored at -20 °C.
6. The sequences of primers used for PCR amplification of the VDR rs1544410 gene are shown in Tables (2-12).

Table 2-12: Specific primers of *BSMI* gene (rs1544410).

Primer	Sequence (5´ –3´)	Allele	Size (bp)	Company
FC	5-AGAACCATCTCTCAGGCTCC-3	C	200	BIONEER/ Korea
FT	5-AGAACCATCTCTCAGGCTCT-3	T	200	BIONEER/ Korea
R	5-CCTCACTGCCCTTAGCTCTG-3	——	200	BIONEER/ Korea

Agarose gel electrophoresis

The presence of amplification was confirmed by agarose gel electrophoresis following PCR amplification. PCR was entirely dependent on the purified DNA standards.

Procedure

One hundred milliliters of a 1.5% agarose solution were prepared by the following steps.

A. Preparation of solution

1X TBE buffer (tris borate EDTA) was prepared by diluting 10X TBE buffer with deionized water (one volume of 10X TBE buffer with 9volumes of deionized water: 1.10 dilution).

Preparation of agarose gel

1. 1.5 grams of agarose were weighed and placed into a conical flask, and then 100 ml of 1X (TBE) buffer was added and mixed gently.
2. The solution was heated on a hot plate until the agarose is dissolved and the solution was clear and then the solution was allowed to cool to about 50°C before pouring.
3. A concentration of 4 µl of ethidium bromide was added to the solution.
4. The comb was placed in the gel chamber about 1 inch from one end of the tray.
5. The gel solution was cast into the chamber and allowed to solidify for about 1 hour at room temperature.
6. The comb was removed, and then the chamber is placed in a horizontal electrophoresis system and covered (just until wells were submerged) with the same TBE buffer that was used to prepare the gel.
7. Samples were loaded (3µl) on each well with extreme caution to avoid damage to the wells and cross-contamination of neighbouring wells.
8. The cathode was connected to the well side of the unit and the anode to the other side.
9. Electrophoresis was done at 60 volts, for 30-35 minutes or until the dye markers have migrated an appropriate distance, depending on the size of the DNA to be visualized.

Loading of samples:

The comb was removed and the glass plates were placed in a vertical electrophoresis chamber, 1 X TBE buffer was added inside the chamber until reaching above the level of wells, and then the samples were loaded into the gel wells by using 10 µl micropipettes.

Electrophoresis conditions:

Negatively charged nucleic acids move across the gel toward the positive (+) electrode as a result of an electric field being given to the system (60 V, 45 mA for 35 min.) after sample loading (anode). Ethidium bromide was used to dye the agarose gel.

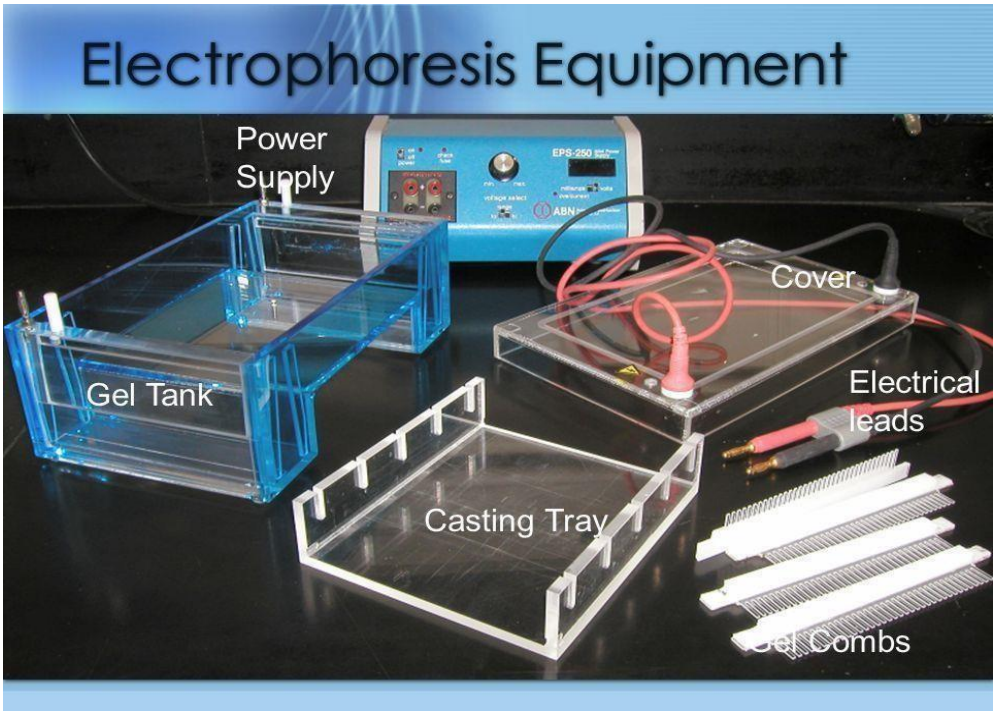


Fig. (2-7): Agarose Gel Electrophoresis

Photo documentation system

Agarose gel was placed above the UV transilluminator device and exposed to UV light. The photos were captured using a digital camera andvisualized by a PC connected to the transilluminator. The UV transilluminator device was covered with a protective shield to avoidexposure to UV light when the light was on.

Statistical analysis

Information obtained by questionnaire from all participants was Interred into a data sheet and assigned a serial identifier number. Multiple entries were used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020). Descriptive statistics were performed on the participants' data of each group. Values were illustrated by n (%) for categorical. The data distribution was checked using the Shapiro-Wilk test as numerical means of assessing normality.

The association between the analyzed factors was estimated using odds ratios (ORs) and a 95% Confidence Interval Range calculated by a non-conditional logistic regression.

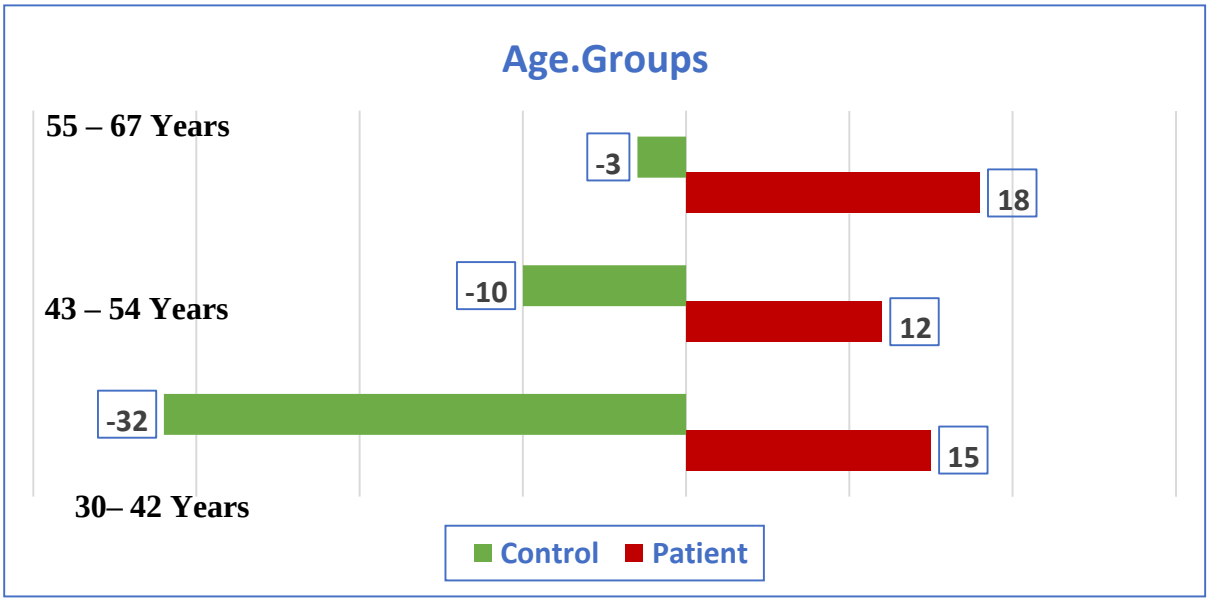
Significant differences in categorical variables among the parameters were confirmed through analytical and statistical tests. Results of all hypothesis test with p-values <0.05 (two-sided) were considered statistically significant.

3. Results

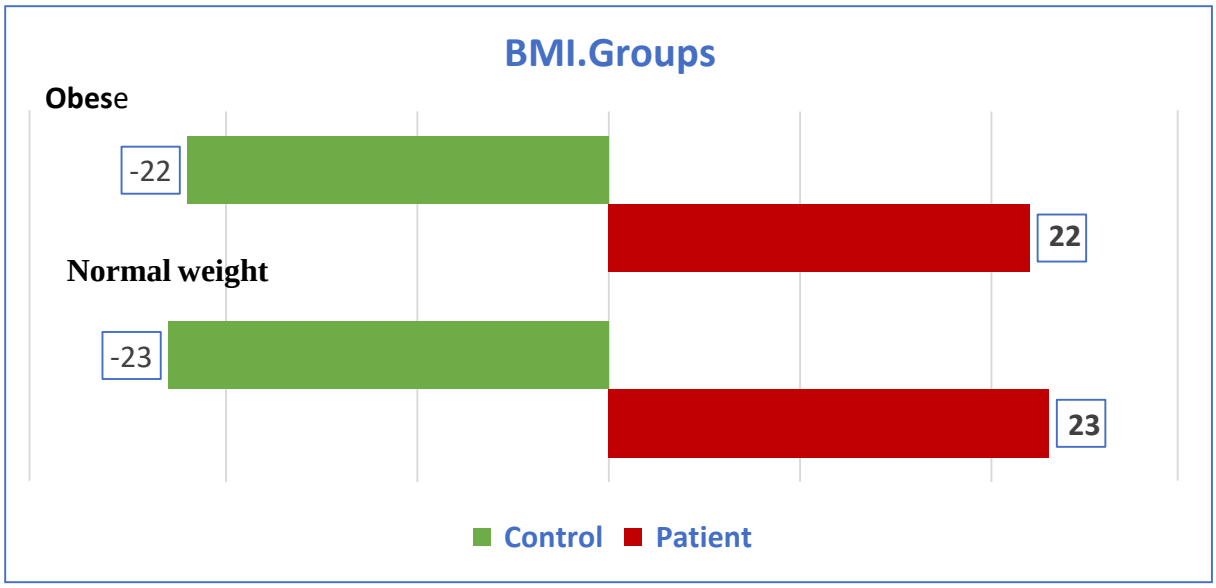
3.1 Demographic and clinical characteristics

The clinical demographic characteristics of patients group were summarized in Figure (3.1). The age range of participants was within (30-67) years old, (33.4%) patient for groups (30- 42), (26.6%) patient for groups (43-54), and (40%) for groups (55-67). The percentage of participants were nonsmokers (98%) and about half of them (55.5%) were reported to have hypertension. Furthermore, 51% of the patient group were within normal weight and 49% were obese. About 95.6% of patients were high score of Waist-to-hip ratio, and both Moderate and low score of Waist-to-hip ratio were 2.2% , data were presented in figures (3.1), (3.2), (3.3), (3.4), and (3.5)

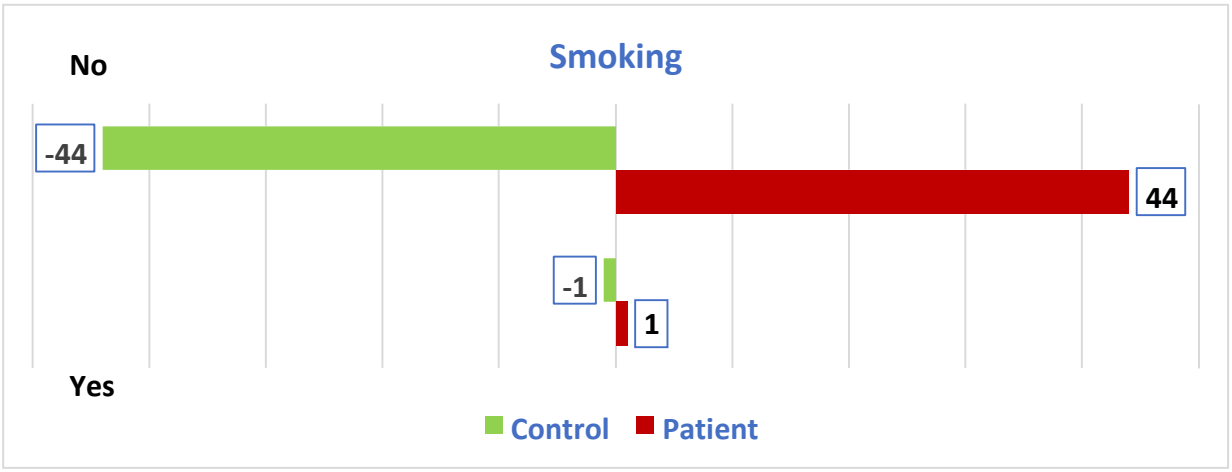
A



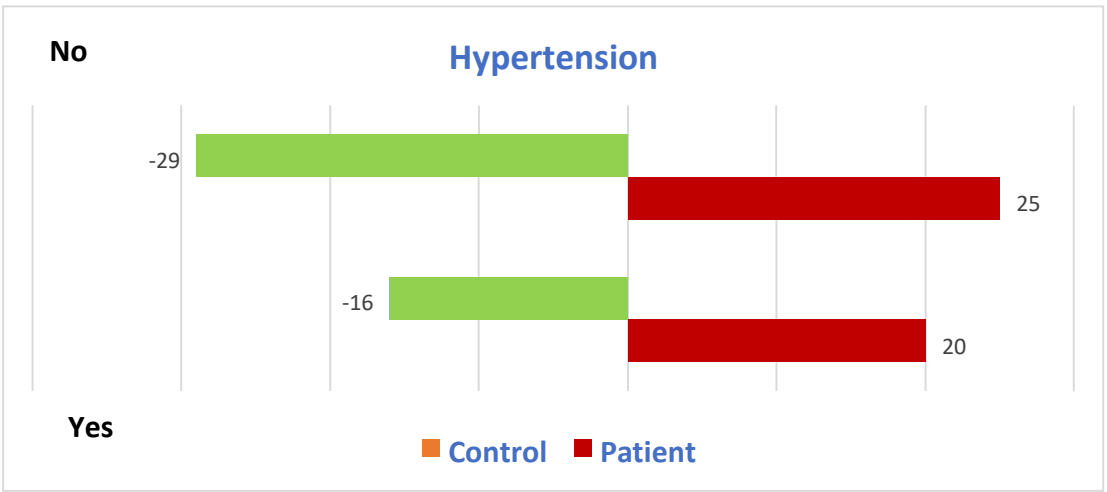
B


BMI.Groups
Obese
-22
22
Normal weight
-23
23
Control
Patient

C


Smoking
No
-44
44
-1
1
Yes
Control
Patient

D


No
Hypertension
-29
25
-16
20
Yes
Control
Patient

E

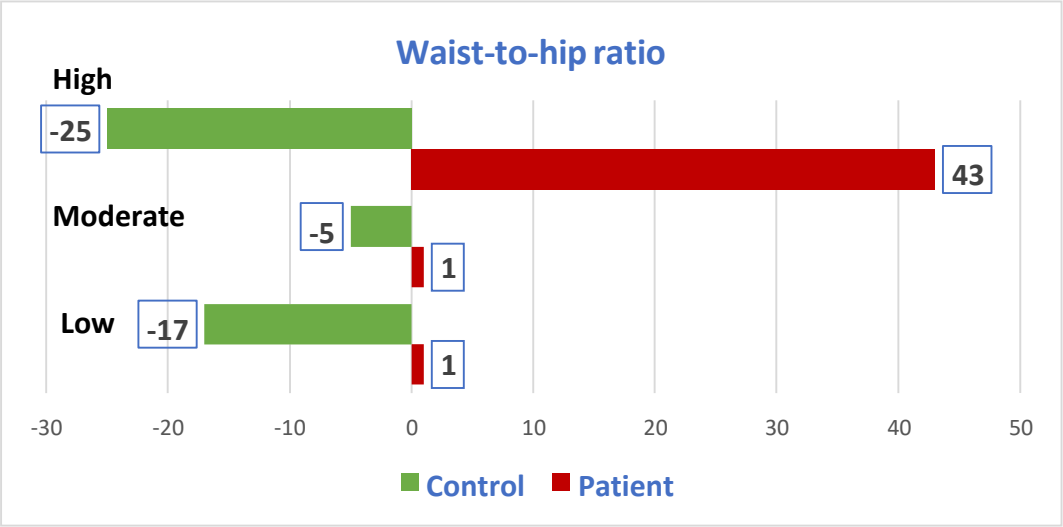


Figure (3.1), (3.2), (3.3), (3.4), and (3.5) Baseline characteristics and Demographic Descriptive of the study population inpatients compared to control group, the number of participant (n= 100): (A) Distribution of Age, (B) Distribution of BMI, (C) Distribution of Smoking status and (D) Distribution of blood pressure and (E) Distribution of Waist-to-hip ratio

Table 3.1: Results of the analysis of basic diabetic characteristics between case and control.

Biomarkers	Patients Mean ± SD N=45	Control Mean ± SD N=45	P value
HSP-70, ng/ml	30.22±6.30	29.11±7.65	0.445[NS]
VDBP, ng/ml	69.21±18.65	73.37±18.94	0.320[NS]
C-peptide, ng/ml	122.82±64.68	163.43±94.15	**0.020[S]**
RBS, mg/dl	269.87±69.43	98.29±12.57	**<0.001[S]**
Insulin, ng/ml	19.85±4.34	3.71±1.44	**<0.001[S]**
HOMA-IR	4.71±1.36	2.27±0.70	**<0.001[S]**
25(OH)D_3, ng/ml	6.72±1.87	20.64±8.29	**<0.001[S]**
HbA1c%	9.58±2.07	4.36±0.69	**<0.001[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05considered significantly different, [S]= Significant, [NS]= non-significant			

Table (3.2) illustrates the result of the biochemical parameters in the Patients and controlgroups according to the Age groups (30-42) years. The sera levels of HSP-70 and VDBP were not showing any significant differences in the patient group with the Age compared to the control. The sera level of insulin was increased significantly in the patient's group compared to the control group; the mean level were (19.92 ± 4.69, 3.41 ± 1.27) respectively. (P.value < 0.05). The sera level of 25(OH)D_3 decreased significantly in the patient's group compared to the control group; the mean levels were (6.48 ± 1.78, 20.30 ± 8.59), respectively (P.value < 0.05). The sera level of C-peptide was decreased significantly in the patient's groupcompared to the control; the mean level were (113.49 ± 27.5 , 159.2 ± 101.4) respectively (P.value < 0.05). While the level of RBS and HbA1c were increased markedly in the patients' group, the same Age range compared to the control. The mean level of RBS and HbA1c in patients were (258.58 ± 41.4, 9.36 ± 1.89) respectively, while in control were (98.1 ± 12.53 and 4.33 ± 0.71) respectively.

Table (3.2)The effect of Age range (30-42) year on the biochemical parameters in Patients and control groups.

Biomarkers	Age range (30-42) year		
	Patients Mean ± SD	**Control Mean** ± SD	**P value**
HSP-70, ng/ml	30.55 ± 5.40	30.64 ± 7.62	0.94[NS]
VDBP, ng/ml	73.48 ± 13.53	73.15 ± 19.94	0.96[NS]
C-peptide, ng/ml	113.49 ± 27.5	159.2 ± 101.4	**0.01[S]**
RBS, mg/dl	258.58 ± 41.4	98.1 ± 12.53	**<0.001[S]**
Insulin, ng/ml	19.92 ± 4.69	3.41 ± 1.27	**<0.001[S]**
HOMA-IR	4.67 ± 1.46	2.03 ± 0.72	**<0.001[S]**
25(OH)D3, ng/ml	6.48 ± 1.78	20.30 ± 8.59	**<0.001[S]**
HbA1c%	9.36 ± 1.89	4.33 ± 0.71	**<0.001[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant			

Table (3.3) the Age group (**43-54**), results indicated no- statistical difference in the sera levels of HSP-70 and VDBP. The sera level of RBS and HbA1c were increased markedly in the patient's group with the same Age range compared to the control. the mean level of RBS and HbA1c% in patients were (290.77±77.94, 9.99 ±2.08) respectively (P.value < 0.05). while in control were (101.83 ± 11.15, 4.57 ± 0.74), respectively (P.value < 0.05). while only the C-Peptide sera level was decreased in the same age rangeof patients compared to the control was, but not significant. The sera level of insulin was increased significantly in the patient's group compared to the control group; the mean level were (18.72 ± 3.05, 4.17 ± 1.34) respectively (P.value < 0.05). The sera level of 25(OH)D_3 decreased significantly in the patient's group compared to the control group; the mean levels were (7.32 ± 1.87, 21.47± 8.96), respectively (P.value < 0.05).

Table (3.3): The effect of Age range (43-54) years on the biochemicalparameters in Patients and control groups.

Biomarkers	Age range (43-54) year		
	Patient Mean ±SD	Control Mean ±SD	P value
HSP-70,ng/ml	31.14 ± 6.42	27.10 ± 6.36	0.17[NS]
C-Peptide ng/ml	151.59 ± 112.1	164.47 ± 61.92	0.72[NS]
VDBP, ng/ml	75.05 ± 17.53	64.55 ± 13.88	0.15[NS]
RBS, mg/dl	290.77 ± 77.94	101.83 ± 11.15	**<0.001[S]**
Insulin, ng/ml	18.72 ± 3.05	4.17 ± 1.34	**<0.001[S]**
HOMA-IR	4.55 ± 1.00	2.78 ± 0.54	**<0.001[S]**
25(OH)D3,ng/ml	7.32 ± 1.87	21.47 ± 8.96	**<0.001[S]**
HbA1c%	9.99 ± 2.08	4.57 ± 0.74	**<0.001[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05considered significantly different, [S]= Significant, [NS]= non-significant			

While in the age group (55-76) in table (3.4), All markers were shown a significant difference among patients compared to the control. The sera level of VDBP was increased significantly in patients compared to the control, the mean levels were (70.51 ± 18.78, 46.62 ± 13.21) respectively, (P value <0.05). The sera level of insulin was increased significantly in the patient's group compared to the control group; the mean level were (18.72 ± 3.05, 4.17 ± 1.34), respectively (P.value < 0.05). The sera levels HSP-70, RBS and HbA1c were also increased significantly in patients group compared to health control. The mean levels in patients were (29.34±7.11, 267.83 ±77.75 and 9.32±2.17) respectively, while in control (19.52±7.11, 101.67±22.50 and 3.83±0.51) respectively, (P value <0.05). Only C-Peptide level was decreased significantly in the patients compared to the control group. The mean level were (107.55±18.25,147.10±47.13) respectively, (P.value<0.05). The sera level of 25(OH)D_3 decreased significantly in the patient's group compared to the control group; the mean levels were (7.32 ± 1.87, 21.47 ± 8.96), respectively (P.value < 0.05).

Table (3.4): The effect of Age range (55 – 76) years on the biochemicalparameters inPatients and control groups.

Biomarkers	Age range (55 – 76) years		
	Patients Mean ± SD	Control Mean ± SD	P value
HSP70,ng/ml	29.34 ± 7.11	19.52 ± 7.11	**0.032[S]**
VDBP, ng/ml	70.51 ± 18.78	46.62 ± 13.21	**0.05[S]**
C- peptide, ng/ml	107.55 ± 18.25	147.10 ± 47.13	**0.013[S]**
RBS, mg/dl	267.83 ± 77.75	101.67 ± 22.50	**0.002[S]**
Insulin, ng/ml	18.72 ± 3.05	4.17 ± 1.34	**<0.001[S]**
HOMA-IR	4.55 ± 1.00	2.78 ± 0.54	**<0.001[S]**
25(OH)D3,ng/ml	7.32 ± 1.87	21.47 ± 8.96	**<0.001[S]**
HbA1c%	9.32 ± 2.17	3.83 ± 0.51	**<0.001[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05considered significantly different, [S]= Significant, [NS]= non-significant			

Table (3.82) illustrated that effect of the biochemical parameters in the Patients control groups according to the BMI groups. The level of HSP70 and VDBP were increased significantly in patients with normal weight group compared to control. The mean levels in normal weight patients and control were (30.31 ± 6.88, 73.61 ± 17.25), and in control (26.15 ± 6.84 and 62.50 ± 18.29) respectively. The level of RBS, HbA1c% were also increased significantly in patients with normal weight group compared to control as presented in Table (3.5). Only C-peptide level was decreased significantly in both patients compared to control, the mean level in patients was 107.82 ±21.23 and in control was (176.44 ± 111.53).

Table (3.5): The effect of BMI groups on the biochemical parameters in the patients and control groups

Variables	Normal Weight		
	Patients Mean ±SD	**Control Mean** ±SD	**P value**
HSP70,ng/ml	30.31 ± 6.88	26.15 ± 6.84	**0.040[S]**
VDBP, ng,ml	73.61 ± 17.25	62.50 ± 18.29	**0.046[S]**
C-Peptide, ng/ml	107.82 ± 21.23	176.44 ± 111.53	**0.004[S]**
Insulin, ng/ml	19.60 ± 4.67	3.65 ± 1.51	**<0.001[S]**
HOMA-IR	4.69 ± 1.40	2.30 ± 0.65	**<0.001[S]**
25(OH)D_3,ng/ml	6.96 ± 1.84	27.07 ± 6.13	**<0.001[S]**
RBS, mg/dl	279.76 ± 59.33	98.04 ± 13.74	**<0.001[S]**
HbA1c%	9.72 ± 2.20	4.13 ± 0.62	**<0.001[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05considered significantly different, [S]= Significant, [NS]= non-significant			

Table (3.6) illustrated that effect of the biochemical parameters in the Patients and control groups according to the BMI groups. The level of RBS, HbA1c were also increased significantly in patients with normal weight group compared to control as presented while the level of HSP70 , VDBP and C-Peptide were no significant , as shown in Table (3.6)

Table (3.6): The effect of BMI groups on the biochemical parameters in the Patients and control groups.

Variables	Obese		
	Patients Mean ±SD	**Control Mean ± SD**	**P value**
HSP70,ng/ml	30.13±5.80	32.12±7.35	0.303[NS]
VDBP,ng/ml	71.77±16.21	67.73±19.32	0.358[NS]
C-Peptide, ng/ml	134.69±83.39	143.11±59.49	0.68[NS]
Insulin, ng/ml	20.10±4.06	3.76±1.39	**<0.001[S]**
HOMA-IR	4.73±1.35	2.24±0.77	**<0.001[S]**
25(OH)D3,ng/ml	6.47±1.91	13.91±3.44	**<0.001[S]**
RBS, mg/dl	260.80±72.38	100.48±11.64	**<0.001[S]**
HbA1c%	9.30±1.85	4.58±0.75	**<0.001[S]**
W.H.R	1.06±0.05	1.02±0.03	**0.006[S]**
T.test. Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05considered significantly different, [S]= Significant, [NS]= non-significant			

Binary logistic regression was performed and forward logistic regression was adopted to analyze the results. It was found that VDBP in normal weight patient was

a protective factor (OR: 0.917; 95% CI: (0.733-1.148) respectively, while in obese groups was (OR: 0.886; 95% CI: (0.996-1.013).

In addition, C- Peptide & HSP70 were shown to be a risk factor for both normal and obese cases. In Normal weight (OR: 1.049 and 1.156, 95% CI: (1.011-1.088), 1.649-1.148) , while in obese (OR: 1.001 and

1.545, 95% CI: (0.992-1.08), (1.011-1.119) as shown in table (3.7).

Table (3.7) Estimation the Associated of analyzed factors in Patients Compared to control group

Variables	Normal weight		Obese	
	OR (Lower– Upper)	P value	OR (Lower–Upper)	P value
HSP70,ng/ml	1.156(1.649-1.148)	0.622	1.545(1.011-1.119)	0.327
C-Peptide	1.049(1.011-1.088)	**0.010**	1.001 (0.992-1.08)	0.763
VDBP,ng/ml	0.917(0.733-1.148)	0.415	0.886 (0.996-1.013)	0.381
HOMA-IR	15.68(3.169-3.196)	**<0.001**	14.878(2.511-88.158)	**0.003**

The mean levels compression of the studied groups of patients and Healthy control according to the BMI subgroups were presented in theFigures (3.2), (3.3) and (3.4) below.

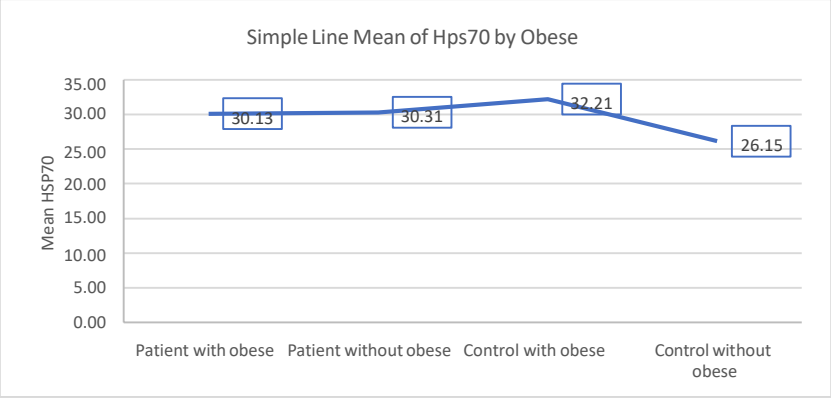


Figure (3.6): Estimation plot of Determination serum level of Hps70 in Patient and control groups

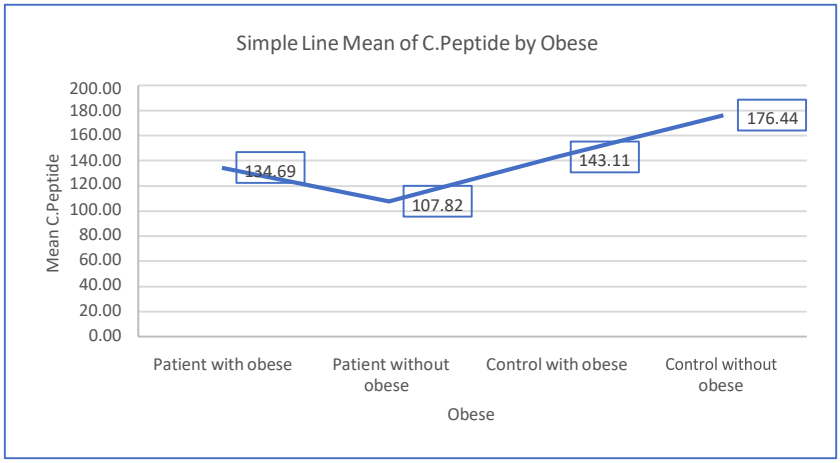


Figure (3.7) Estimation plot of Determination serum level of c-peptide in patient and control groups

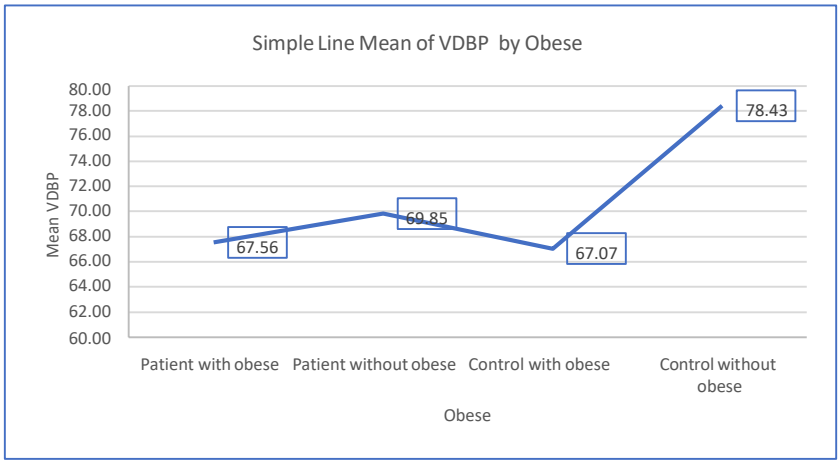


Figure (3.8) Estimation plot of Determination serum level of VDBP in patient and control groups

Receiver operating curve (ROC) curve of serum HSP70, C-peptide and VDBP levels for diagnosis of DM cases.

Furthermore, the analysis of the optimal diagnostic points in cases of DM was performed. Results were indicated that C-peptide was demonstrated the most interesting significant prediction DM in obese cases. Diagnostic thresholds of such cases were presented in figures (3.5), and table (3.8). For C-peptide levels: (sensitivity = 93%, specificity = 88%) at a level = 134.7646, p-values of the AUC for C- peptide was <0.001 and statistically significant.

Table (3.8) Receiver operating characteristic curve showing sensitivity and specificity of Hps70, VDBP **& C-peptide in patients compared to control group**

Test Result Variable(s)	HSP-70	C-peptide	VDBP
AUC	53.6%	70.2%	54%
Sensitivity %	90%	93%	96%
Specificity %	87%	88%	78%
Youden index	0.2	0.378	0.175
Cut-off points	29.086	134.7646	56.423
CI (95%)	0. 416-0.657	0.594-0.810	0.429 – 0.661
PPV	59.57%	20.68%	41%
NPV	60.46%	8.88%	25%
P value	<0.001	<0.001	0.05

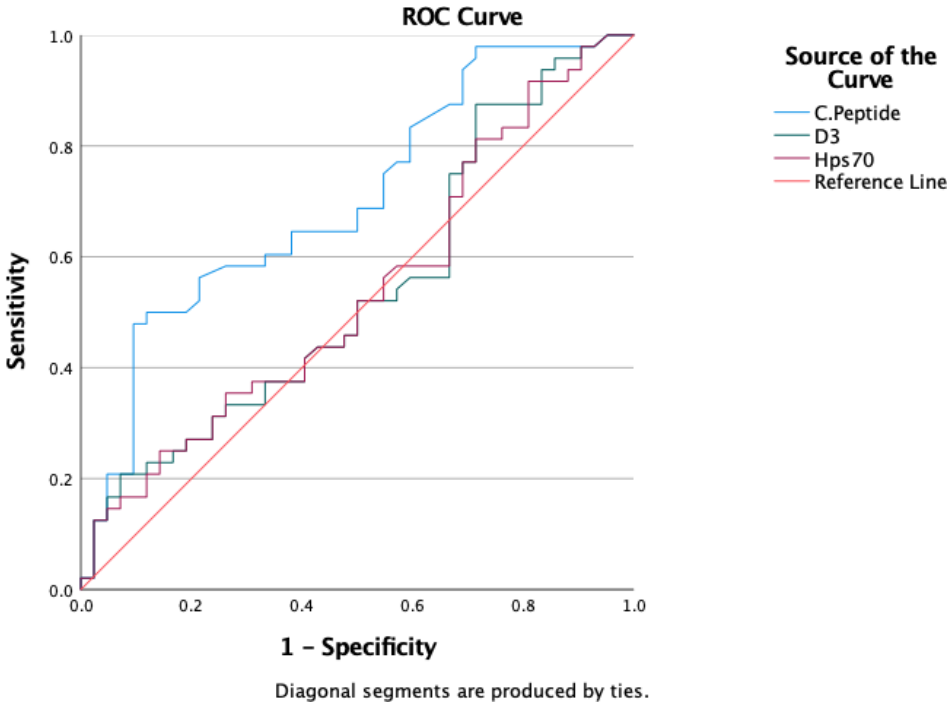


Fig. (3.9): **ROC curves for HSP-70, C-peptide and VDBP in patients to analyse the optimal diagnostic points for predicting of DM cases compared tocontrol group.**

3.2 Results of genetic variants of VDR

Results of genetic variants of VDR

The results of variants Genotype (rs 1544410) was a clear band with a molecular size 200 bps. Figure (3.10). The size of amplicon was determined by compare with DNA ladder 100 - 1500 bp. gene polymorphisms

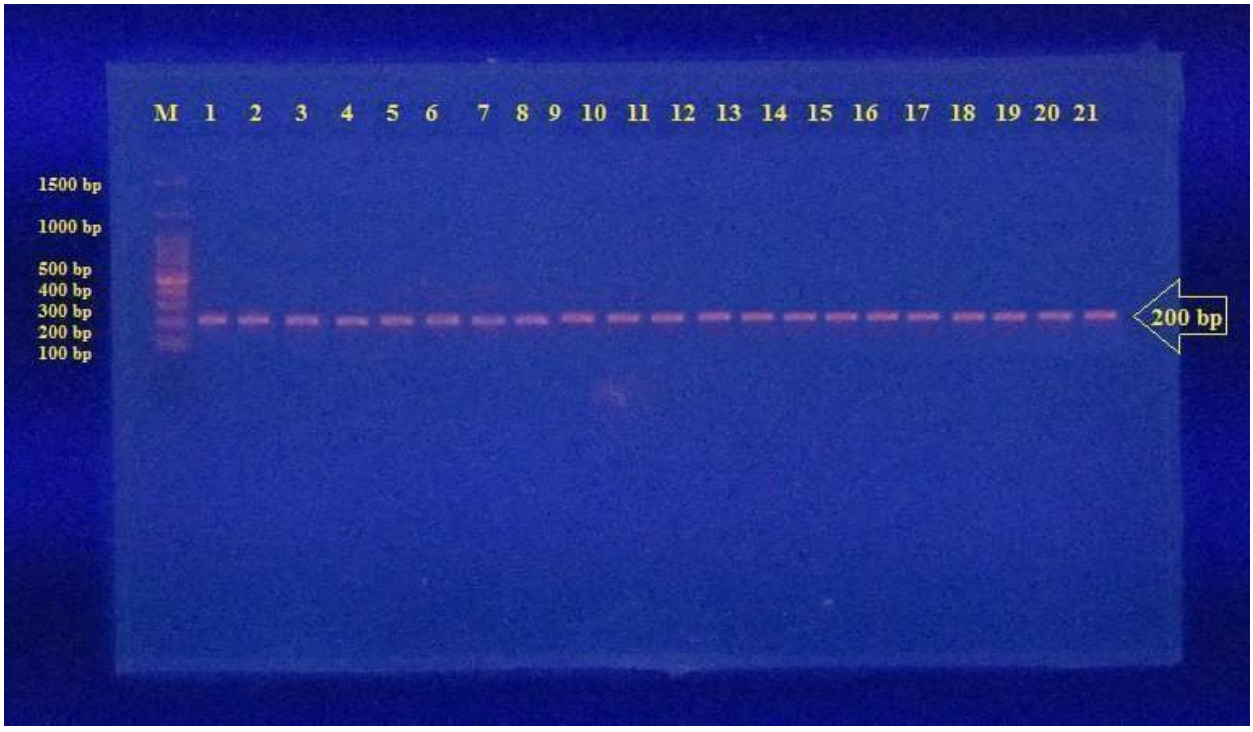
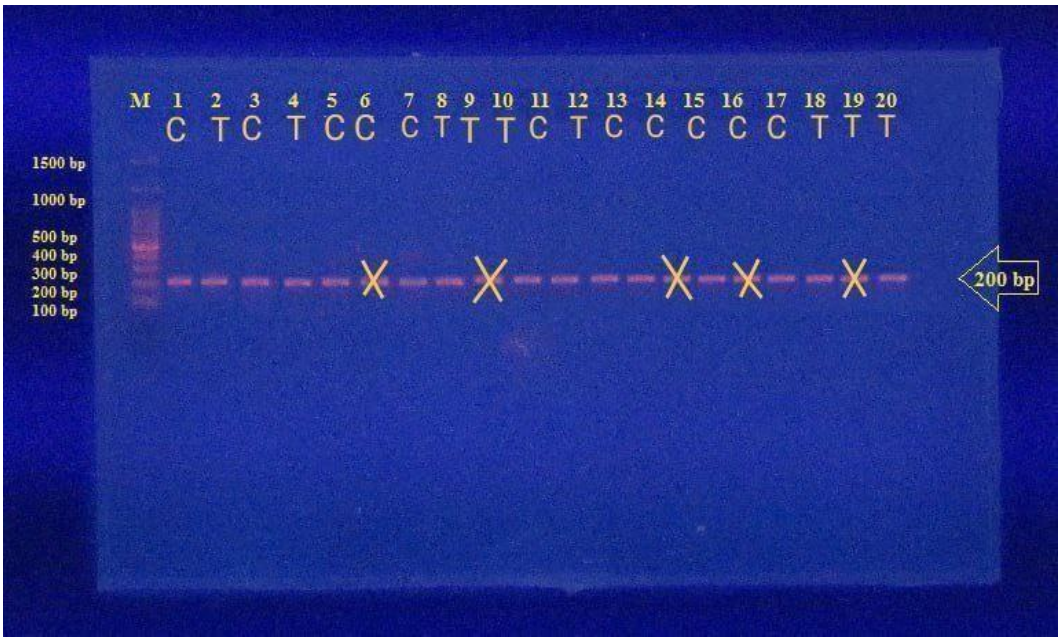


Fig.(3-10): Genotyping of gene variants of VDR **(rs**1544410**)**

Genotype variants of VDR of gene (rs 1544410) SNP which were classified into three genotypes:

1. The major genotype group (CC) homozygous for the allele C.
2. The minor genotype group (TT) homozygous for the allele T.
3. Heterozygous (CT).



Lane 1- 1,2 3,4 7,8 11,12 17,18: Presented CT (Hetero) allele of VDR gene where showed in 200bp

Lane 2- 5,6 13,14 15,16: Presented CC (Wild) allele of VDR gene where showed in 200bp

Lane 3- 9,10 19,20: Presented TT (Homo) allele of VDR gene where showed in 200bp

The distribution of genotyping groups of patients shows in table (3.9).

Table 3.9: Distribution of gene variants of VDR **Genotype (rs** 1544410**) different genotypes in studied groups**

Variable	Group	Frequency	Percentage
Genotype	CC (wild)	18	40
	CT (hetero)	12	24
	TT (homo)	15	36
Data Presented by numbers and percentage			

Results of the comparison between observed and anticipated values for SNIP with (rs1544410) in the tested population were shown in Figure (3.7), and table (10). The distribution and percentage of individuals having (rs1544410) differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected, which were: CC (18, 12.8); TT (15,9.8); CT (12, 22.4) (goodness-of-fit $\chi 2$ for (rs1544410), 19.397, P= < 0.001 and therefore it was statistically significant.

Table (3.10) Hardy–Weinberg equilibrium for (rs1544410) genotype in patient groups

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			C	T	
Genotype N= 100	Frequency	%	0.533	0.467	19.397 P < **0.001** [S]
CC (Wild Type)	18	40			
CT (heterozygous mutant type)	12	24			
TT (homozygous mutant type)	15	36			

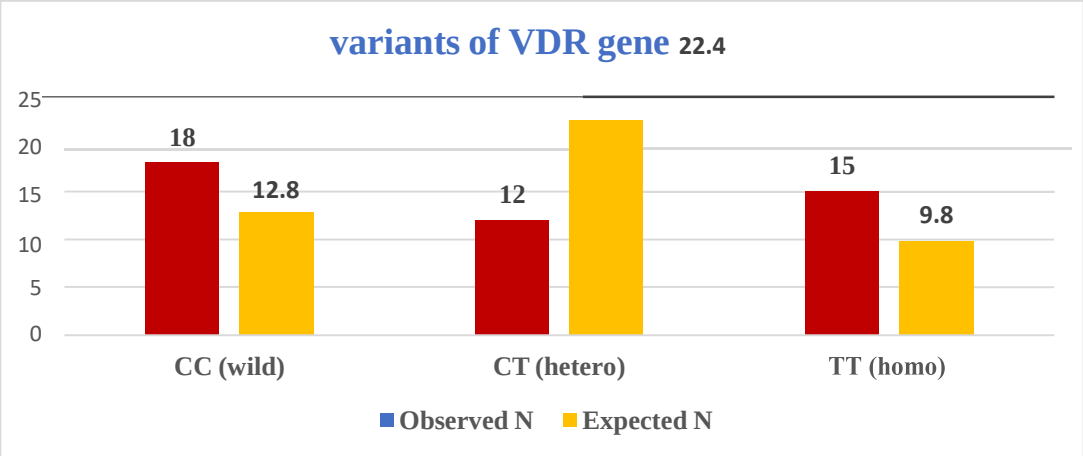


Figure (3.11): variants of VDR Observed (Obs.) vs expected (Exp.) genotype frequencies % ofrs 1544410 among individuals' sample

Relationship between demographic characteristics and (rs 1544410) SNP

The difference between demographic characteristics and (rs1544410) SNP table (3-11), was performed using one-way ANOVA test to compare the age, BMI and groups of study. No significant difference was found between all groups.

Table (3-11) difference between demographic characteristics in (rs1544410) SNP in patient groups

Demographic parameters		rs 1544410 (N= 45)			P-Value
		CC (N=18)	CT (N=12)	TT (N=15)	
Age		42. 33±8. 89	46. 75±12. 32	47. 07±12. 08	0.156 [NS]
BMI		29. 35±6. 39	28. 45±6. 19	27. 18±5. 74	0.363[NS]
Group	Patient	18	12	15	0.903[NS]
Study Group	Patient with obese	10	6	6	0.953[NS]
	Patient without obese	8	6	9	
Bp	Yes	12	11	13	0.563[NS]
	No	24	13	17	
Smoking	Yes	1	1	0	0.564[NS]
	No	35	23	30	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant					

Mean Difference of biomarkers and Genotype (rs 1544410) SNP in Obese groups :

The difference between biomarkers and rs(1544410) SNP table(3-12) was performed using one-way ANOVA test to compare the mean levels of HSP-70, VDBP, C. Peptide , RBC& while blood test , HbA1c was shown all no significant difference among the variants of VDBP Genotype (rs1544410) in obese (Patients & Control) studied groups, p value > 0.05.

Table (3-12) Difference between alleles of variants of VDR Genotype (rs1544410) with mean levels of biomarkers in obese studied groups

Biomarkers	rs 1544410(N= 50)			P value
	CC (N=10)	CT (N=12)	TT (N=28)	
HSP-70	30.22±6.28	28.88±7.06	29.63±7.88	0.769 [NS]
VDBP	73.16±21.42	72.34±16.58	68.94±17.75	0.669 [NS]
C-Peptide	139.72±68.61	163.72±126.68	130.73±46.30	0.334 [NS]
Insulin	3.81±1.39	2.87±1.06	4.26±1.52	0.037[S]
HOMA-IR	2.38±0.77	1.94±0.62	2.41±0.62	0.165[NS]
25(OH)D3	7.51±1.74	6.12±1.79	6.26±1.86	0.06[NS]
RBS	184.19±96.81	197.46±113.47	173.23±92.75	0.678 [NS]
HbA1c	7.19±3.06	7.28±3.69	6.45±2.40	0.521 [NS]
W.H.R	0.97±0.11	0.94±0.11	0.93±0.12	0.337 [NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant				

Estimation of risk in VDR Genotype (rs1544410) SNP with biomarkers

The odds ratios of the detected genotypes of the (rs1544410) of the patients with levels of biomarkers. The logistic analysis of the (rs 1544410) SNP of the patients concluded that HSP-70 , VDBP, C- Peptide level was no significantly related to the Also C-Peptide, was shown to be related risk factor to the both CT and CT alleles (1.003, p

> 0.05) in comparison with CC alleles. Furthermore, HbA1c level was demonstrated to be related risk factor for the CG allele in comparison with CC & GG alleles (1.009, $p < 0.05$).

Table (3-13): The odds ratios of VDR Genotype (rs1544410) with biomarkers level

Variables	SNIP	OR (95% CI)	p value
HSP-70	CC	1[a]	-
	CT	0.972(0.901 - 1.048)	0.464 [NS]
	TT	0.988(0.921 - 1.059)	0.732 [NS]
VDBP	CC	1[a]	-
	CT	0.998(0.969 - 1.027)	0.875 [NS]
	TT	0.988(0.961 - 1.015)	0.384 [NS]
C-Peptide	CC	1[a]	-
	CT	1.003(0.997 - 1.009)	0.338 [NS]
	TT	0.998(0.990 - 1.006)	0.593 [NS]
HbA1c%	CC	1[a]	-
	CT	1.009 (0.853 - 1.193)	0.917 [NS]
	TT	0.918 (0.778 - 1.084)	0.315 [NS]
RBS	CC	1[a]	-
	CT	1.001 (0.996 - 1.006)	0.618 [NS]
	TT	0.999 (0.994 - 1.004)	0.648 [NS]
	CC	1[a]	-
Insulin	CT	0.551(0.288-1.054)	0.072
	TT	1.264(0.767-2.082)	0.358
HOMA-IR	CC	1[a]	-
	CT	0.361(0.109-1.198)	0.096
	TT	1.064(0.389-2.911)	0.903
25(OH)D3	CC	1[a]	-
	CT	0.632(0.400-0.996)	0.048[S]
	TT	0.661(0.431-1.013)	0.05[S]

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference category

4. Discussion

4.1. Biochemical studies

According to figure 1; The findings of this results were similar with other studies conducted in other middle east and even in some western countries. A study in Lebanon found the prevalence of normal weight and obesity were 14% and 17% among the population. In Kuwait the overweight and obesity prevalence were found to be 32% and 8.9% respectively. Whereas in the United States and United Arab Emirates the prevalence of overweight and obesity were 35% among the college students (**Musaiger, 2003**). Different studies across the globe reported various prevalence rate of normal weight and obesity and that may be due to the differences in sample size and culture.

Vitamin D3 bioavailability has garnered much attention from scientists as they investigate and find potential new contributing factors. Vitamin D-binding protein (VDBP) is responsible for binding the vast majority of circulating 25(OH)D3 and, after activation in the kidneys, 1,25(OH)2D3. Only about 1-2% of it is not linked to albumin and circulates freely in the blood **(Christakos *et al.*, 2016)**.

Results from the current study were inconsistent with those from the works of **Oluwabukola et al., Jiao *et al.*, and Yosria *et al* (Ala *et al.*, 2017)**. Resistance of cells in target tissues to insulin is the most critical pathophysiological event leading to the development of T2DM, and this resistance is often associated with abnormal insulin production in addition to the decline in the function of B-cells. In a clinical setting, IR causes a rapid increase in insulin levels to counteract the effects of hyperglycemia. Insufficient insulin signaling

from the insulin receptor to the final insulin action substrates, involved in a wide range of metabolic and mitogenic processes are the underlying cause of insulin resistance at the cellular level **(Saini, 2010)**.

These results were agreed with other studies such as, **Alhumaidi *et al* (Alhumaidi *et al.*, 2013)**. Who investigated vitamin D deficiencyin type 2 diabetes and discovered low levels of 25(OH)D in both patients and controls. In yet another cross-sectional investigation **(Hidayat *et al.*, 2010)**. Which looked at the link between vitamin D and T2DM in the elderly and found similar rates of deficiency inboth patients and controls (78%), suggesting that body mass index (BMI), sun protection use, and gender all play a role in determining whether or not someone has low vitamin D levels. The research conducted by **Al-Tu'ma *et al* (Al-Tu'ma & Yosuf, 2015)**. VitaminD deficiency has been shown in a recent study to be associated with an elevated risk of type 2 diabetes. Additional evidence comes froma research by **Boyuk *et al* (Boyuk *et al.*, 2017)**.

Demonstrated that vitamin D deficiency was common among both T2DM patients and healthy adults **(Bayani *et al.*, 2014)**.

25-hydroxyvitamin D levels are lower in the obese. It might be due to inefficient exposure to sun light because decreasing activity, or because more of their 25-hydroxyvitamin D is being stored as fat **(Salih *et al.*, 2021)**.

Low vitamin D (insufficient and deficient) was significantly more prevalent in female patients; they explain that the deficiency of vit D may be due to social behavior and religious reasons, as women are required to cover their entire bodies and don a hijab when they go

outside. Consequently, 25(OH) D synthesis and levels are reduced **(Salih *et al.*, 2021)**.

Vitamin D may have a positive effect on insulin secretion and sensitivity, either directly via its role in pancreatic beta-cellactivation and sensitive organs, or indirectly via calcium homeostasisregulation **(Bayani *et al.*, 2014)**. The vit D has been shown to increase the expression of insulin receptors **(Sacerdote *et al.*, 2019)**.

Vitamin D-binding protein (VDBP) is responsible for binding the vast majority of circulating 25(OH)D3 and, after activation in the kidneys, 1,25$(OH)_2$D3. Only about 1-2% of it is not linked to albumin and circulates freely in the blood **(Christakos *et al.*, 2016)**.VDBP is crucial in regulating the levels of both total and free 25(OH)D in the body **(Chun *et al.*, 2014)**.

Vitamin D3 plays a function in insulin secretion and sensitivity, as evidenced by the discovery of a receptor for (1,25$(OH)_2$D3) in the pancreatic cells **(Alvarez & Ashraf, 2010)**. Larger adipocytes are thought to be less efficient at sequestering and mobilizing vitamin D, leading to reduced vitamin D bioavailability **(Liel *et al.*, 1988)**. It has also been hypothesized that altered hepatic production and harmful feedback mechanisms contribute to the lower 25(OH)D3 levels in obese people **(Compston *et al.*, 1981)**. The rapid rise in diabetes rates calls for attention to VDBP because of the correlation between vitamin D insufficiency and glucose homeostasis**(Saraswathi *et al.*, 2019)**.

Insulin secreting-cell dysfunction, and glucose metabolism are all thought to be influenced by variations in vitamin D-binding proteins **(Wang *et al.*, 2014)**. Insulin secretion and sensitivity have been linked

to vitamin D-binding proteins **(Mohammed *et al.*, 2023)**. The role of VDBP in insulin dynamics is yet unclear. No significant link was found between VDBP and insulin resistance in other study **(Naderpoor *et al.*, 2018)**. While others found a negative and independent relationship between VDBP concentration and several parameters (lipid, c-peptide, HA1c) in those with metabolic syndrome and the free and bioavailable fraction linked with specific components of the disease **(Pelczyńska *et al.*, 2017)**. Another analysis of the connections between these factors HSP-70 , D3 and VDR found that VDBP was linked to insulin resistance, with the strength of the link appearing to be influenced by racial background **(Ashraf *et al* 2014**). found a negative correlation between insulin resistance and insulin level, and they found the same thing for VDBP concentrations. These results also suggested that VDBP levels might need to shift to keep bioavailable 25(OH)D3 levels stable **(Ashraf *et al.*, 2014)**. Based on the findings that VDBP levels can rise in response to inflammation, specifically to interleukin-6, it stands to reason that the prevalence of systemic inflammation in obese and overweight individuals would cause VDBP levels to rise **(Guha *et al.*, 1995)**.

Inflammation is positively correlated with serum HSP70 levels, and elevated insulin resistance may be a mechanism by which HSP70 contributes to the aetiology of T2DM **(Nakhjavani *et al.*, 2013)**. By binding to Toll-like Receptor-2 and Toll-like Receptor-4 (TLR-2,TLR-4), HSP70 may stimulate inflammatory responses **(Vabulas *et al.*, 2002)**. In the past decade, some studies have shown the significant

role of a chaperone family. HSP70 belongs to a family of chaperones that have been found to play a crucial role in the pathophysiology of insulin resistance, the underlying cause of T2DM, in recent years. Insulin resistance and T2DM may result from a confluence of biological mechanisms. Increasing HSP70 expression in the brain has been shown to directly affect insulin sensitivity and glycemic regulation.

The role of HSP-70 in T2DM patients has been validated by several investigations, and these results are compatible with those of previously reported studies. There are multiple mechanisms through which HSP-70 influences inflammation and insulin resistance. The formation of reactive oxygen species (ROS) may rise in obese people. The rate of ROS generation may influence the expression of HSP-70 **(Archer *et al.*, 2018)**.Serum HSP70 levels are positively linked with inflammatory parameters, according to investigations of T2DM and older patients; endogenous HSP-70 (eHSP-70) may contribute to the aetiology of T2DM by increasing insulin resistance

(Nakhjavani *et al.*, 2013). Patients with T2DM and healthy people show significant positive correlation between HSP70 and BMI. Our results raise the possibility that HSP70 plays **(Vabulas *et al.*, 2002)**.

C-Peptide level considerably decreased in patient groups that are subdivided based on gender and BMI compared to the controls. Chronic malfunction of the pancreatic -cells results in an inadequate supply of C-peptide and insulin entering the bloodstream simultaneously. More and more research suggests that c-peptide via its antioxidant, vasoprotective, antiapoptotic, anti-inflammatory, and necrotic factor NF-b modulatory actions, has a protective potential **(Yaribeygi *et al.*, 2019)**. Various studies demonstrate that C-Peptide protects vascular networks by reducing NF-b translocation and activity in diabetics **(Cifarelli *et al.*, 2008)**. Others show that C-peptide could slow down the development of DM by inhibiting NF-kB activity and, in turn, its subsequent effects **(Li *et al.*, 2018)**. Renal damage from diabetes can be reduced through NF-b regulation, according to research by Hills

and colleagues **(Hills *et al.*, 2010)**. C-Peptide's anti-apoptotic actions were shown to depend on NF-b modulatory potentials in renal tissues, as shown by research conducted by others which hypothesized that NF-b activation produced by C-peptide shields renal cells from tissue necrotic factor TNF- damage in a diabetic environment **(Al-Rasheed *et al.*, 2006)**. In 2008, Luppi and

colleagues discovered that the C-peptide treatment improves diabetes-induced endothelial dysfunction **(Luppi *et al.*, 2008)**.

VDBP was found to be protective in patients of normal weight, Furthermore, C-peptide and HSP-70 were investigated to be a risk factors for both normal and obese individuals. The VDBP was reported to have links to glucose levels. Having a higher BMI and being overweight are both known risk factors **(Vasilopoulos *et al.*, 2013)**.

ROC analysis was carried out as a multivariable clinical diagnostic model that incorporates clinical aspects of diabetes in obese individuals and is also used to analyse the ideal diagnosis points in instances of DM, with the goal of determining the sensitivity

C-peptide has been shown to have potential as a biomarker for use in assisting in the risk stratification of diabetic complications **(Maddaloni *et al.*, 2022)**.

4.2 Genetics variants of VDR genotype

Previous research has demonstrated a robust association between VDR polymorphisms and metabolic markers related to type 2 diabetes **(Zaki *et al.*, 2017)**.

In a nutshell, the human VDR gene sits at 12q13.1 on the human chromosome. The VDR gene is made up of spliced-together coding and non-coding exons **(Gyapay *et al.*, 1996)**. Polymorphisms in the VDR gene may contribute to increased secretion from cellsand be linked to type 1 and type 2 diabetes **(Mackawy & Badawi, 2014)**. Homozygous dominant model analysis was used to verify the role of the important allele in the VDR gene's connection to type 2 diabetes.

Homozygous CC was discovered in 40% of the groups, with 26% finding CT and 33% finding TT. Recessive model analysis alsohelped shed light on the impact of the minor allele on the VDR gene's connection with T2DM.

In this study, the A-allele of the VDR gene's rs1544410 polymorphism was linked to higher insulin secretion, as evaluated bya disposition index, in women with a history of DM. As a transcriptional regulator, VDR belongs to the nuclear receptor superfamily. It is found in a variety of cell types across the body and,specifically, in pancreatic beta cells, where it exists as a heterodimer with retinoid X receptors (RXRs) **(Bouillon *et al.*, 2008)**. Consistentwith previous research, this study found that vitamin D may play a role in a variety of health problems **(Wang *et al.*, 2017)**.

Potentially beneficial effects on insulin secretion and sensitivity have been postulated. Vitamin D receptor (VDR) activation on pancreatic beta cells and insulin-sensitive organs and calcium homeostasis modulation may mediate the effects **(Wang *et al.*, 2017)**.

Researchers have shown an inverse association between vitamin D levels in the blood and the likelihood of developing diabetes, metabolic syndrome, impaired insulin production, or insulin resistance **(Knekt *et al.*, 2008)**.

Polymorphisms in the VDR gene have been linked to diabetes and insulin resistance in multiple studies, including a meta-analysis and those conducted by **Malik *et al* (Malik *et al.*, 2018)**.The VDR gene has been linked to around 25 distinct polymorphisms thus far. These VDR polymorphisms have been linked to type 2 diabetes and altered insulin secretion in a number of studies **(Shaat *et al.*, 2017)**. Metabolic syndrome, which includes abnormalities in metabolism related to obesity, is also associated with VDR polymorphisms **(Ignell *et al.*, 2013)**. According to the results of this study, Pakistani patients with the genotype of the VDR gene variant rs1544410 are more likely to develop T2DM and obesity.

In people with vitamin D deficiency, the (rs10735810) polymorphism of the VDR gene was discovered to be an additional independent driver of insulin secretion. In addition, the ability to secrete insulin depends on VDR mRNA levels **(Ogunkolade *et al.*, 2002)**. Recently, DNA polymorphisms in the VDR gene have been linked to an increased risk of developing metabolic syndrome (MetS) **(Uitterlinden *et al.*, 2004)**. Several studies found that variations in the vitamin D receptor, gene was associated with the risk of cluster disorder **(Al-Daghri *et al.*, 2014)**.

However, there is room for improvement in the consistency of the findings; this may be attributed to the fact that study participants are of different ethnicities. It still needs to be apparent what role VDR polymorphisms have at the molecular level in the pathogenic context of MetS. Moreover, the linkage disequilibrium (LD) and haplotypes of the four VDR above SNPs and their relationships with MetS in the Thai population have not been published **(Karuwanarint *et al.*, 2018)**. Other researchers have proved that the underlying pathophysiological processes of these correlations are still unclear. Since VDR is found In pre-adipocytes, it is plausible that vitamin D has an indirect effect on adipocyte development and metabolism**(Kamei *et al.*, 1993)**. It has been shown in vitro that vitamin D inhibits the differentiation of pre-adipocytes and promotes adipocytes' completion of their differentiating process andlipoprotein lipase production and release.

In Caucasian patients, a negative correlation was found between body mass index and vitamin D3 levels in the blood **(Vasilopoulos *et al.*, 2013)**.Moreover, it was discovered that VDR polymorphisms affected the amount of vitamin D in the blood. The modification of insulin secretion may possibly play a role in the connection of VDR polymorphisms with obesity in type 2 diabetic people, which is a compelling theory to make. Obese individuals frequently have hyperinsulinemia, which may result from a B-cell compensatory response to insulin resistance. Alternately, it could be brought on by a primary over-secretion of insulin due to cellular malfunction, leading to the accumulation of extra fat and the emergence of insulin resistance. In the population we analysed, the association between these genotypes and insulin levels could not be determined because every participant received insulin or oral hypoglycaemic medication. If their insulin levels were measured, they would mainly represent how well they managed their diabetes and/or the harmful consequences of persistent hyperglycaemia (glucotoxicity) on B-cell activity. These SNPs were discovered to affect how much insulin a group of non-diabetic Asians secretes in response to an oral glucose tolerance test **(Hitman *et al.*, 1998)**.

Conclusions and Recommendation

1. Conclusions

1. There was a significant increase in sera levels of heat shockprotein-70 in T2DM patients as compared with the control group (p-value < 0.05). only a the age range (55-67) years
2. Vit D3 level shows a significant decline in the patient's group in comparison to the control group (p-value < 0.05).
3. VDBP levels were changed variably depending upon the patients age.
4. C-peptide level shows a significant decline in the patient's group in comparison to the control group (p-value < 0.05), and that might be due to elevated insulin level and its feedback effect on insulin production.
5. VDR gene analysis revealed that the SNP obtained as follows (40% wild or CC, 26.7% indicate heterogenotype or CT and 33.3% indicate homogenotyes or TT) of VDR BsmI genotytpe.

Conclusions and Recommendation

2. Recommendations

According to all previous knowledge, we can recommend the following:

1. Study the relationship between HSP-70 with long-term complications of type 2 diabetes (retinopathy, kidneys, nerves, and arteries and veins).
2. Studying the relationship between advanced glycemic end products (AGE) with (VDBP, D3, and HSP-70).
3. Study of the level of insulin, C-peptide in the case of pre-diabetes, and whether these markers can be used as a diagnosis of type 2 diabetes and in pre-diabetes.
4. Study the effect of exercise for (30-45) minutes a day on the level of heat shock protein-70 and insulin.
5. In forthcoming research, C-peptide testing can be effectively applied in some settings, such as in persons with predominant insulin resistance. C-peptide testing helps sort out muddled diagnosis and keeps tabs on a select group of people over time.
6. Study various genetic variants of VDR and other genotypes in T2DM and in obese individuals to investigate its associations with trhe disease pathogenesis.

References

- Abbas, K. M., Alaaraji, S. F., & Alâ, R. S. (2020). A study of the association between IL-17 and HOMA-IR in Iraqi type 2 diabetic patients. Iraqi Journal of Science, 491-498 .

- Abusaib, M., Ahmed, M., Nwayyir, H. A., Alidrisi, H. A., Al-Abbood, M., Al- Bayati, A., Al-Ibrahimi, S., Al-Kharasani, A., Al-Rubaye, H., & Mahwi, T. (2020). Iraqi experts consensus on the management of type 2 diabetes/prediabetes in adults. Clinical Medicine Insights: Endocrinology and Diabetes, 13, 1179551420942232 .

- Ahmad, S., Verma, P., Gupta, A., Srivastava, A. K., & Mohan, R. (2021). A REVIEW ON DIABETES MELLITUS .

- Akai, R., Hosoda, A., Yoshino, M., & Iwawaki, T. (2015). Constitutive role of GADD 34 and CR eP in cancellation of phospho-eIF 2α-dependent translational attenuation and insulin biosynthesis in pancreatic β cells. Genes to Cells, 20(11), 871-886 .

- Ala, O. A., Akintunde, A. A., Ikem, R. T., Kolawole, B. A., Ala, O. O., & Adedeji, T. (2017). Association between insulin resistance and total plasma homocysteine levels in type 2 diabetes mellitus patients in south west Nigeria. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 11, S803-S809.

- Al-Daghri, N. M., Mohammed, A. K., Al-Attas, O. S., Ansari, M. G. A., Wani, K., Hussain, S. D., Sabico, S., Tripathi, G., & Alokail, M. S. (2017). Vitamin D receptor gene polymorphisms modify cardiometabolic response to vitamin D supplementation in T2DM patients. Scientific Reports, 7(1), 8280 .

- Al-Daghri, N. M., Al-Attas, O. S., Alkharfy, K. M., Khan, N., Mohammed, A. K., Vinodson, B., Ansari, M. G. A., Alenad, A., & Alokail, M. S. (2014). Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. Gene, 542(2), 129-133.

- Al-Hazmi, A. S., Al-Mehmadi, M. M., Al-Bogami, S. M., Shami, A. A., Al-Askary, A. A., Alomery, A. M., Al-Shehri, S. S., Dahlawi, H ,.Abdulrazag, K., & Ali, T. (2017). Vitamin D receptor gene polymorphisms as a risk factor for obesity in Saudi men. Electronic physician, 9(10), 5427 .

- Alhumaidi, M., Adnan, A., & Dewish, M. (2013). Vitamin D deficiency in patients with type-2 diabetes mellitus in southern region of Saudi Arabia. Maedica, 8(3), 231.

- Al-Rasheed, N. M., G.B. Willars, and N.J. Brunskill, C-Peptide Signals via Gαi to Protect against TNF-α–Mediated Apoptosis of Opossum Kidney Proximal Tubular Cells. Journal of the American Society of Nephrology, 2006. 17(4): p. 986-995 .

- Alvarez, J. A., & Ashraf, A. (2010). Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. International journal of endocrinology, 2010.

- Al-Shoumer, K. A., & Al-Essa, T. M. (2015). Is there a relationship between vitamin D with insulin resistance and diabetes mellitus? World journal of diabetes, 6(8), 1057 .

- Al-Tu'ma, F., & Yosuf, L. M. Z. (2015). Vitamin D deficiency and hypertension in type 2 diabetic Iraqi patients. J Contemp Med Sci, 1(1), 17-20.

- Al-Zuhaeri, A., Al-Shakour, A., & Ali Mansour, A. (2022). Serum level of heat shock protein 70 in patients with type 2 diabetes mellitus in Basrah, Iraq. Archives of Razi Institute, 77(5), 1837-1844 .

- Al Mheid, I., & Quyyumi, A. A. (2017). Vitamin D and cardiovascular disease: controversy unresolved. Journal of the American College of Cardiology, 70(1), 89-100 .

- Alam, U., Arul-Devah, V., Javed, S., & Malik, R. A. (2016). Vitamin D and diabetic complications: true or false prophet? Diabetes therapy, 7, 11-26 .

- Ali, S. M., & Jebor, M. A. (2021). Vitamin-D Receptor (VDR) Gene Polymorphisms (Apai&Bsmi) In Iraqi Osteoporosis Patients. Annals of the Romanian Society for Cell Biology, 13842-13852 .

- Almubarak ,F. (2016). The Association Between Known Risk Factors for Type 2 Diabetes, and the Body Mass Index of Diabetic Adults. University of Arkansas .

- Anderson, P. H. (2017). Vitamin D activity and metabolism in bone. Current Osteoporosis Reports, 15, 443-449 .

- Anuradha ,C. V. (2013). Phytochemicals targeting genes relevant for type 2 diabetes. Canadian Journal of Physiology and pharmacology, 91(6), 397-411.

- Apidechkul, T., Chomchoei, C., & Upala, P. (2022). Epidemiology of undiagnosed type 2 diabetes mellitus among hill tribe adults in Thailand. Scientific Reports, 12(1), 3969 .

- Apovian, C. M., Aronne, L. J., Bessesen, D. H., McDonnell, M. E., Murad, M. H., Pagotto, U., Ryan, D. H., & Still, C. D. (2015). Pharmacological

management of obesity: an endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism, 100(2), 342-362 .

- Archer, A. E., Von Schulze, A. T., & Geiger, P. C. (2018). Exercise, heat shock proteins and insulin resistance. Philosophical transactions of the royal society B: biological sciences, 373(1738), 20160529 .

- Ashraf, A. P., Huisingh, C., Alvarez, J. A., Wang, X., & Gower, B. A. (2014). Insulin resistance indices are inversely associated with vitamin D binding protein concentrations. The Journal of Clinical Endocrinology & Metabolism, 99(1), 178-183 .

- ATLAS, I. (2019). 2017: http://www. diabetesatlas. org. Accessed on Feb 10th .

- Bayani, M. A., Akbari, R., Banasaz, B., & Saeedi, F. (2014). Status of Vitamin-D in diabetic patients. Caspian journal of internal medicine, 5(1), 40.

- Becheva, M. S. V., & Kirkova-Bogdanova, A. G. (2022). Prophylactics of Type 2 Diabetes and Diabetic Foot. Iranian Journal of Public Health .2370 ,)10(51 ,

- Bellini, S., Barutta, F., Mastrocola, R., Imperatore, L., Bruno, G., & Gruden, G. (2017). Heat shock proteins in vascular diabetic complications: review and future perspective. International journal of molecular sciences, 18(12), 2709 .

- Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Chang, A. R., Cheng, S., & Das, S.

R. (2019). Heart disease and stroke statistics—2019 update: a report from the American Heart Association. Circulation, 139(10), e56-e528 .

- Bigagli, E., & Lodovici, M. (2019). Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. Oxidative Medicine and Cellular Longevity, 2019 .

- Bikle, D., & Christakos, S . (2020) .New aspects of vitamin D metabolism and action—Addressing the skin as source and target. Nature reviews endocrinology, 16(4), 234-252 .

- Bikle, D. D. (2020). Vitamin D: Newer concepts of its metabolism and function at the basic and clinical level. Journal of the Endocrine Society, 4(2), bvz038 .

- Bikle, D. D., & Schwartz, J. (2019). Vitamin D binding protein, total and free vitamin D levels in different physiological and pathophysiological conditions. Frontiers in endocrinology, 10, 317 .

- Bischoff-Ferrari ,H., Borchers, M., Gudat, F., Dürmüller, U., Stähelin, H., & Dick, W. (2004). Vitamin D receptor expression in human muscle tissue decreases with age. Journal of Bone and Mineral Research, 19(2), 265-269 .

- Bolland, M. J., Grey, A. B., Ames, R. W., Horne ,A. M., Mason, B. H., Wattie,

- D. J., Gamble, G. D., Bouillon, R., & Reid, I. R. (2007). Age-, gender-, and weight-related effects on levels of 25-hydroxyvitamin D are not mediated by vitamin D binding protein. Clinical endocrinology, 67(2), 259-264 .

- Bouillon, R., Schuit, F., Antonio, L., & Rastinejad, F. (2020). Vitamin D binding protein: a historic overview. Frontiers in endocrinology, 10, 910 .

- Bouillon, R., Carmeliet, G., Verlinden, L., Van Etten, E., Verstuyf, A., Luderer,

H. F., Lieben, L., Mathieu, C., & Demay, M. (2008). Vitamin D and human health: lessons from vitamin D receptor null mice. Endocrine reviews, 29(6), 726-776.

- Boyuk, B., Atalay, H., Değirmencioğlu, Ş., Altay, M., Güzel, S., Çelebi, A., & Ayar, Y. (2017). Association between vitamin D level and microvascular complications in patients with type 2 diabetes. Eurasian Journal of Medicine and Oncology.

- Brown, I. R. (2007). Heat shock proteins and protection of the nervous system. Annals of the New York Academy of Sciences, 1113(1), 147-158 .

- Butt, J., Ishtiaq, S., Ijaz, B., Mir, Z. A., Arshad, S., & Awais, S. (2018). Authentication of polyherbal formulations using PCR technique. Ann Phytomed, 7(1), 131-139 .

- Calderwood, S. K., Mambula, S. S., Gray Jr, P. J & ,.Theriault, J. R. (2007). Extracellular heat shock proteins in cell signaling. FEBS letters, 581(19), 3689- 3694 .

- Cancienne, J. M., Brockmeier, S. F., & Werner, B. C. (2018). Association of perioperative glycemic control with deep postoperative infection after shoulder arthroplasty in patients with diabetes. JAAOS-Journal of the American Academy of Orthopaedic Surgeons, 26(11), e238-e245 .

- Carlberg, C., & Muñoz, A. (2022). An update on vitamin D signaling and cancer. Seminars in cancer biology ,

- Chacon Cortes, D. F., & Griffiths, L. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine, 2014(2), 1-9 .

- Chang, S.-W., & Lee, H.-C. (2019). Vitamin D and health-The missing vitamin in humans. Pediatrics & Neonatology, 60(3), 237-244 .

- Charoenngam, N., Shirvani, A., & Holick, M. F. (2019). Vitamin D for skeletal and non-skeletal health: What we should know. Journal of clinical orthopaedics and trauma, 10(6), 1082-1093 .

- Chehade, J. M., Gladysz, M., & Mooradian, A. D. (2013). Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management. Drugs, 73(4), 327-339 .

- Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., & Wei, Y. (2020 .)Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet, 395(10223), 507-513 .

- Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L., & Carmeliet, G. (2016). Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiological reviews, 96(1), 365-408 .

- Chun, R. F. (2012). New perspectives on the vitamin D binding protein. Cell biochemistry and function, 30(6), 445-456 .

- Chun, R. F., Peercy, B .E., Orwoll, E. S., Nielson, C. M., Adams, J. S., & Hewison, M. (2014). Vitamin D and DBP: the free hormone hypothesis revisited. The Journal of steroid biochemistry and molecular biology, 144, 132- 137 .

- Chun, R. F., Shieh, A., Gottlieb, C., Yacoubian, V ,.Wang, J., Hewison, M., & Adams, J. S. (2019). Vitamin D binding protein and the biological activity of vitamin D. Frontiers in endocrinology, 10, 718 .

- Chung, J., Nguyen, A.-K., Henstridge, D. C., Holmes, A. G., Chan, M. S., Mesa,

- J. L., Lancaster, G .I., Southgate, R. J., Bruce, C. R., & Duffy, S. J. (2008). HSP72 protects against obesity-induced insulin resistance. Proceedings of the National Academy of Sciences, 105(5), 1739-1744 .

- Cifarelli, V., Luppi, P., Hubert, M. T., He, J., Piganelli, J., & Trucco, M. (2008). Human proinsulin C-peptide reduces high glucose-induced proliferation and NF- κB activation in vascular smooth muscle cells. Atherosclerosis, 201(2), 248-257 .

- Compston, J. E., Vedi, S., Ledger, J. E., Webb, A., Gazet, J.-C., & Pilkington ,T.

- (1981). Vitamin D status and bone histomorphometry in gross obesity. The American journal of clinical nutrition, 34(11), 2359-2363 .

- Costa, M. F. F. L., Peixoto, S. W. V., & Collaboration, N. R. F. (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population- based studies with 4.4 million participants .

- Dilaveris, P., Antoniou, C.-K., Manolakou, P., Tsiamis, E., Gatzoulis, K., & Tousoulis, D. (2019). Biomarkers associated with atrial fibrosis and remodeling. Current medicinal chemistry, 26(5), 780-802 .

- Dixon, A. E., & Peters, U. (2018). The effect of obesity on lung function. Expert review of respiratory medicine, 12(9), 755-767 .doi.org/10.1111/dom.14785 .

- Donini, L. M., Pinto, A., Giusti, A. M., Lenzi, A., & Poggiogalle, E .)2020(.

Obesity or BMI paradox? Beneath the tip of the iceberg. Frontiers in Nutrition, 7, 53 . e Souza, C. L., de Sá, L. B. P. C., Rocha, D. R. T. W., & Arbex, A. K.

(2016). Vitamin D and diabetes mellitus: a review. Open Journal of Endocrine and Metabolic Diseases, 6(01), 1 .

- Emdin, C. A., Khera, A. V., Natarajan, P., Klarin, D., Zekavat, S. M., Hsiao, A. J., & Kathiresan, S. (2017). Genetic association of waist-to-hip ratio with

cardiometabolic traits, type 2 diabetes, and coronary heart disease. Jama , .634-626 ,6(317)

- Frydrych, L. M., Bian, G., O'Lone, D. E., Ward, P. A., & Delano, M. J. (2018). Obesity and type 2 diabetes mellitus drive immune dysfunction, infection development, and sepsis mortality. Journal of Leukocyte Biology, 104(3), 525- 534 .

- Gaborit, B., Abdesselam, I., Kober, F., Jacquier, A., Ronsin, O., Emungania, O., Lesavre, N., Alessi, M.-C., Martin, J., & Bernard, M. (2015). Ectopic fat storage in the pancreas using 1H-MRS: importance of diabetic status and modulation with bariatric surgery-induced weight loss. International journal of obesity, 39(3), 480-487 .

- Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus .International journal of molecular sciences, 21(17), 6275.

- Gasbjerg, L. S., Helsted, M. M., Hartmann, B., Jensen, M. H., Gabe, M. B., Sparre-Ulrich, A. H., Veedfald, S., Stensen, S., Lanng, A. R., & Bergmann, N.

C. (2019). Separate and combined glucometabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals. Diabetes, 68(5), 906-917 .

- Gil, Á., Plaza-Diaz, J., & Mesa, M. D. (2018). Vitamin D: classic and novel actions. Annals of Nutrition and Metabolism, 72(2), 87-95 .

- Gillett, M. J. (2009). International expert committee report on the role of the A1c assay in the diagnosis of diabetes: diabetes care 2009; 32 (7): 1327–1334. The Clinical Biochemist Reviews, 30(4), 197 .

- Giustina, A., Adler, R. A., Binkley, N., Bouillon, R., Ebeling, P. R., Lazaretti-Castro, M., Marcocci, C., Rizzoli, R., Sempos, C. T., & Bilezikian, J. P. (2019). Controversies in vitamin D: summary statement from an international conference. The Journal of Clinical Endocrinology & Metabolism, 104(2), 234- 240 .

- Glovaci, D., Fan, W., & Wong, N. D. (2019). Epidemiology of diabetes mellitus and cardiovascular disease. Current cardiology reports, 21(4), 1-8 .

- Graham, C. H., Ferrier, S., Huettman, F., Moritz, C., & Peterson, A .T. (2004). New developments in museum-based informatics and applications in biodiversity analysis. Trends in ecology & evolution, 19(9), 497-503 .

- Grammatiki, M., Rapti, E., Karras, S., Ajjan, R., & Kotsa, K. (2017). Vitamin D and diabetes mellitus: Causal or casual association? Reviews in Endocrine and Metabolic Disorders, 18(2), 227-241 .

- Green, H. J., Jones, L. L., Hughson, R. L., Painter, D. C., Farrance, B. W. J. M., Sports, S. i., & Exercise. (1987). Training-induced hypervolemia: lack of an effect on oxygen utilization during exercise. 19(3), 202-206 .

- Guha, C., Osawa, M., Werner, P. A., Galbraith, R. M., & Paddock, G. V. (1995). Regulation of human Gc (vitamin D—binding) protein levels: hormonal and cytokine control of gene expression in vitro .Hepatology, 21(6), 1675-1681 .

- Gyapay, G., Schmitt, K., Fizames, C., Jones, H., Vega-Czarny, N., Spillett, D., Muselet, D., Prud'Homme, J.-F., Dib, C., & Auffray, C. (1996). A radiation hybrid map of the human genome. Human Molecular Genetics, 5(3), 339-346.

- Hanel, A., & Carlberg, C. (2020). Vitamin D and evolution: Pharmacologic implications. Biochemical pharmacology, 173, 113595 .

- Health, E. P. o. I. G. f. C., Children, R. R. i., & Adolescents. (2011). Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics, 128(Supplement_5), S213-S256 .

- Heise, E. A., & Fort, P. E. (2011). Impact of diabetes on alpha-crystallins and other heat shock proteins in the eye. Journal of ocular biology, diseases, and informatics, 4, 62-69 .

- Heitkamp, M., Siegrist, M., & Halle, M. (2021). Consideration of Sex Differences in Children With Obesity—Reply. JAMA pediatrics, 175(7), 748- 749 .

- Herrick, K. A., Storandt, R. J ,.Afful, J., Pfeiffer, C. M., Schleicher, R. L., Gahche, J. J., & Potischman, N. (2019). Vitamin D status in the United States, 2011–2014. The American journal of clinical nutrition, 110(1), 150-157 .

- Hills, C. E., N.J. Brunskill, and P.E. Squires, C-peptide as a therapeutic tool in diabetic nephropathy. American journal of nephrology, 2010. 31(5): p. 389-397 .

- Himanshu, D., Ali, W., & Wamique, M. (2020). Type 2 diabetes mellitus: pathogenesis and genetic diagnosis. Journal of Diabetes & Metabolic Disorders, 19, 1959-1966 .

- Hidayat, R., Setiati, S., & Soewondo, P. (2010). The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. Age, 42, 123-129.

- Horst-Sikorska, W., Dytfeld, J., Wawrzyniak, A., Marcinkowska, M., Michalak, M., Franek, E., Napiórkowska, L., Drwęska, N., & Słomski, R. (2013). Vitamin D receptor gene polymorphisms, bone mineral density and fractures in postmenopausal women with osteoporosis. Molecular biology reports, 40, 383- 390 .

- Hou, Y.-C., Wu, C.-C., Liao, M.-T., Shyu, J.-F., Hung, C.-F., Yen, T.-H., Lu, C.-L., & Lu, K.-C. (2018). Role of nutritional vitamin D in osteoporosis treatment. Clinica Chimica Acta, 484 .191-179 ,

- Howard, M., Roux, J., Lee, H., Miyazawa, B., Lee, J.-W., Gartland, B., Howard, A. J., Matthay, M. A., Carles, M., & Pittet, J.-F. (2010). Activation of the stress protein response inhibits the STAT1 signalling pathway and

iNOS function in alveolar macrophages: role of Hsp90 and Hsp70. Thorax, 65(4), 346-353 .

- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., & Gu, X. (2020). Clinical features of patients infected with 2019 novel

- coronavirus in Wuhan, China. The Lancet, 395(10223), 497-506 .

- Hyppönen, E., & Power, C. (2007). Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. The American journal of clinical nutrition, 85(3), 860-868 .

- Ignell, C., Shaat, N., Ekelund, M., & Berntorp, K. (2013). The impact of ethnicity on glucose homeostasis after gestational diabetes mellitus. Acta diabetologica, 50, 927-934.

- Index, H. E. (2019 .)Food and Nutrition Service. US Department of Agriculture. Available online: https://www. fns. usda. gov/resource/healthy-eating-index-hei (accessed on 12 November 2019 .)

- Jain, S. K., & Micinski, D. (2013). Vitamin D upregulates glutamate cysteine ligase and glutathione reductase, and GSH formation, and decreases ROS and MCP-1 and IL-8 secretion in high-glucose exposed U937 monocytes. Biochemical and biophysical research communications, 437(1), 7-11 .

- James, S. L., Abate, D., Abate, K. H., Abay, S. M ,.Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., & Abdelalim, A. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017 :a

systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 392(10159), 1789-1858 .

- Jee, H. (2016). Size dependent classification of heat shock proteins: a mini-review. Journal of exercise rehabilitation, 12(4), 255 .

- Jorde, R .)2019(.The role of vitamin D binding protein, total and free 25-hydroxyvitamin D in diabetes. Frontiers in endocrinology, 10, 79 .

- Kähm, K., Laxy, M., Schneider, U., Rogowski, W. H., Lhachimi, S. K., & Holle, R. (2018). Health care costs associated with incident complications in patients with type 2 diabetes in Germany. Diabetes care, 41(5), 971-978 .

- Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 444(7121), 840-846.

- Kamei, Y., Kawada, T., Kazuki, R., Ono, T., Kato, S., & Sugimoto, E. (1993). Vitamin D receptor gene expression is up-regulated by 1, 25-dihydroxyvitamin D3 in 3T3-L1 preadipocytes. Biochemical and biophysical research communications, 193(3), 948-955.

- Karuwanarint, P., Phonrat, B., Tungtrongchitr, A., Suriyaprom, K., Chuengsamarn, S., & Tungtronchitr, R. (2018). Genetic variations of vitamin D receptor gene in metabolic syndrome and related diseases in the Thai population. Asia Pacific Journal of Clinical Nutrition, 27(4), 935-944.

- Karonova, T., Grineva, E., Belyaeva, O., Bystrova, A., Jude, E. B., Andreeva, A., Kostareva, A., & Pludowski, P. (2018). Relationship between vitamin D status and vitamin D receptor gene polymorphisms with markers of metabolic syndrome among adults. Frontiers in endocrinology, 9, 448 .

- Khan, M. A. B., Hashim, M. J., King, J. K., Govender, R. D., Mustafa, H., & Al Kaabi, J. (2020). Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. Journal of epidemiology and global health, 10(1), 107 .

- Knekt, P., Laaksonen, M., Mattila, C., Härkänen, T., Marniemi, J., Heliövaara, M., Rissanen, H., Montonen, J., & Reunanen, A. (2008). Serum vitamin D and subsequent occurrence of type 2 diabetes. Epidemiology, 666-671.

- Kim, D.-H., Meza, C. A., Clarke, H., Kim, J.-S., & Hickner, R. C. (2020). Vitamin D and endothelial function. Nutrients, 12(2), 575 .

- Kim, O., & Drapkina, O. (2022). Obesity epidemic through the prism of evolutionary processes. Cardiovascular Therapy and Prevention, 21(1), 3109.

- Kirtland, K. A., Cho, P., & Geiss, L. S. (2015). Diabetes among Asians and Native Hawaiians or other Pacific Islanders—United States, 2011–2014. Morbidity and Mortality Weekly Report, 64(45), 1261-1266 .

- Kow, M., Akam, E., Singh, P., Singh, M., Cox, N., Bhatti, J. S., Tuck, S. P., Francis, R. M., Datta, H., & Mastana, S. (2019). Vitamin D receptor (VDR)

gene polymorphism and osteoporosis risk in White British men. Annals of Human Biology, 46(5), 430-433 .

- Krause, M., Bock, P .M., Takahashi, H. K., Homem De Bittencourt Jr, P. I., & Newsholme, P. (2015). The regulatory roles of NADPH oxidase, intra-and extra- cellular HSP70 in pancreatic islet function, dysfunction and diabetes. Clinical Science, 128(11), 789-803 .

- Krause, M., Heck, T. G., Bittencourt, A., Scomazzon, S. P., Newsholme, P., Curi, R., & Homem de Bittencourt, P. I. (2015). The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. Mediators of inflammation, 2015 .

- Kumar, A., Mansour, H. M., Friedman, A., Blough, E. R., & Press, C. (2013).

Nanomedicine in drug delivery. CRC Press Boca Raton, FL, USA .:

- Kumar, R., Saha, P ,.Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A Review on Diabetes Mellitus: Type1 & Type2. World Journal of Pharmacy and Pharmaceutical Sciences, 9(10), 838-850 .

- Landecho, M. F., Tuero, C., Valentí, V., Bilbao, I., de la Higuera, M., & Frühbeck, G. (2019). Relevance of leptin and other adipokines in obesity-associated cardiovascular risk. Nutrients, 11(11), 2664 .

- Le Marchand, L., & Wilkens, L. R. (2008). Design considerations for genomic association studies: importance of gene-environment interactions. Cancer Epidemiology Biomarkers & Prevention, 17(2), 263-267 .

- Lee, X. Y. B., Yusof, N. W., Pillai, N. K., Yap, C. G., & Jahan, N. K. (2022). Association between Social Network and Obesity among Adult Population. Open Journal of Endocrine and Metabolic Diseases, 12(2), 20-46 .

- Leighton, E., Sainsbury, C. A., & Jones, G. C. (2017). A practical review of C- peptide testing in diabetes. Diabetes therapy, 8, 475-487 .

- Lemieux, P., Weisnagel, S. J., Caron, A. Z., Julien, A.-S., Morisset, A.-S., Carreau, A.-M., Poirier, J., Tchernof, A., Robitaille, J., & Bergeron, J.(2019). Effects of 6-month vitamin D supplementation on insulin sensitivity

and secretion: a randomised, placebo-controlled trial. European journal of endocrinology, 181(3), 287-299 .

- Leung, M.-Y. M., Pollack, L. M., Colditz, G. A., & Chang, S.-H. (2015). Life years lost and lifetime health care expenditures associated with diabetes in the US, National Health Interview Survey, 1997–2000. Diabetes care, 38(3), 460- 468 .

- Li, Y., Li, X., He, K., Li, B., Liu, K., Qi, J., Wang, H., Wang, Y., & Luo, W. (2018). C-peptide prevents NF-κB from recruiting p300 and binding to the inos promoter in diabetic nephropathy. The FASEB Journal, 32(4), 2269-2279.

- Liel, Y., Ulmer ,E., Shary, J., Hollis, B. W., & Bell, N. H. (1988). Low circulating vitamin D in obesity. Calcified tissue international, 43, 199-201 .

- Lin, Y., & Sun, Z. (2010). Current views on type 2 diabetes. The Journal of endocrinology, 204(1), 1 .

- Little, P., Everitt, H., Williamson, I., Warner, G., Moore, M., Gould, C., Ferrier, K., & Payne, S. (2001). Observational study of effect of patient centredness and positive approach on outcomes of general practice consultations. Bmj, 323(7318), 908-911 .

- Lotta, L. A ,.Wittemans, L. B., Zuber, V., Stewart, I. D., Sharp, S. J., Luan, J. a., Day, F. R., Li, C., Bowker, N., & Cai, L. (2018). Association of genetic variants related to gluteofemoral vs abdominal fat distribution with type 2 diabetes, coronary disease, and cardiovascular risk factors. Jama, 320(24), 2553-2563 .

- Lu, L., Bennett, D. A., Millwood, I. Y., Parish, S., McCarthy, M. I., Mahajan, A., Lin, X., Bragg, F., Guo, Y., & Holmes, M. V. (2018). Association of vitamin D with risk of type 2 diabetes: a Mendelian randomisation study in European and Chinese adults. PLoS medicine, 15(5), e1002566 .

- Lui, C., Cady, N. C., & Batt, C. A. (2009). Nucleic acid-based detection of bacterial pathogens using integrated microfluidic platform systems. Sensors, 9(05), 3713-374 .4

- Luppi, P., et al., Human C-peptide antagonises high glucose-induced endothelial dysfunction through the nuclear factor-κB pathway. Diabetologia, 2008. 51(8): p. 1534 .

- Luppi, P., Cifarelli, V., Tse, H., Piganelli, J., & Trucco, M. (2008). Human C- peptide antagonises high glucose-induced endothelial dysfunction through the nuclear factor-κB pathway. Diabetologia, 51, 1534-1543.

- Mahdi, S. A. (2021). Frequency Of Vitamin D3 Receptor Gene Polymorphism Among Iraqi Individuals. NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO, 13178-13188 .

- Manchanda, P., & Bid, H. (2012). Vitamin D receptor and type 2 diabetes mellitus: Growing therapeutic opportunities. Indian Journal of Human Genetics, 18(3), 274 .

- Mangemba ,N. T., & San Sebastian, M. (2020). Societal risk factors for overweight and obesity in women in Zimbabwe: a cross-sectional study. BMC public health, 20(1), 1-8 .

- Martins, A. R., Nachbar, R. T., Gorjao, R., Vinolo, M. A., Festuccia, W. T., Lambertucci ,R. H., Cury-Boaventura, M. F., Silveira, L. R., Curi, R., &

Hirabara, S. M. (2012). Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. Lipids in health and disease, 11(1), 1-11 .

- Mayer-Davis, E. J., Lawrence, J. M., Dabelea, D., Divers, J., Isom, S., Dolan, L., Imperatore, G., Linder, B., Marcovina, S., & Pettitt, D. J. (2017). Incidence trends of type 1 and type 2 diabetes among youths, 2002–2012. N Engl J Med, 376, 1419-1429 .

- Mackawy, A. M., & Badawi, M. E. (2014). Association of vitamin D and vitamin D receptor gene polymorphisms with chronic inflammation, insulin resistance and metabolic syndrome components in type 2 diabetic Egyptian patients. Meta Gene, 2, 540-556.

- Maddaloni, E., Bolli, G. B., Frier, B. M., Little, R. R., Leslie, R. D., Pozzilli, P., & Buzzetti, R. (2022). C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective. Diabetes, Obesity and Metabolism, 24(10), 1912-1926.

- Malik, R., Farooq, R., Mehta, P., Ishaq, S., Din, I., Shah, P., & Majid, S. (2018). Association of Vitamin D receptor gene polymorphism in adults with type 2 diabetes in the Kashmir Valley. Canadian journal of diabetes, 42(3), 251-256.

- Mohammed, A. A., El-Matty, D. M. A., Abdel-Azeem, R., Raafat, K., Hussein, M. A., El-Ansary, A. R., Hafez, W., Hassan, H. A., Nassar, N. A., & Selim, N. M. (2023). Allelic Discrimination of Vitamin D Receptor Polymorphisms and Risk of Type 2 Diabetes Mellitus: A Case-Controlled Study. Healthcare,

- McKeigue PM, S. A., McGurnaghan S, et al. Persistent C-peptide secretion in type 1 diabetes and its relationship to the genetic architecture of diabetes. BMC Med. 2019, & 17(1):165 .

- Medvedev, M. G., Bushmarinov, I. S., Sun, J., Perdew, J. P., & Lyssenko, K. A . .)2017(Density functional theory is straying from the path toward the exact functional. Science, 355(6320), 49-52 .

- Metus, P., Ruzzante, N., Bonvicini, P., Meneghetti, M., Zaninotto, M., & Plebani, M. J. J. o. c. l. a. (1999). Immunoturbidimetric assay of glycated hemoglobin. 13(1), 5-8 .

- Mitchell, F. (2020). Vitamin-D and COVID-19: do deficient risk a poorer outcome? The lancet Diabetes & endocrinology, 8(7), 570 .

- Mohajan, D., & Mohajan, H. K. (2023). Obesity and Its Related Diseases: A New Escalating Alarming in Global Health. Journal of Innovations in Medical Research, 2(3), 12-23 .

- Morris, A. P. (2018). Progress in defining the genetic contribution to type 2 diabetes susceptibility. Current opinion in genetics & development, 50, 41-51 .

- Morteza, A ,.Nakhjavani, M., Larry, M., Nargesi, A. A., & Esteghamati, A. (2013). Heat shock protein 70 and albuminuria in patients with type 2 diabetes: a matched case control study. Cell Stress and Chaperones, 18, 815-819 .

- Mosaad, Y. M., Hammad, E. M., Fawzy, Z ,.Aal, I. A. A., Youssef, H. M., ElSaid, T. O., Monir, R., & El-Deek, B. S. (2014). Vitamin D receptor gene

polymorphism as possible risk factor in rheumatoid arthritis and rheumatoid related osteoporosis. Human immunology, 75(5), 452-461 .

- Moser, S. C & ,.van der Eerden, B. C. (2019). Osteocalcin—A versatile bone-derived hormone. Frontiers in endocrinology, 9, 794 .

- Mridha, M. (2015). Prospects of moringa cultivation in Saudi Arabia. J. Appl. Environ. Biol. Sci, 5(3), 39-46 .

- Murray, C. J., Aravkin, A. Y ,.Zheng, P., Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., & Abdollahpour, I. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990– 2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 396(10258), 1223-1249 .

- Naderpoor, N., Shorakae, S., Abell, S. K., Mousa, A., Joham, A. E., Moran, L. J., Stepto, N. K., Spritzer, P. M., Teede, H. J., & de Courten, B. (2018). Bioavailable and free 25-hydroxyvitamin D and vitamin D binding protein in polycystic ovary syndrome: Relationships with obesity and insulin resistance. The Journal of steroid biochemistry and molecular biology, 177, 209-215 .

- Nakhjavani, M., Morteza, A., Khajeali, L., Esteghamati, A., Khalilzadeh, O., Asgarani, F., & Outeiro, T. F. (2010). Increased serum HSP70 levels are

associated with the duration of diabetes. Cell Stress and Chaperones, 15, 959-964 .

- Nakhjavani, M., Morteza, A., Nargesi, A. A., Mostafavi, E., & Esteghamati, A. (2013). Appearance of leptin–HSP70 correlation, in type 2 diabetes. Meta Gene, 1, 1-7 .

- Nasser, F. A., Algenabi, A. A., & Kadhim, A. M. (2019). Relationship of body mass index (BMI) to dyslipidemia in Type2 diabetes mellitus. Al-Kufa University Journal for Biology, 1 .)1(1

- Newsholme, P., Keane, K. N., Elahy, M., & Cruzat, V. F. (2017). l-Arginine, Pancreatic Beta Cell Function, and Diabetes: Mechanisms of Stimulated Insulin Release and Pathways of Metabolism. L-Arginine in Clinical Nutrition, 85-94 .

- Nosratabadi, R ,.Arababadi, M., Salehabad, V., Shamsizadeh, A., Mahmoodi, M., Sayadi, A., & Kennedy, D. (2010). Polymorphisms within exon 9 but not intron 8 of the vitamin D receptor are associated with the nephropathic complication of type-2 diabetes. International journal of immunogenetics, 37(6), 493-497 .

- Obied, M. R., Al-Tu'ma, F. J., & Al-jameel, H. H. (2019). Role of polymorphism (rs1024611) in monocyte chemoattractant protein-1 gene in diabetic foot ulcer of Iraqi patients. Gene Reports, 17, 100502 .

- Oh, K.-J., Lee, D. S., Kim, W. K., Han, B. S., Lee, S. C., & Bae, K.-H. (2016).

Metabolic adaptation in obesity and type II diabetes: myokines, adipokines and hepatokines. International journal of molecular sciences, 18(1), 8 .

- Ojha, A., Ojha, U., Mohammed, R., Chandrashekar, A., & Ojha, H. (2019). Current perspective on the role of insulin and glucagon in the pathogenesis and treatment of type 2 diabetes mellitus. Clinical pharmacology: advances and applications, 57-65 .

- Olden, J. D., Jackson, D. A., & Peres-Neto, P .R. (2001). Spatial isolation and fish communities in drainage lakes. Oecologia, 127, 572-585 .

- Organization, W. H. (2016). World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization .

- Ortlepp, J ,.Metrikat, J., Albrecht, M., Von Korff, A., Hanrath, P., & Hoffmann,

R. (2003). The vitamin D receptor gene variant and physical activity predicts fasting glucose levels in healthy young men. Diabetic Medicine, 20(6), 451-454 .

- Ogunkolade, B.-W., Boucher, B. J., Prahl, J. M., Bustin, S. A., Burrin, J. M., Noonan, K., North, B. V., Mannan, N., McDermott, M. F., & DeLuca, H. F. (2002). Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes, 51(7), 2294-2300.

- Ota, T. (2015). Obesity-induced inflammation and insulin resistance. Frontiers Media SA .

- Owens, D. J., Allison, R., & Close, G. L. (2018). Vitamin D and the athlete: current perspectives and new challenges. Sports medicine, 48, 3-16 .

- Padhi, S., Nayak, A. K., & Behera, A .)2020(.Type II diabetes mellitus: A

review on recent drug based therapeutics. Biomedicine & Pharmacotherapy, 131, 110708 .

- Pelczyńska, M., Grzelak, T., Sperling, M., Bogdański, P., Pupek-Musialik, D., & Czyżewska, K. (2017). Impact of 25-hydroxyvitamin D, free and bioavailable fractions of vitamin D, and vitamin D binding protein levels on metabolic syndrome components. Archives of Medical Science, 13(4), 745-752 .

- Pike, J. W., Meyer, M. B., Benkusky, N. A., Lee, S. M., John, H. S., Carlson, A., Onal, M., & Shamsuzzaman, S. (2016). Genomic determinants of vitamin D- regulated gene expression. Vitamins & hormones, 100, 21-44 .

- Pilz, S., Zittermann, A., Trummer, C., Theiler-Schwetz, V., Lerchbaum, E., Keppel, M. H., Grübler, M. R., März, W., & Pandis, M .)2019(.Vitamin D testing and treatment: a narrative review of current evidence. Endocrine connections, 8(2), R27 .

- Pischon, T., Boeing, H., Hoffmann, K., Bergmann, M., Schulze, M. B., Overvad, K., Van der Schouw, Y., Spencer, E., Moons, K., & Tjønneland, A. (200 .)8General and abdominal adiposity and risk of death in Europe. New England Journal of Medicine, 359(20), 2105-2120 .

- Pittas, A. G., Dawson-Hughes, B., Sheehan, P., Ware, J. H., Knowler, W. C., Aroda, V. R., Brodsky, I., Ceglia, L., Chadha, C., & Chatterjee, R. (2019). Vitamin D supplementation and prevention of type 2 diabetes. New England Journal of Medicine, 381(6), 520-530 .

- Pittas, A. G., Lau, J., Hu, F. B., & Dawson-Hughes, B. (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. The Journal of Clinical Endocrinology & Metabolism, 92(6), 2017-2029 .

- Posa, F., Di Benedetto, A., Colaianni, G., Cavalcanti-Adam, E. A., Brunetti, G., Porro, C., Trotta, T., Grano, M., & Mori, G. (2016). Vitamin D effects on osteoblastic differentiation of mesenchymal stem cells from dental tissues. Stem Cells International, 2016 .

- Punthakee, Z., Goldenberg, R., & Katz, P. (2018). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian journal of diabetes, 42, S10-S15 .

- Radons, J. (2016). The human HSP70 family of chaperones: where do we stand?

Cell Stress and Chaperones, 21(3), 379-404 .

- Rahmadhani, R., Zaharan, N. L., Mohamed, Z., Moy, F. M., & Jalaludin, M. Y. (2017). The associations between VDR BsmI polymorphisms and risk of vitamin D deficiency, obesity and insulin resistance in adolescents residing in a tropical country. PloS one, 12(6), e0178695 .

- Rajagambeeram, R., Malik, I., Vijayan, M., Gopal, N., & Ranganadin Jr, P. (2020 .)Evaluation of serum electrolytes and their relation to glycemic status in patients with T2DM. Int. J. Clin. Biochem. Res, 6, 10 .

- Rhodes, J. M., Subramanian, S., Laird, E., Griffin, G., & Kenny, R. A. (2021). Perspective: Vitamin D deficiency and COVID 19-severity–plausibly linked by latitude, ethnicity, impacts on cytokines, ACE2 and thrombosis. Journal of internal medicine, 289(1), 97-115 .

- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., & Ogurtsova, K. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice, 157, 107843 .

- Safadi, F. F., Thornton, P., Magiera, H., Hollis, B. W., Gentile, M., Haddad, J. G., Liebhaber, S. A., & Cooke, N. E. (1999). Osteopathy and resistance to

vitamin D toxicity in mice null for vitamin D binding protein. The Journal of clinical investigation .251-239 ,)2(103 ,

- Saibil, H. (2013). Chaperone machines for protein folding, unfolding and disaggregation. Nature reviews Molecular cell biology, 14(10), 630-642 .

- Saintonge, S., Bang, H., & Gerber, L. M. (2009). Implications of a new definition of vitamin D deficiency in a multiracial us adolescent population: the National Health and Nutrition Examination Survey III. Pediatrics, 123(3), 797- 803 .

- Sacerdote, A., Dave, P., Lokshin, V., & Bahtiyar, G. (2019). Type 2 diabetes mellitus, insulin resistance, and vitamin D. Current diabetes reports, 19, 1-12.

- Saini, V. (2010). Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World journal of diabetes, 1(3), 68.

- Salih, Y. A., Rasool, M. T., Ahmed, I. H., & Mohammed, A. A. (2021). Impact of vitamin D level on glycemic control in diabetes mellitus type 2 in Duhok. Annals of Medicine and Surgery, 64, 102208.

- Sakurai, Y., Kubota, N., Yamauchi, T., & Kadowaki, T. (2021). Role of insulin resistance in MAFLD. International journal of molecular sciences, 22(8), 4156 .

- Saraswathi, S., Al-Khawaga, S., Elkum, N., & Hussain, K. (2019). A systematic

review of childhood diabetes research in the Middle East region. Frontiers in endocrinology, 10, 805 .

- Sassi, F., Tamone, C & ,.D'Amelio, P. (2018). Vitamin D: nutrient, hormone, and immunomodulator. Nutrients, 10(11), 1656 .

- Schauer, P. R., Bhatt, D. L., Kirwan, J. P., Wolski, K., Aminian, A., Brethauer, S. A., Navaneethan, S. D., Singh, R. P., Pothier, C. E., & Nissen, S. E. (20 .)17 Bariatric surgery versus intensive medical therapy for diabetes—5-year outcomes. N Engl J Med, 376, 641-651 .

- Schopf, F. H., Biebl, M. M., & Buchner, J. (2017). The HSP90 chaperone machinery. Nature reviews Molecular cell biology, 18(6), 345-360 .

- Sharma, D., Martineau, C. N., Le Dall, M.-T., Reidy, M., Masison, D. C., & Kabani, M. (2009). Function of SSA subfamily of Hsp70 within and across species varies widely in complementing Saccharomyces cerevisiae cell growth and prion propagation. PloS one, 4 ,)8(e6644 .

- Sherman, M. Y., & Goldberg, A. L. (2001). Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron, 29(1), 15-32 .

- Shaat, N., Ignell, C., Katsarou, A., & Berntorp, K. (2017). Glucose homeostasis, beta cell function, and insulin resistance in relation to vitamin D status after gestational diabetes mellitus. Acta obstetricia et gynecologica Scandinavica, 96(7), 821-827.

- Siderova, M. (2019). Type 2 Diabetes: From Pathophysiology to Modern Management. BoD–Books on Demand .

- Singh, R. G., Yoon, H. D., Wu, L. M., Lu, J., Plank, L. D., & Petrov, M. S. (2017). Ectopic fat accumulation in the pancreas and its clinical relevance: a

systematic review, meta-analysis, and meta-regression. Metabolism, 69 .13-1 ,

- St. John, H. C., Bishop, K. A., Meyer, M. B., Benkusky, N. A., Leng, N., Kendziorski, C., Bonewald, L. F., & Pike, J. W. (2014). The osteoblast to osteocyte transition: epigenetic changes and response to the vitamin D3 hormone. Molecular endocrinology, 28(7), 1150-1165 .

- Stamatakis, E., Gale, J., Bauman, A., Ekelund, U., Hamer, M., & Ding, D. (2019). Sitting time, physical activity, and risk of mortality in adults. Journal of the American College of Cardiology, 73(16), 2062-2072 .

- Sun, J. (2018). Dietary vitamin D, vitamin D receptor, and microbiome. Current opinion in clinical nutrition and metabolic care, 21(6), 471 .

- Sung, C.-C., Liao, M.-T., Lu, K.-C., & Wu, C.-C. (2012). Role of vitamin D in insulin resistance. Journal of biomedicine and biotechnology, 2012 .

- Szymczak-Pajor, I., & Śliwińska, A. (2019). Analysis of association between vitamin D deficiency and insulin resistance. Nutrients, 11(4), 794 .

- Talbot, H., Coleman, L., Crimin, K., Zhu, Y., Rock, M., Meece, J., Shay, D., Belongia, E & ,.Griffin, M. (2012). Association between obesity and vulnerability and serologic response to influenza vaccination in older adults. Vaccine, 30(26), 3937-3943 .

- Tan, G. H. (2015). Diabetes care in the Philippines. Annals of global health, 81(6), 863-869 .

- Tchernof, A., & Després, J.-P. (2013). Pathophysiology of human visceral obesity: an update. Physiological reviews .

- Theis, T., Skurray, R. A., & Brown, M. H. (2007). Identification of suitable internal controls to study expression of a Staphylococcus aureus multidrug resistance system by quantitative real-time PCR. Journal of microbiological methods, 70(2), 355-362 .

- Thorens, B. (2015). GLUT2, glucose sensing and glucose homeostasis.

Diabetologia, 58(2), 221-232 .

- Tokarz, V. L., MacDonald, P. E., & Klip ,A. (2018). The cell biology of systemic insulin function. Journal of Cell Biology, 217(7), 2273-2289 .

- Trinder, P. J. A. o. c. B. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. 6(1), 24-27 .

- Uitterlinden, A. G., Fang, Y., Van Meurs, J. B., Pols, H. A., & Van Leeuwen, J.

P. (2004). Genetics and biology of vitamin D receptor polymorphisms. Gene, 338(2), 143-156 .

- Usategui-Martín, R., Luis-Román, D., Fernández-Gómez, J. M., Ruiz-Mambrilla, M., & Pérez-Castrillón, J.-L. (2022). Vitamin D receptor (VDR) gene polymorphisms modify the response to vitamin D supplementation: A systematic review and meta-analysis. Nutrients, 14(2), 360 .

- Vabulas, R. M., Ahmad-Nejad, P., Ghose, S., Kirschning, C. J., Issels, R .D., & Wagner, H. (2002). HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. Journal of Biological Chemistry, 277(17), 15107-15112 .

- Vasilopoulos Y., S. T., Kotsa K., Papadimitriou M., Goutzelas Y., Stamatis C., Bagiatis V., Tsekmekidou X., Yovos J.G., Mamuris Z. VDR TaqI is associated with obesity in the Greek population. Gene. 2013, & 512:237–239.

- Vasudevan, D. M., Sreekumari, S., & Vaidyanathan, K. (2013). Textbook of biochemistry for medical students. JP Medical Ltd .

- Wang ,B., Li, R., Lu, Z., & Huang, Y. (2020). Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. Aging (albany NY), 12(7), 6049 .

- Wang, G., Li, Y., Li, L., Yu, F., Cui, L., Ba, Y., Li, W., & Wang, C. (2014).

Association of the vitamin D binding protein polymorphisms with the risk of type 2 diabetes mellitus: a meta-analysis. BMJ open, 4(11), e005617 .

- Wang, H., Chen, W., Li, D., Yin, X., Zhang, X., Olsen, N., & Zheng, S. G. (2017). Vitamin D and chronic diseases. Aging and disease, 8(3), 346.

- Wang, X., Bao, W., Liu, J., OuYang, Y.-Y., Wang, D., Rong, S., Xiao, X., Shan, Z.-L., Zhang, Y., & Yao, P. (2013). Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes care, 36(1), 166-175.

- Wang, L., Zhang, C., Song, Y., & Zhang, Z. (2020). Serum vitamin D deficiency and risk of gestational diabetes mellitus :a meta-analysis. Archives of Medical Science, 16 .)1(

- Wang, X., Bao, W., Liu, J., OuYang, Y.-Y., Wang, D., Rong, S., Xiao, X., Shan, Z.-L., Zhang, Y., & Yao, P. (2013). Inflammatory markers and risk of

type 2 diabetes: a systematic review and meta-analysis. Diabetes care, 36(1), 166-175 .

- Wang, X., Zhang, Y., Fei, S., Awais, M. M., Zheng, H., Feng, M., & Sun, J. (2021). Heat Shock Protein 75 (TRAP1) facilitate the proliferation of the Bombyx mori nucleopolyhedrovirus. International Journal of Biological Macromolecules, 175, 372-378 .

- Wei, W., Liu, Q., Tan, Y., Liu, L., Li, X., & Cai, L. (2009). Oxidative stress, diabetes, and diabetic complications. Hemoglobin, 33(5), 370-377 .

- Włodarczyk, M., & Nowicka, G. (2019). Obesity, DNA damage, and development of obesity-related diseases. International journal of molecular sciences, 20(5), 1146 .

- Wongdee, K., & Charoenphandhu, N. (2015). Vitamin D-enhanced duodenal calcium transport. Vitamins & hormones, 98, 407-440 .

- Wysoczańska-Klaczyńska, A., Ślęzak, A., Hetman, M., & Barg, E. (2018). The impact of VDR gene polymorphisms on obesity, metabolic changes, bone mass disorders and neoplastic processes. Pediatric Endocrinology Diabetes and Metabolism, 24(2), 96-105 .

- Xia, Y., Kelton, C. M., Guo, J. J., Bian, B., & Heaton, P. C. (2015). Treatment of obesity: Pharmacotherapy trends in the U nited S tates from 1999 to 2010. Obesity, 23(8), 1721-1728 .

- Yang, Y., Shields, G. S., Guo, C., & Liu, Y. (2018). Executive function performance in obesity and overweight individuals: A meta-analysis and review. Neuroscience & Biobehavioral Reviews, 84, 225-244 .

- Yaribeygi, H., Maleki, M., Sathyapalan, T., & Sahebkar, A. (2019). The effect of C-peptide on diabetic nephropathy: A review of molecular mechanisms. Life sciences, 237, 11 .6950

- Ye, J. (2013). Mechanisms of insulin resistance in obesity. Frontiers of medicine, 7(1), 14-24 .

- Zaccardi, F., Webb, D. R., Yates, T., & Davies, M. J. (2016). Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. Postgraduate medical journal, 92(1084), 63-69 .

- Zaki, M., Basha, W., El-Bassyouni, H. T., El-Toukhy, S., & Hussein, T. (2018). Evaluation of DNA damage profile in obese women and its association to risk of metabolic syndrome, polycystic ovary syndrome and recurrent preeclampsia. Genes & diseases, 5(4), 367-373 .

- Zaki, M., Kamal, S., Basha, W. A., Youness, E., Ezzat, W., El-Bassyouni, H., & Amr, K. (2017). Association of vitamin D receptor gene polymorphism (VDR) with vitamin D deficiency, metabolic and inflammatory markers in Egyptian obese women. Genes & diseases, 4(3), 176-182.

- Zhao, L., Wang, M., Li, J., Bi, Y., Li, M., & Yang, J. (2019). Association of circulating branched-chain amino acids with gestational diabetes mellitus: A meta-analysis. International Journal of Endocrinology and Metabolism, 17 .)3(

- Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature reviews endocrinology, 14(2), 88-98 .

- Zhu, J., Bing, C., & Wilding, J. P. (2018). Vitamin D receptor ligands attenuate the inflammatory profile of IL-1β-stimulated human white preadipocytes via modulating the NF-κB and unfolded protein response pathways. Biochemical and biophysical research communications, 503(2), 1049-1056 .

- Zierath, J. R. (2019 .)Major advances and discoveries in diabetes-2019 in review. Current diabetes reports, 19(11), 1-9 .

- Zou, Q., Qu, K., Luo, Y., Yin, D., Ju, Y., & Tang, H. (2018). Predicting diabetes mellitus with machine learning techniques. Frontiers in genetics, 9, 51.5

الملاحق

Characteristics of Vitamin D3 Receptor Genotypes in T2DM of Iraqi Obese Women

Baraa Abdul-Kareem Mutar[1], Thikra Ali Allwsh[2], Ammar Gany Yassin[1], Fadhil Jawad Al-Tu'ma[1,*], Hassanien S. Taghi[3]

[1]Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq.
[2]Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.
[3]Department of Pharmaceutics, College of Pharmacy, Al-Bayan University, Iraq.
*Correspondence to: Fadhil Jawad Al-Tu'ma (E-mail: fadhil.jawad@uokerbala.edu.iq)
(Submitted: 10 April 2023 – Revised version received: 29 April 2023 – Accepted: 12 May 2023 – Published online: 26 June 2023)

Abstract

Objectives: This study aims to examine the association between the (rs1544410) polymorphism of the VDR gene with the pathogenesis of T2DM in Iraqi obese women.

Methods: A case-control study was performed on 50 patients with T2DM and 50 apparently healthy subjects who were admitted to Al-Hussein Teaching Hospital and Al-Hassan Center of Diabetes and Endocrinology unit/Kerbala health directorate—Iraq during (April 2022–March 2023). The T2DM groups were divided into two groups, 25 obese and 25 non-obese; the control group was divided into 25 obese and 25 non-obese as apparently healthy groups. The ELISA Kit was used to measure serum 25(OH)D3, heat shock protein-70, VDBP, insulin and C-peptide. Also HbA1c% and insulin resistance (HOMA-IR) were evaluated. The vitamin D3 receptor gene (VDR) variant and the SNP (rs1544410) polymorphism was determined using allele specific polymerase chain reaction, 1.5% agarose gel electrophoresis and then visualized by gel photo-documentation system.

Results: The result of vitamin D3 variants genotype (rs 1544410) was a clear band with a molecular size of 200 bps. The size of the amplicon was determined by compare with DNA ladder 100–1500 bp. The result of the comparison between observed and anticipated values for SNIP with (rs 1544410) in the tested population was statistically significant, $P = < 0.001$ and the difference between demographic characteristics and (rs 1544410) SNP, age and BMI shows non-significant difference among all groups. The difference between biomarkers and (rs1544410) SNP was performed using one-way ANOVA test to compare the mean levels of HSP-70, VDBP, C-peptide, RBC and HbA1c% which shown a non-significant difference among the variants of VDBP Genotype (rs1544410) in obese women (patients and control) studied groups, P value > 0.05.

Conclusion: The logistic analysis of the (rs1544410) SNP of the patients concluded that HSP-70, VDBP, and C-peptide level was no significantly related to the also C-peptide, was shown to be a related risk factor to both CT and CT alleles (1.003, $P > 0.05$) in comparison with CC alleles. Furthermore, HbA1c% level was demonstrated to be related as a risk factor for the CG allele in comparison with CC and GG alleles (1.009, $P < 0.05$).

Keywords: Diabetes mellitus, type 2, receptors, calcitriol, vitamin D-binding protein, HSP-70, obesity

Introduction

Diabetes mellitus (DM) is a chronic metabolic condition that causes blood glucose levels to rise as a result of either reduced insulin production or body cells that do not react to the effects of insulin (insulin resistance).[1] Although there are various forms of diabetes, type 2 diabetes mellitus (T2DM) is the most frequent type and accounts for 90–95% of cases of diagnosed (DM). T2DM is more common in adults and elderly[2] resulting in anomalies in the metabolism of carbohydrates, lipids, and proteins, as well as disturbance of the regulatory systems that control the storage and mobilization of metabolic fuel.[3] Obesity is mostly measured by means of body mass index (BMI), but anthropometric classification systems do not reflect the presence or severity of comorbidities.[4] Vitamin D3 receptor gene factor nuclear transcription by the mediation of 1,25(OH)$_2$D3, VDR influences calcium absorption, bone remodeling, and the rate of mineralization. There are 11 exons in the 3q11 region on the long arm of chromosome 12, and 2 to 9 of them are actively transcribed.[5] Numerous investigations have revealed that the nuclear receptor superfamily's VDR gene, which is found on chromosome 12's long arm (12q13.11), plays a significant role in the etiology of osteoporosis.[6]

The majority of research on VDR polymorphisms has been done in Caucasian populations and has concentrated on five SNPs: (1) rs10735810 or *FokI* in exon 2; (2) rs1544410 or *BsmI* in intron 8; (3) rs731236 or *TaqI* in exon 9; (4) rs7975232 or *ApaI* in intron 8 and (5) rs757343.[7] Vitamin D receptor gene sigle nucleotide polymorphism of BsmI variant modulate glucose intolerance, insulin secretion and sensitivity.[8] VDR gene's variants can alter insulin secretion, leading to insulin resistance, as well as vitamin D3 biosynthesis, transportation, and action.[9] Reported about T2DM patients have a considerably higher prevalence of the *BsmI* SNP. Several studies showed a similar connection between *BsmI* polymorphism and T2DM in other groups.[10] *BsmI* SNP and risk of T2DM in various ethnic groups are thus not conclusively linked. SNP is connected to therapeutic responsiveness and illness susceptibility.[11] T2DM) and VDR polymorphisms are still not clearly linked. Many genetic VDR polymorphisms have been identified in studies carried out in different places with varying populations of people. Just one research has examined the relationship between the VDR *BsmI* (rs1544410) polymorphism and vitamin D3 insufficiency, obesity, and insulin resistance among non-diabetic subjects across various age groups to date, and it was conducted in the central area of Malaysia.[12] Accordingly, the aforementioned study had found that the BsmI (rs1544410) polymorphism was associated with increased risk for vitamin D deficiency and insulin resistance among the Malaysian population.[12] The risk of metabolic

سومین همایش بین المللی تحقیقات در علوم و فناوری نانو

المؤتمر الدولي الثالث لبحوث في علوم و تكنولوجيا النانو

University of Tehran Aras International Campus

3rd International Conference on Researches in Nanotechnology & Nanoscience

Certificate of Paper Presentation

This is to certify that

Baraa Abdul-Kareem Mutar

Presented a paper as **Poster** titled

Association between Heat Shock Protein-70 and Vitamin D3 Binding Protein in Iraqi Obese Type 2 Diabetic Women

at the **3rd Intl. Conference on Nanotechnology & Nanoscience**

held by Aras International Campus (UTAIC), University of Tehran

Tehran, Iran - 26th April 2023

Authors: Baraa Abdul-Kareem Mutar, Fadhil Jawad Al-Tu'ma, Thikra Ali Allwsh, Israa Khalil Ibrahim Al-Yassiri, Ammar Ghani Yassin

Dr. Zahra Emam Djomeh

Dean of Aras Intl. Campus (UTAIC)

Conference Scientific Director

Address: End of Qods St., Enghelab Sq., Tehran, Iran.

Cell: 0098 935 438 9168 **Fax:** 00982189776220

Website: www.Utnano.ir **E-Mail:** Info@Utnano.ir

سومین همایش بین المللی پژوهش های دانشجویی در علوم و فناوری نانو

المؤتمر الدولي الثالث لطلاب الدراسات العليا في تكنولوجيا و علوم النانو

University of Tehran
Aras International Campus

3rd International Conference on Researches in Nanotechnology & Nanoscience

Certificate of Paper Presentation

This is to certify that

Baraa Abdul-Kareem Mutar

Presented a paper as **Poster** titled

Association between Nesfatin-1 Levels and C-Peptide in Sera of Obese / Non-Obese Type 2 Diabetic Women

at the **3rd Intl. Conference on Nanotechnology & Nanoscience**

held by Aras International Campus (UTAIC), University of Tehran

Tehran, Iran - 26th April 2023

Authors: Israa Khalil Ibrahim Al-Yassiri, Fadhil Jawad Al-Tu'ma, Maher Abbood Mukheef, Khosay Baqir Jawad, Baraa Abdul-Kareem Mutar

Dr. Zahra Emam Djomeh
Dean of Aras Intl. Campus (UTAIC)
Conference Scientific Director

Address: End of Qods St., Enghelab Sq., Tehran, Iran.
Cell: 0098 935 438 9168 **Fax:** 00982189776220
Website: www.Utnano.ir **E-Mail:** info@Utnano.ir

University of Kerbala – College of Medicine

Department of Chemistry and Biochemistry

Student Name: Baraa Abdul-Kareem Mutar Hussein

Supervisors:

Prof. Dr. Fadhil Jawad Al-Tu'ma - College of Medicine – University of Kerbala

Prof. Dr. Thikra Ali Allwsh – College of Science – University of Mosul.

((Experimental Data of the Case-Control Study))

Role of Heat Shock Protein-70 and Vitamin D3 Binding Protein in Relation to its Receptor Gene Variants among Iraqi Obese Type 2 Diabetic Women		
Sample No.:	**Smoking:** Yes or No **[If Yes (Heavy – Moderate – Slightly)]**	**BMI :** **Age:**
Disease Description:		
Blood Pressure: Diastolic: Systolic:		
Exclusion Criteria: Renal Diseases, thyroid diseases, liver disease, heart diseases		
Inclusion Criteria: Obese Women of Type 2 Diabetes Mellitus with/without Hypertension.		

Biomarkers Descriptions

Biomarkers	Levels	Units
Vitamin D3		
Vitamin D3 Binding Protein		
Heat-Shock Protein-70		
Serum Insulin		
Serum HbA1c%		
HOMA-IR		
Serum Glucose		
C-Peptide		
Vitamin D3 Receptor Gene Variant *Bsm* I Analysis (rs1544410)		

الخلاصـــــة

اشتملت الدراسة الحالية على 45 سيدة مصابة بداء السكري من النوع 2 من مراجعي مستشفى الحسين التعليمي ومركز الامام الحسن لأمراض السكر والغدد الصماء / مديرية صحة كربلاء العراق، اذ تراوحت أعمارهن بين (30_67) عام، وتم تقسيمهن إلى مجموعتين. اعتمادًا على مؤشر كتلة الجسم (BMI)، فقد شملت مجموعة النساء البدينات بمتوسط كتلة جسم يزيد عن 30 كجم / م2، ومجموعة النساء ذوات الوزن الطبيعي بمتوسط كتلة جسم أقل من 30 كجم / م 2 ، استمرت فترة أخذ العينات من بداية أبريل (2022) إلى مارس (2023). بالإضافة إلى 45 امرأة تتمتع بصحة جيدة ولا تعاني من أي مشكلة صحية وفي نفس أعمار مجموعة البروتينات الضابطة. أشارت النتائج التي لوحظت إلى أن مستويات HSP-70و VDBP أظهرت انخفاضًا غير معنوي في جميع مجموعات مرضى السكري من النوع 2 مقارنةً بمجموعة السيطرة على ما يبدو، بينما انخفض الببتيد C بشكل ملحوظ في مرضى السكري من النوع 2 عند مقارنته مع مجموعة السيطرة الضابطة. بالإضافة إلى ذلك ، أظهر كل من الجلوكوز العشوائي ونسبة HbA1c في الدم ارتفاعًا ملحوظًا في مرضى السكري من النوع 2 مقارنةً بمجموعات التحكم السليمة ظاهريًا. أشارت البيانات المرصودة إلى أن مستويات HSP70 و VDBP و C-peptide عند المقارنة بين مرضى السكري من النوع 2 والتحكم في مجموعات السمنة أظهرت عوامل خطر اعتمادًا على النسبة الفردية الملاحظة (OR = 1.156 (1.649-1.148) ، 0.917 ((0.733-1.148) و 1.049 (1.088-1.011)) على التوالي ، ولكن تم إثبات أهمية ببتيد C فقط. في مؤشر كتلة الجسم ، كانت مستويات HSP-70 و VDBP و C-peptide ، كما هو موضح ، HSP70و VDBP و -C الببتيد غير مهمة في مجموعات السمنة ، بينما أظهر HSP-70 VDBP و -C الببتيد بشكل كبير في مجموعات الوزن الطبيعي . كانت نقاط التشخيص المثلى لـ HSP70 هي (الحساسية = 90٪ ، النوعية = 87٪) عند مستوى (نقاط القطع) = 29.086 ، بينما مستويات) :C-Peptide الحساسية = 93٪ ، النوعية = 88٪) عند مستوى (نقاط القطع) = 134.76 ، و) VDBP الحساسية = 96٪ ، النوعية = 78٪) عند مستوى (نقاط القطع) = 56.423 جميع العلامات لها قيم p من AUC كانت <0.001 وذات دلالة إحصائية. وبناءً عليه ، تم التوصل إلى وجود علاقة معنوية بين مستويات VDBP المتداولة و .T2DM يبدو أن VDBPقادرة على المساهمة في علاج السمنة ومرض السكري لما لها من آثار فقدان الشهية ومضادات ارتفاع السكر في الدم. بالإضافة إلى ذلك ، فإن -C الببتيد هو علامة بيولوجية معروفة لمقاومة الأنسولين ووظيفة خلايا بيتا. تم الحصول على نتائج تحليل عالية الدقة والحساسية من خلال تحليل ROC لكلا الواسمتين في T2DM ونتيجة التركيب الجيني, ، بالإضافة إلى ذلك ، عند مقارنة VDR مع البوليمرات الجينية في المرضى أظهروا زيادة في ترددات :HSP-70الأنماط الجينية CT + TT و TT في (rs1544410ـل (0.901 - 1.048) CT (OR = 0.972 ،p = 0.464 ؛ TT (OR = 0.988

(0.921 - 1.059)، p = 0.734 ، على التوالي) ، الأنماط الجينية VDBP: CT + TT و TT في (
rs1544410) من أجل (0.969 - 1.027) CT (OR = 0.998 ، p = 0.875 ؛ TT (OR = 0.988
(0.961 - 1.015)، p = 0.384 ، الببتيد- C : الأنماط الجينية CT + TT و TT في (rs1544410)
لـ (0.997 - 1.009) CT (OR = 1.003 ، p = 0.338 ؛ (0.990 - 1.006) TT (OR 0.998 ،
p = 0.593 ، فقط فروق ذات دلالة إحصائية في 25 (OH)D3

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

دور بروتين الصدمة الحرارية – 70 (HSP-70) والبروتينات المرتبطة بفيتامين D3 (VDBP) وعلاقته بجينات مستقبلات فيتامين D3 بين النساء البدينات المصابات بداء السكر النوع الثاني

رسالة ماجستير

مقدمة الى مجلس كلية الطب – جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل
براء عبد الكريم مطر حسين

(بكالوريوس تقي تحليلات مرضية – كلية الصفوة الجامعة 2017)

باشراف

الأستاذ الدكتور	الاستاذ الدكتورة
فاضل جواد ال طعمة	ذكرى علي علوش
كلية الطب – جامعة كربلاء	كلية العلوم – جامعة الموصل

حزيران 2023 ميلادية
ذي القعدة 1444 هجري