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Estimation the levels of (coenzyme Q10) and Respiratory Factor Erythroid 2–Related Factor 2 (Nrf-2) in Pre diabetes and type 2 diabetes mellitus Patients

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

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بسم الله الرحمن الرحيم

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Supervisor - Certification

we certify that this thesis entitled **(Estimation the levels of (coenzyme Q10) and Respiratory Factor Erythroid 2–Related Factor 2 (Nrf-2) in Pre diabetes and type 2 diabetes mellitus Patients).** " Was prepared by **(Hayder Abdulhussaain Taher)** under our supervision at the college of medicine, university of karbala , as a partial fulfillment of the requirement for the Degree of Master in (Clinical Chemistry).

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Dedication

- To the people closest to my heart at all times, the Great Messenger Muhammad ibn Abdullah (Allah Blessing be upon Mohammed and his Immsdiate family) and his pure household.
- To the dearest people to my heart, my parents, who raised me well.
- To my martyred brother Ali, his sons, and every martyr.
- To my brother, may Allah have mercy on him, Salam, my dear sisters, and their sons.
- To every friend and companion who has supported me.

I dedicate this work with all love and gratitude.

Hayder

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Summary

Type 2 Diabetes Mellitus (T2DM) and its precursor, pre-diabetes, are characterized by chronic oxidative stress and metabolic dysfunction. Coenzyme Q10 (CoQ10), a vital mitochondrial antioxidant, and Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), a master regulator of antioxidant defenses, are crucial in cellular protection. This study aimed to estimate the levels of serum CoQ10 and Nrf2 in T2DM patients, pre-diabetic individuals, and healthy controls, and to evaluate their diagnostic potential.

Methods: A case control was conducted from September 2024 to July 2025 in Iraq , study was carried out at Imam Hassan Center for Endocrinology and Diabetes /Internal Medicine Consultant and Imam Al – Hassan Al Mujtaba teaching hospital in Kerbala city/ Internal Medicine Consultant. This study involved 88 individuals, including 46 type II diabetes patients , pre diabetes patients (12) and 30 volunteers as a control, with inclusion criteria including fasting blood sugar and haemoglobin A1c results. Blood samples were collected from both control and patient groups after 12 hours of fasting.

Data were collected via a structured questionnaire covering sociodemographics, medical history, and lifestyle, complemented by clinical evaluations by specialist physicians. Serum lipid profile were measured using fully automatic chemistry analyzer (SMART-120, Geno TEK, United States of America). ELISA system was used for the detection of Nrf2 level. Q10levels were measured quantitatively by high-performance liquid chromatography.

Results: The study groups were well-differentiated by HbA1c and lipid profiles, with DM and pre-DM groups exhibiting typical glycemic and dyslipidemic patterns. Serum CoQ10 levels showed a clear and significant progressive decline from healthy controls (median 1.6 U/L) to the pre-diabetic group (median 0.89 U/L) and lowest in DM patients (median 0.38 U/L). In contrast, median serum Nrf2 levels were remarkably similar and largely overlapping across all three groups (medians: Healthy 26, Pre-DM 30, DM 29), indicating no significant difference.

ROC curve analysis revealed excellent diagnostic accuracy for CoQ10: an Area Under the Curve (AUC) of 0.9724 ($p<0.0001$) for differentiating DM patients from healthy controls, with an optimal cut-off of <1.275 U/L. For identifying pre-diabetic individuals, CoQ10 showed an AUC of 0.9013 ($p<0.0001$), with an optimal cut-off of <1.120 U/L. Correlation analysis revealed a significant positive correlation between CoQ10 and Nrf2 in both DM ($r=0.4$, $p=0.05$) and pre-DM ($r=0.65$, $p=0.001$) groups. Notably, CoQ10 was strongly negatively correlated with HbA1c in the pre-DM group ($r=-0.920$, $p=0.027$), and positively correlated with HDL in the DM group ($r=0.332$, $p=0.005$).

Conclusion: Serum CoQ10 levels progressively decrease with the advancement of metabolic dysregulation and serve as a highly accurate biomarker for diagnosing both pre-diabetes and Type II Diabetes Mellitus. Its strong associations with glycemic control and lipid profiles highlight its integral role in metabolic health. Conversely, circulating Nrf2 levels do not appear to be a reliable diagnostic marker for these conditions. These findings underscore the potential of CoQ10 as a valuable non-invasive tool for screening and monitoring in the context of impaired glucose metabolism.

List of Abbreviations	
Abbreviations	Definition
4-AAP	4-aminoantipyrine
ABEI	N-4-Aminobutyl-N-ethylisoiuminol
ABO	blood type
ACADS	acetyl-CoA dehydrogenase
ADA	American Diabetes Association
AGEs	Advanced glycation end products
AGEs	Advanced glycation end products
ARE	antioxidant response element
AS160	It is part of the TBC1D4 family.It is a key protein in insulin-related signaling and regulating GLUT4 activity, making it a key player in regulating blood glucose levels.
AUC	area under the curve
BCODh (B F)	branched chain 2-oxoacid dehydrogenase
BGLs	Blood glucose levels
C18 – ODS-2	column (C18 It means 18 carbon long carbon group and ODS-2 means Octadecylsilane encapsulating the column)
CAT	catalase
CHE	Cholesterase
CHOD	cholesterol oxidase
CI	Confidence Interval
complex I (I F, I Q)	The first complex in the electron transport chain is NADH: Ubiquinone oxidoreductase, and unit I F Flavin mononucleotide) oxidation, unit I Q (the part responsible for transferring electrons to ubiquinone Q reduction)
complex II (II F)	Succinate: Ubiquinone oxidoreductase (Convert succinate to formate and FAD to FADH2

CoQ10	2,3-Dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone
CRIF1	CR6-interacting factor1
DHODh (D Q)	dihydroorotate dehydrogenase
DKA	diabetic ketoacidosis
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic Acid) anticoagulant)
ELISA	Enzyme-Linked Immunosorbent Assay
ER	endoplasmic reticulum
ETF	electron transfer flavoprotein (in mitochondria)
ETF:Q	ETF:Q oxidoreductase
FAM129B	Family with Sequence Simiarity 129 Member B
FAs	Fatty acids
FBG	Fasting blood glucose
FPG	fasting plasma glucose
G6PD	glucose-6-phosphate dehydrogenase
GCLC	glutamate-cysteine ligase catalytic subunit
GDM	Gestational diabetes mellitus
Gk	Glycerol kinase
GLUT4	Glucose Transporter Type 4
GPO	glycerol phosphate oxidase
GSK3-B–	Glycogen Synthase Kinase3 beta
GSK-3β	glycogen synthase kinase-3β
GSVs	Glucose Storage Vesicles
HbA1c	Hemoglobin A1
HbA1cADA	Hemoglobin A1 according to the American Diabetes Association standards

HbA1cIEC	Hemoglobin A1 according to the International Expert Committee standards
HDAOS	[N-(2-hydroxy-3-sulfopropyl)- 3,5-dimethoxyaniline
HDL-C	High Density Lipoprotein Cholesterol
HMG CoA	hydroxyl-methylglutaryl coenzyme A
HO-1	heme oxygenase 1
HOMA	homeostatic model assessment
HRD1	HMG-CoA Reductase Degredation protein
HRP	Horseradish peroxidase
IDF	International Diabetes Federation
IDF	International Diabetes Federation
IDH1	isocitrate dehydrogenase 1
IEC	International Expert Committee
IFG	impaired fasting glucose
IFGADA	impaired fasting glucose according to the American Diabetes Association standards
IFGWHO	impaired fasting glucose according to the World Health Organization standards
IGT	impaired glucose tolerance
IMM	inner mitochondrial membrane
IR	insulin resistance
IRS-1	Insulin receptor substrate 1
Keap1	Kelch-like ECH-associated protein 1
KEAP1-CUL3-RBX1	Kelch-like ECH-associated protein1,Cullin-3 ,RING-box protein 1
LDL	low-density lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
LPL	Lipoproteinlipase

MafA	Mast cell associated function associated antigen
ME1	malic enzyme 1
ME-1	malic-enzyme 1
MENA	Middle East and North Africa
MS	metabolic syndrome
mtGPDh	mitochondrial glycerol-3-phosphate dehydrogenase
NADPH	Nicotinamude adenine dinucleotide phosphate
Nrf1	nuclear respiratory factor 1
Nrf2	Nuclear factor erythroid 2-related factor 2
Nrf-2	Nuclear factor erythroid 2-related factor 2
OADh (A F)	2-oxoadipate dehydrogenase
OD	optical density
OGDh (O F)	2-oxoglutarate dehydrogenase
OGTT	oral glucose tolerance test
OR	Odds Ratio
p62	Sequestosome1 protein
PALB2	Partner and localizerof BRCA2
PBS	Phosphste Buffered Saline
PDh (P F)	pyruvate dehydrogenase
PDK	Phosphatidylinositol trisphosphate (PIP3)-dependent kinase
PERK	Protein kinase RNA-like ER kinase
PGD	phosphogluconate dehydrogenase
PI3	Phosphatidylinositol 3
PIP2	Phosphatidylinositol bisphosphate
PIP3	Phosphatidylinositol trisphosphate
PKC	protein kinase C
POD	peroxidase
PPAR	peroxisome proliferator-activated receptor

PTEN	Phosphatase and tensin homolog
Rab10 GAP	Ras-related protein Rab-10 and GAP is a GTPase activating protein,regulators of Rsb GTP proteins
RAGE	receptor for advanced glycation end products
ROS	reactive oxygen species
RPM	revolutions per minute
SCF/b-TRCP	Skp1-Cullin1-F-box ,B-Transducin repeat Containing protein
SIAH2	Seven in Absentia Homolog
sMaf	small musculoaponeurotic fibrosarcoma
SOD	superoxide dismutase
SOD	superoxide dismutase
T1DM	type One diabetes mellitus
T2DM	type two diabetic mellitus
TC	total cholesterol
TG	triglycerides
TGF-β	transforming growth factor-beta
TNF-α	tumor necrosis factor-alpha
TOOS	Thymidylate synthase
VDBP	Vitamin D3 binding protein
VDD	Vitamin D Receptor deficiency
VDR	Vitamin D Receptor
VLDL- C	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organization
WTX	Gene mutation

Chapter One
Introduction
and
Literature Review

1.1. Introduction

Diabetes Mellitus (DM) is a major metabolic illness marked by increased blood glucose levels, arising from a complex interaction of hereditary and environmental influences [Huang D-D, et al., 2020]. The traditional classification of the disorder as either type 1(T1DM) (β-cell autoimmunity-mediated) or type 2 (T2DM) (insulin resistance and β-cell dysfunction-mediated) belies a more heterogeneous pathophysiology that, in some individuals, exhibit features of more than one type. For example, a recent study showed that up to 41% of individuals with T2DM exhibit evidence of autoimmunity (islet β-cell-specific T-cell reactivity) with higher frequency of β-cell dysfunction [Brooks-Worrell,et al, 2022]. In 2020, the International Diabetes Federation (IDF) reported that approximately 463 million individuals globally are afflicted with diabetes mellitus, while in the Middle East and North Africa (MENA), which encompasses 21 countries including Iraq, diabetes is responsible for 373,557 fatalities. According to a WHO report (2018), over 1.4 million Iraqis have diabetes [World Health Organization, Geneva, 2018]. Diabetes is scientifically divided into three types: type one diabetes mellitus (T1DM), type two diabetic mellitus (T2DM), and gestational diabetes mellitus (GDM) [Aranaz, et al, 2023].

Type II diabetes: The pancreas produces insulin, but in inadequate amounts to meet the requirements of the body, or the cells exhibit an improper response to insulin [Huang D-D, et al., 2020].

T2DM is a disease characterized by a nonautoimmune heterogeneously progressive loss of adequate islet β cell insulin secretion frequently in the presence of insulin resistance (IR) and metabolic syndrome [Samimi F., et al., 2019].

There are several factors that can be considered risk factors for type 2 diabetes, including obesity, high blood pressure, lifestyle, age, nutrition, physical activity, In addition to the genetic factor [Antwi, et al,2023; American Diabetes Association et al., 2018]. Many serious complications resulting from diabetes can be divided into acute complications such as diabetic ketoacidosis (DKA) and hyperglycemia [American Diabetes Association,2017] or chronic complications, which are divided into micro-complications (diabetic retinopathy, diabetic nephropathy , and diabetic neuropathy) and macro-complications (peripheral vascular disease and cardiovascular disease) [Yang, Shaoling et al., 2017].

The 2,3-Dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone (CoQ10) is an antioxidant and vitamin-like substance that is fat-soluble due to its nonpolar structure. Therefore, it should be taken with a fatty meal when used as a dietary supplement. It is located in mitochondria, facilitates energy transfer via the electron transport chain, and significantly influences the

oxidation-reduction state within the cell, in addition to regulating gene expression. It is a prominent supplement employed in the treatment of diverse health conditions, including cardiovascular diseases, diabetes, neurological disorders, and metabolic abnormalities such as hyperlipidemia and hypertension, along with mitochondrial diseases [Samimi F, et al, 2019].

Patients with Type II diabetes demonstrate elevated blood lipid levels, which are targeted by reactive oxygen species, thus leading to a significant decrease in intracellular antioxidant levels. This depletion subsequently enhances oxidative stress, contributing to the problems associated with Type II diabetes. Antioxidant therapy is an effective and important approach to manage the advancement of type II diabetes [Zhao S, et al, 2022].

The nuclear factor erythroid 2-related factor 2 (Nrf2). It is located in the nucleus. A crucial transcription factor plays an essential role in mitigating oxidative stress , Any dysfunction in this pivotal antioxidant pathway plays a pivotal role in the development of diabetes and its various complications [Ngo, et al, 2022; Cai, et al, 2023].

1.2.Diabetes Mellitus (DM)

1.2.1.Definition

Diabetes mellitus is the predominant metabolic syndrome illness marked by persistent hyperglycemia and disruption of carbohydrate, lipid, and protein metabolism resulting from an absolute, or relative deficit in insulin secretion [WHO, 2024]. Diabetes are developed by two primary pathologic processes: first a reduction in insulin secretion by pancreatic beta cells and second insulin resistance in many target organs, including muscle, liver, and adipose tissue [Prabhakar et al, 2016]. The manifestations of diabetes mellitus encompass, loss of weight, polydipsia, polyuria, and occasionally polyphagia [ADA, et al., 2017]. Uncontrolled hyperglycemia results in damage to essential structures, including the basement membrane and endothelial tissue [Robin, et al., 2013].

1.2.2. Epidemiology

Type II diabetes mellitus is among the most widespread chronic conditions [Goldman L, et al., 2016]. Research Studies suggest that the prevalence of T2DM is anticipated to increase over the next two decades, particularly among individuals aged 45 to 64 [Gujral, et al., 2021; IDF ,2025]. In 2020, the International Diabetes Federation reported that around 589 million (11.1 %) individuals globally are afflicted with diabetes, as seen in Figure (1.1) [IDF DIABETES ATLAS 11th 2025; Brussels, Belgium 2020].

In Iraq, two million inhabitants, constituting 13.4 % of the entire population, were projected to be impacted , In 2024, there were 2,669,400 registered cases of diabetes in Iraq [IDF DIABETES ATLAS, 2024]. Globally, 589 million individuals are affected by diabetes, with over 39 million in the Middle East North Africa (MENA) region. By 2050, this number is projected to increase to 67 million. Iraq is one of the 19 countries and territories in the Middle East North Africa (MENA) region identified by the International Diabetes Federation (IDF) [IDF ,2025].

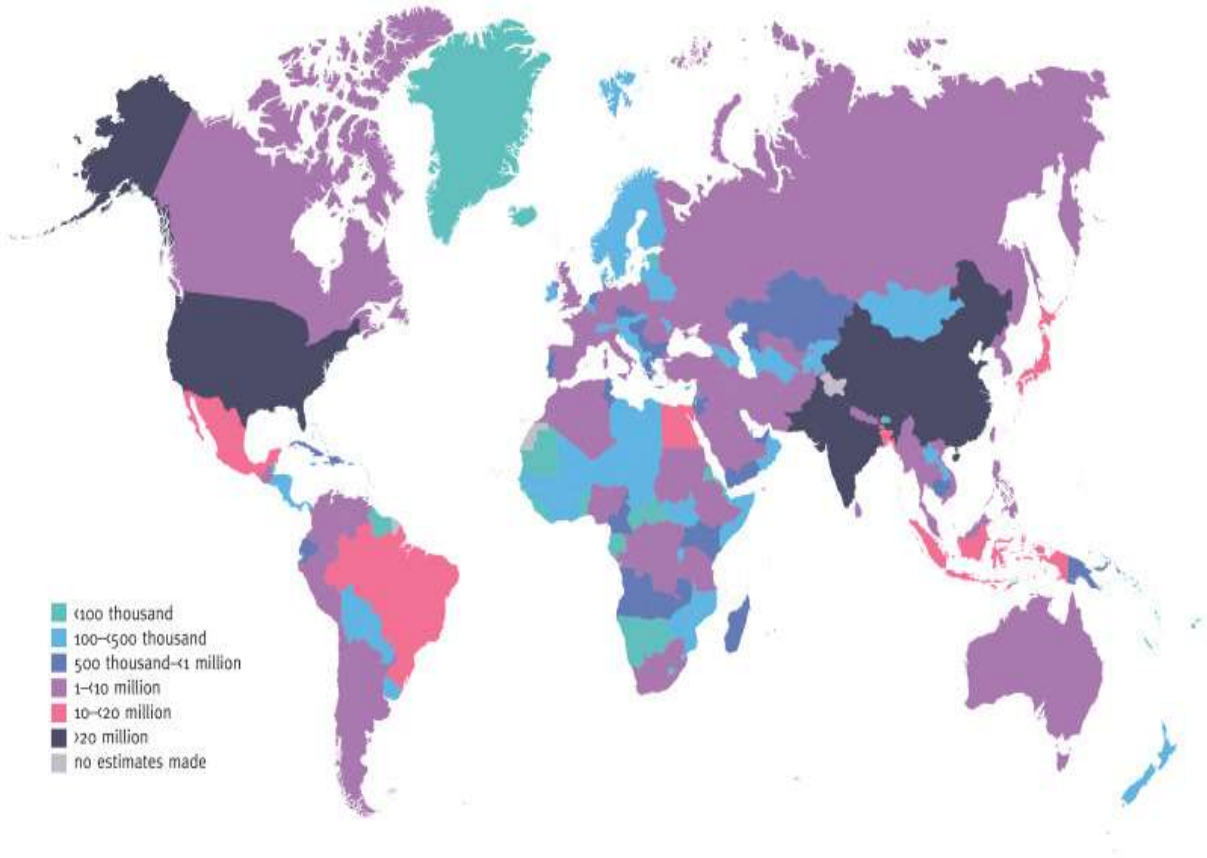


Fig. (1.1) prevalence of impaired glucose tolerance in adults (20–79 years) in 2021, adapted from International Diabetes Federation [Magliano,et al, 2021].

1.2.3. Classification of Diabetes

Different forms of diabetes arise from genetic or environmental factors, as well as lifestyle choices. Diabetes is classified into several types based on how it develops. Type 1 diabetes (DM) is also known as juvenile diabetes or insulin-dependent diabetes mellitus (IDDM), while type 2 diabetes (T2DM) results from insulin deficiency or insulin resistance [WHO, 2024].

Diabetes can be classified into the following general categories:

1.2.3.1. Type 1 Diabetes Mellitus (T1DM)

When you have type 1 diabetes, your immune system mistakenly treats the beta cells in your pancreas that create insulin as foreign invaders and destroys them. When enough beta cells are destroyed, your pancreas can't make insulin or makes so little of it that you need to take insulin to live [Santiago,et al,2023;IDF,2023]. Most of the time, type 1 diabetes is diagnosed in young people, but it can develop in anyone at any age. Scientists and researchers today aren't sure how to prevent type 1 diabetes or what triggers it, Individuals with type 1 diabetes mellitus exhibit

polyuria, polydipsia, polyphagia, weight loss, lethargy, visual impairment, and ketoacidosis, necessitating lifelong need on insulin therapy [ADA,2021].

1.2.3.2. Type 2 diabetes mellitus (T2DM)

Diabetes is a chronic, metabolic disease characterized by high levels of glucose (sugar) in the blood, which, over time, can lead to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. Of the various types of diabetes, type 2 diabetes is the most common and occurs when the body becomes resistant to insulin or does not produce enough of it [ADA,2021]. Diabetes is a chronic metabolic disease characterized by high levels of glucose (sugar) in the blood, which can, over time, lead to serious damage to the heart, blood vessels, eyes, kidneys, and nerves.

Diagnosis can be delayed because symptoms are often mild and unnoticeable, leading to the disease being discovered only after complications have developed [WHO, 2024-2025].

Type 2 is primarily caused by insulin resistance, where the body does not fully respond to insulin. Over time, this leads to persistently high glucose levels, prompting the pancreas to produce increasing amounts of insulin. However, over time, the beta cells of the pancreas may fail to meet the demand, leading to further declines in production and increased hyperglycemia. There are clear risk factors, such as being overweight, lack of physical activity, and genetic factors, which can be partially prevented or delayed through lifestyle changes [WHO, 2024-2025].

According to the International Diabetes Federation (IDF) Global Clinical Recommendations for the Management of Type 2 Diabetes 2025 Edition, type 2 diabetes accounts for approximately 90% to 95% of all diabetes cases worldwide. In 2024, the number of adults living with diabetes was approximately 589 million (aged 20 to 79), and this number is expected to rise to 853 million by 2050. More than half of those with this disease are not yet diagnosed [IDF, 2025].

1.2.3.3.Gestational diabetes mellitus (GDM)

It is categorized as varying degrees of glucose intolerance identified during pregnancy, impacting 4 to 18% of pregnant women across diverse diagnostic and ethnic factors [Madan, et al,2023]. The pathophysiological mechanism of gestational diabetes mellitus (GDM) resembles that of type 2 diabetes mellitus (T2DM), characterized by insulin resistance, oxidative stress, and systemic inflammation, and is diagnosed in the second or third trimester of pregnancy without evident overt diabetes [ElSayed, et al ,2024].

1.3. Pre diabetes

The term "prediabetes" denotes persons with glucose levels that are elevated above normoglycemia yet remain below the diagnostic threshold for diabetes, characterized by impaired glucose metabolism. Laboratory markers for identifying prediabetes include fasting blood glucose (FBG), 2-hour post load blood glucose, or HbA1c [American Diabetes Association,2022]. The term prediabetes originated in 1979, when the National Diabetes Data Group characterized impaired glucose tolerance (IGT) based on 2-hour post-glucose load measurements in a 75-g oral glucose tolerance test (OGTT) ranging from 140 to 199 mg/dL or 7.8 to 11.1 mmol/L. This definition was endorsed by the American Diabetes Association (ADA) by the World Health Organization (WHO) .In 1997, the ADA and in 1998, the WHO established impaired fasting glucose (IFG) as an additional category based on fasting blood glucose levels of 110–125 mg/dL or 6.1–6.9 mmol/L (IFG^{WHO}). In 2003, the ADA lowered the fasting plasma glucose (FPG) threshold, expanding the range from 110 to 125 mg/dL to 100–125 mg/dL or 5.6–6.9 mmol/L (IFG^{ADA}).

In 2009, the International Expert Committee (IEC) proposed a supplementary definition of prediabetes based on HbA1c levels ranging from 6.0% to 6.4% or 42–46 mmol/mol ($HbA1c^{IEC}$). In 2010, the American Diabetes Association (ADA) expanded this range to 5.7%–6.4% or 39–46 mmol/mol ($HbA1c^{ADA}$). Nevertheless, the World Health Organization and the International Endocrine Congress did not support the reduced diagnostic thresholds for fasting plasma glucose and HbA1c levels [Richter B, et al, 2018; Echouffo-Tcheugui JB, et al,2021].

Consequently, five definitions now exist for the diagnosis of prediabetes (Table 1.1). In addition to these categorical impediments, the many glycaemic metrics do not consistently indicate the same persons at risk. A recent report from the U.S. National Health and Nutrition Examination Survey 2005–2016 estimated that clinically significant discrepancies could result in HbA1c-related misdiagnosis for up to 30 million individuals in the USA, potentially leading to detrimental outcomes from improper management [Staimez LR, et al, 2022].

Table (1.1) Criteria for the definition of prediabetes.

Test	FPG		2-hPG		HbA1c	
Panel	**mg/dL**	**mmol/ L**	**mg/dL**	**mmol/L**	**%**	**mmol/mol**
ADA	**100 -125**	**5.6 -6.9**	**140 -199**	**7.8 -11.1**	**5.7– 6.4**	**39 -46**
WHO	**110 -125**	**6.1 -6.9**	**140 -199**	**7.8 -11.1**	**n.a.**	
IEC	**n.a.**		**n.a.**		**6.0 -6.4**	**42 -46**

Abbreviations: ADA, American Diabetes Association; FPG, fasting plasma glucose; IEC, International Expert Committee; n.a., not applicable; 2-h PG, plasma glucose after 2 h in 75-g oral glucose tolerance test (OGTT); World Health Organization

1.4. Pathogenesis of Type 2 Diabetes Mellitus in Older Adults

T2DM is characterized by hyperglycaemia, which results from a progressive deterioration of insulin secretory β- cell function, typically combined with varying degrees of insulin resistance. These two key pathogenetic mechanisms are usually accompanied by other glucoregulatory disturbances such as inappropriate hyperglucagonaemia and an impaired incretin response (Fig. 1.2) [American Diabetes Association, 2021]. There is also a study that says “The serum level of adiponectin , A hormone secreted by adipose tissue that helps improve insulin sensitivity and inflammation. Low levels of adiponectin are associated with several conditions, including obesity, type 2 diabetes, and atherosclerosis and also the serum level of TNF was increased significantly in T2DM patients. the adiponectin and TNF can used to distinguished the T2DM patients. The deficiency of adiponectin regareded as a risk for developing T2DM” [Mohammed, et al,2019].

Type 2 diabetes has been found to indirectly affect the bones and joints, i.e. osteoarthritis, in women after the age of forty. Through this study, it is found that there are many components that change due to diabetes, and their variation is evidence of the presence of arthritis in the same patient[Akaash, et al.,2024].

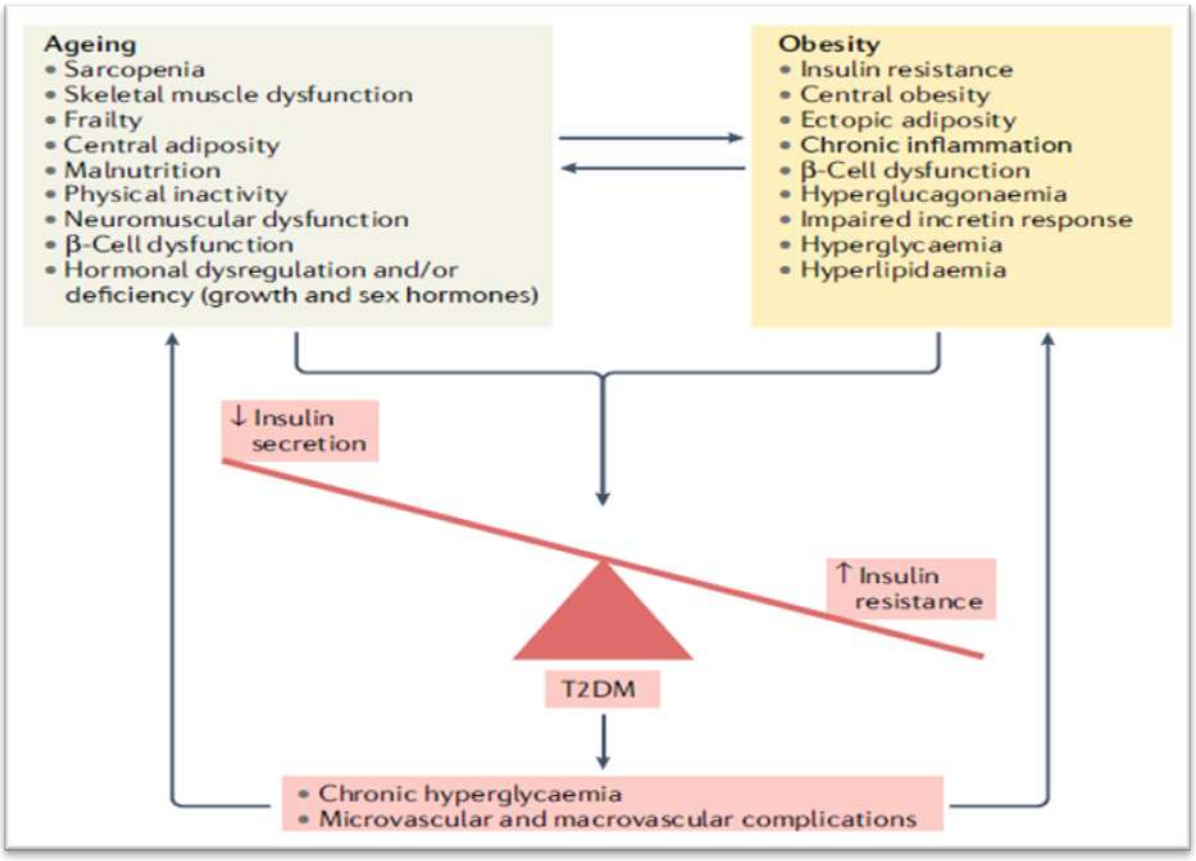


Fig.(1.2) Pathophysiological links between ageing, obesity and T2dM. [American Diabetes Association, 2021; DeFronzo, R. A., 2004].

1.5. Insulin

Insulin is a polypeptide with 51 amino acids, consisting of Chain A (21 amino acids) and Chain B (30 amino acids) [Seong J, et al., 2019]. It is produced by pancreatic beta cells as a single chain of 110 amino acids referred to as preproinsulin. Preproinsulin loses the amino-terminal signal peptide, leading to the action of proteolytic enzymes that generate proinsulin. The cleavage of a fragment of internal proinsulin (C-peptide) results in insulin, which consists of two chains (α and β) linked by two disulfide bridges and a single intrachain bridge within the alpha chain, as illustrated in figure (1-3) [Joshi, et al., 2007].

The half-life of insulin is short, ranging from 4 to 6 minutes. [Steiner, D.F., et al. 2009]. Insulin penetrates the target cell and binds to a receptor on the cell surface that possesses

intrinsic tyrosine kinase activity. It promotes the auto-phosphorylation of the insulin receptor's tyrosine residues by binding to the α-subunit, so facilitating the phosphorylation of the insulin receptor, which activates proteins through several mechanisms figure (1-4) [Salltiel A. R. A, 2001; Kohn Aimee D., et al. 1996].

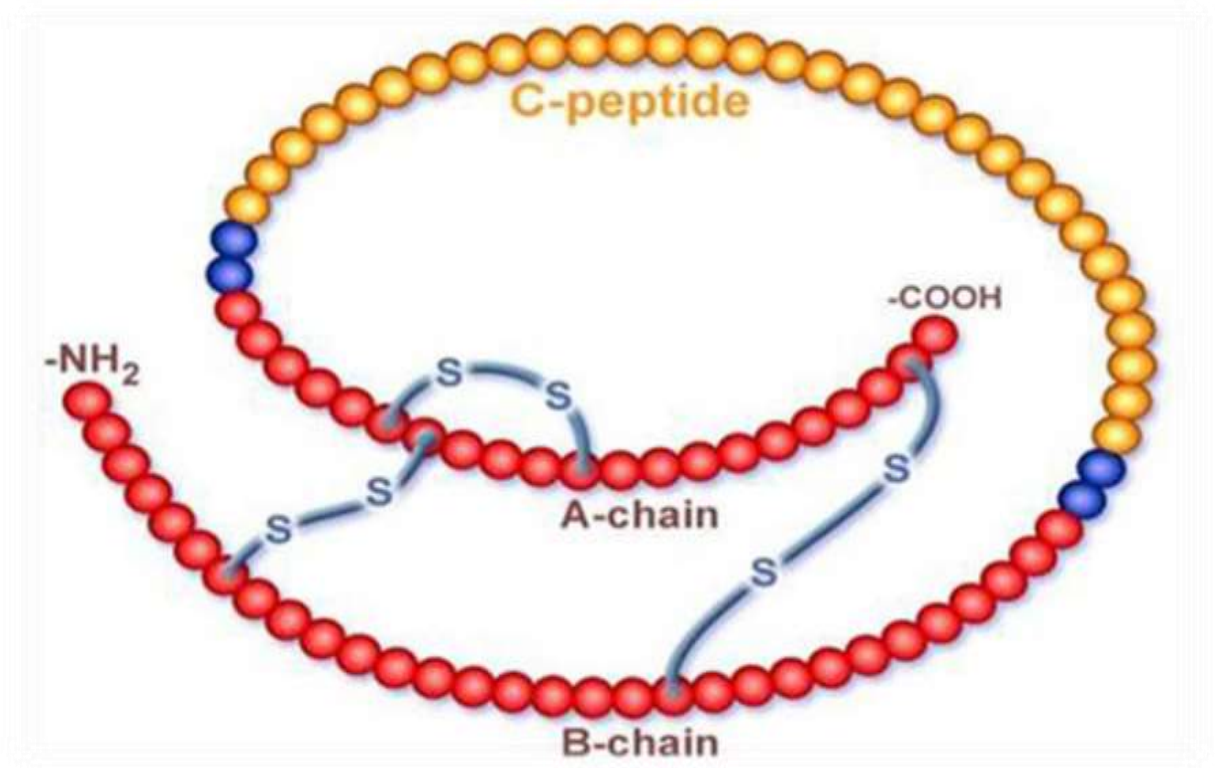


Figure (1.3): *Structure of proinsulin, the yellow is c-peptide and red is two chain insulin bind by disulfide bridges blue in color* [Joshi, et al., 2007].

The insulin receptor is a receptor tyrosine kinase, which undergoes dimerisation and autophosphorylation on insulin binding. The phosphorylated receptor recruits and phosphorylates the insulin receptor substrate 1 (IRS-1) on tyrosine residues, which then recruits dimeric PI3 kinase via SH2 domains on the p85 subunit. PI3 kinase catalyses the phosphorylation of phosphatidylinositol bisphosphate (PIP2) at the plasma membrane to PIP3 (reversed by PTEN), which then recruits PIP3-dependent kinase (PDK) and Akt, allowing PDK to phosphorylate and activate Akt. Activated Akt phosphorylates and inactivates the Rab10 GAP, AS160, allowing sustained Rab10 activation which plays a critical role in trafficking of GLUT4 storage vesicles (GSVs) to the plasma membrane and surface expression of GLUT4. High levels of free fatty acids lead to an accumulation of lipid-derived second messengers, e.g.

diacylglycerol and ceramide, which can inhibit the pathway at several different stages [Carmichael, R.E,et al.,2019].

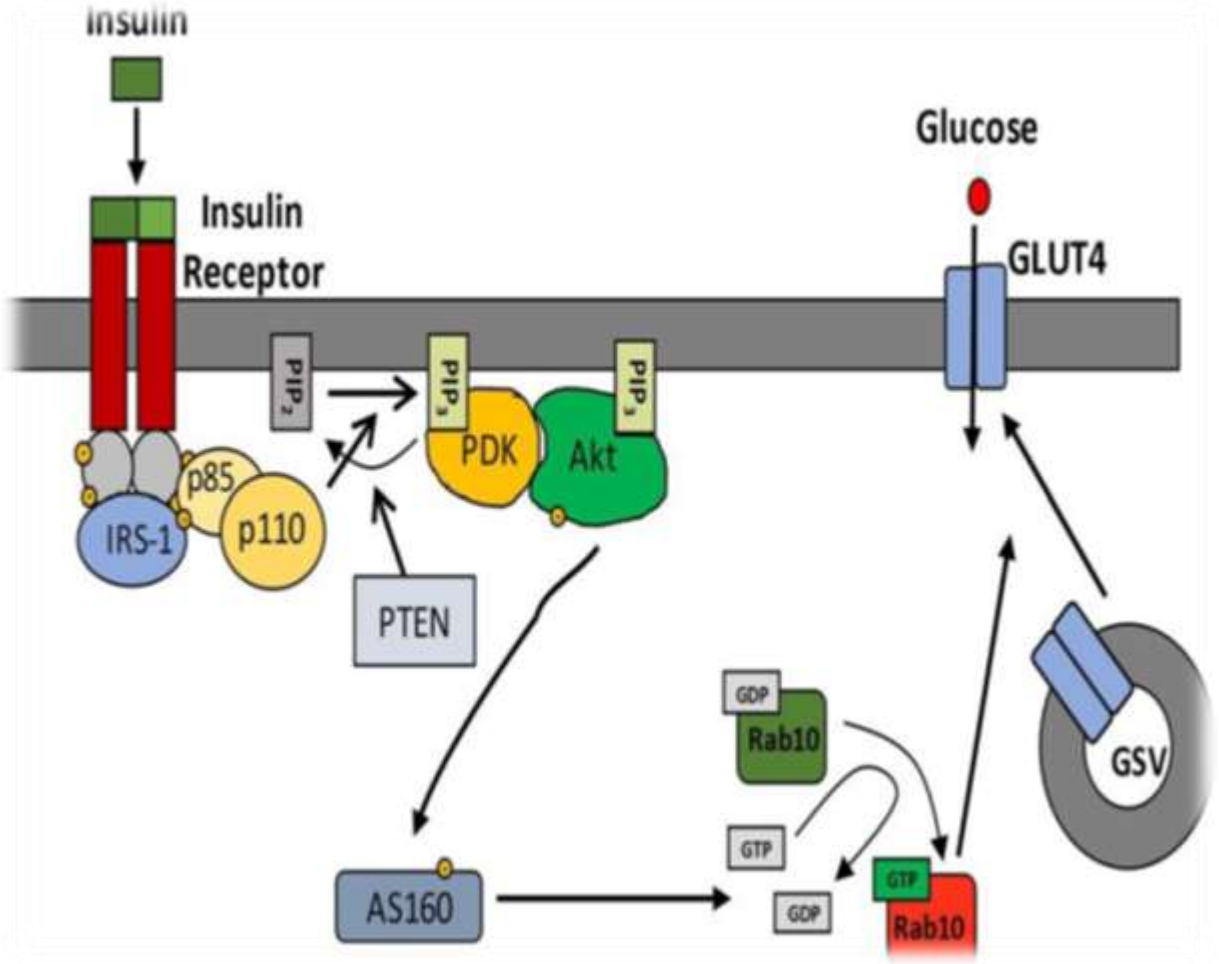


Fig. (1.4): *Schematic of the Insulin Signalling Pathway and insulin resistance. [Carmichael, R.E,et al.,2019].*

1.5.1. Insulin Resistance

Insulin resistance (IR) is a poor biological response of target tissues, particularly the liver, adipose tissue, and skeletal muscle, leading to decreased insulin sensitivity and consequently impaired glucose tolerance [Lee, et al, 2022; Seong J, et al., 2019] . This has significant implications for patients, who are unable to obtain the energy needed from glucose to maintain cellular metabolic functions. Over the past two to three decades, it has been established that inflammation, with increased immune cell accumulation and inflammatory polarization, occurs in various tissues, including adipose tissue, skeletal muscle, liver, intestine, pancreatic islets, and brain, and may contribute to obesity-related metabolic dysfunctions, leading to insulin resistance

and type 2 diabetes [Lee, et al, 2022]. Therapies targeting inflammation have shed light on some obesity-related diseases, including type 2 diabetes and atherosclerotic cardiovascular disease, but they still need to be further tested and confirmed in clinical trials. Wu, H., & Ballantyne, C. M. (2020) focused in their review on inflammation in adipose tissue and its potential role in obesity-associated insulin resistance. Insulin resistance is of global importance due to its association with numerous chronic conditions, such as type 2 diabetes, obesity, cardiovascular disease, and liver cirrhosis [Zygmunt, et al.2025].

Insulin resistance primarily involves a decrease in the number of receptors and their stimulatory effectiveness, attributed to various factors such as mutations, alterations, or changes in insulin receptors, as well as a decreased metabolic rate [Olivares-Reyes J. A. 2012].

1.5.2. Determination of Insulin Resistance

The evaluation of insulin resistance can be conducted using the homeostatic model assessment (HOMA). In 1985, David Matthews and colleagues released the model, as referenced by Nakell AN in 2012. The HOMA model was mathematically formulated by investigating the correlation between fasting blood sugar and fasting insulin levels [Abstracts of the 20th International Congress of Nutrition, 2013]. These correlations can quantify beta-cell activity and insulin resistance [WIKSTRÖM, Jakob D. 2010]. The subsequent formulas are utilized:

HOMA-IR = (fasting blood insulin (µU/ml) × fasting blood glucose (mg/d L) / 405 = Kg / m2 [Matthews David R., et al.,1985].

1.5.3. Diagnostic Assessments for Diabetes Mellitus

Diabetes mellitus is diagnosed according to the following criteria. The World Health Organization (WHO) recommends that fasting blood sugar (FBS) and HbA1c tests are more convenient and effective methods for diagnosing Type 2 Diabetes Mellitus (T2DM) and pre-diabetes.

- Testing fasting blood sugar (FBS) ≥ 7.0 mmol/L or 126 mg/dL. As demonstrated in table (1.1) [IDF,2023].
- HbA1c (≥ 6.56%) Glycated hemoglobin indicates the glycemic status over a period of 90-129 days, corresponding to the lifespan of red blood cells [Mellitus. Diagnosis and classifications of diabetes mellitus, 2012; IDF,2023].
- The glucose tolerance test (GTT) is a diagnostic method that evaluates the body's ability to metabolize glucose. This involves providing a designated dosage of glucose to a fasting person and then testing glucose levels in the blood and urine at set intervals.

This test is mostly employed for the identification of diabetes mellitus [Khan HA, et al., 2014].

1.6. Risk Factors for Type 2 Diabetes Mellitus

1.6. 1. Obesity

Obesity is the primary factor contributing to the tendency to Type 2 Diabetes Mellitus. Specifically, genetic predisposition. Numerous studies denote that moderate chronic inflammation is a key contributor to diabetes mellitus and obesity [Saltiel et al., 2017]. Adipocytes in obese patients harbor significant quantities of pro-inflammatory cytokines, including interleukin 6 and interleukin 1 beta [Basanta-ALARIO et al., 2016]. In 2014, the overall prevalence of overweight and obesity was 52%, although the projected incidence was over 90% [World Health Organization, Obesity and Overweight. July 2016]. A weight loss of 5 to 10 percent is associated with significant health benefits, including improved lipid profiles, glycemic control, and blood pressure regulation [BRAY, et al., 2016].

1.6.2. Age

Type 2 diabetes mellitus (T2DM) is projected to impact 10% to 15% of those aged 65 years and older, and 20% of those between 65 and 80 years [Ismail, et al,2021]. representing a prevalence more than eightfold higher than that of individuals aged 18 to 44 years, which stands at 2.4% [Díaz Ana, et al,2019]. This seems to have happened due to aging, which results in an annual increase of 1 to 2 milligrams percent in FBS. These modifications would be associated with variations in peripheral insulin sensitivity or changes in pancreatic islet function [Huang, et al, 2018].

1.6.3. Nutrition and Physical Activity

The American Diabetes Association recommends stringent glycemic control as an effective strategy to prevent microvascular consequences of Type 2 Diabetes Mellitus through modifications in pharmacotherapy and lifestyle choices [American Diabetes Association, 2017]. It is debated whether diets with a high proportion of ultra-processed food (UPF) pose health risks beyond the nutritional quality of the diet [Tobias, et al,2021]. Obesity and the progression of Type 2 Diabetes Mellitus are correlated with insufficient aerobic activity and suboptimal dietary habits[Astrup, et al,2022].The implementation of a congenial diet and consistent physical exercise is essential not only for the prevention and treatment of diabetes mellitus but also for the maintenance of physical and mental health [Khemayanto, et al., 2014]

1.6.4. Hypertension

The coexistence of diabetes and hypertension influences clinical outcomes in both microvascular and macrovascular diseases. The etiology of hypertension in diabetes entails maladaptive changes and intricate interactions between the autonomic nervous system and mechanical forces [FRIMPONG, et al, 2020]. The primary etiological factors for atherosclerosis and Type 2 Diabetes Mellitus (T2DM) include hypertension, which encompasses myocardial infarctions and cerebrovascular accidents. Diabetes mellitus and hypertension exhibit substantial overlap, indicating a significant correlation in their etiology and pathophysiological mechanisms [Naseri, et al,2022].

1.6.5. Genetic Factor

Type 2 diabetes mellitus (T2DM) has a distinct genetic foundation.In monozygotic twins, the concordance rate for (T2DM) is approximately 70%, compared to 20-30% in dizygotic twins [Kaprio, J., et al. 1992]. Research indicates that a first-degree family history is linked to a twofold chance of developing future Type 2 Diabetes Mellitus [Lyssenko, V., et al, 2008].The interaction between environmental factors and a significant genetic predisposition leads to T2DM. Investigate the heritability of Type 2 Diabetes Mellitus and the historical context of genomic and genetic studies in this domain. The advent of genome-wide association studies has led to the identification of numerous genes. Several of them were previously unrecognized for any role in T2DM [Goyal, et al, 2023]. as illustrated in figure (1-5) which depicts the interplay between genetics, epigenetics, and environment [Hu, et al., 2014].

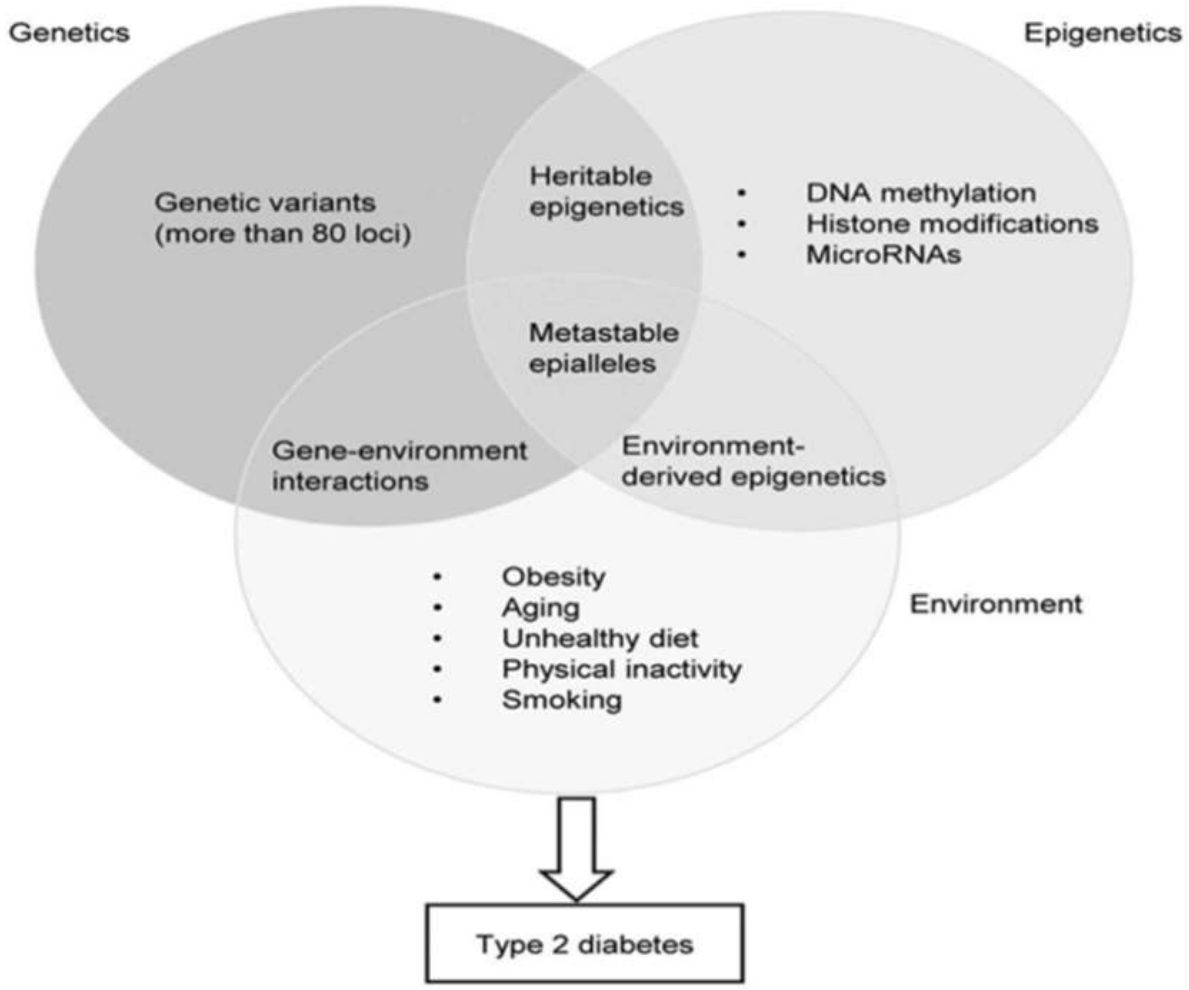


Fig.(1-5): *Roles of, genetics, epigenetics, and, the environment in the development, of T2DM [Hu,et al.,2014].*

1.6.6. Additional Factors

Stigmatization connected with type 2 diabetes mellitus is a significant issue. Lower educational attainment, divorce or widowhood, and unemployment are socio-demographic characteristics correlated with increased stigmatization. It is recommended to implement an educational program for health care personnel, family members, and friends of diabetic patients as an intervention to reduce stigma [Taher, et al.,2023].Vitamin D3 receptor polymorphisms (VDR) may have a crucial role in the occurrence of T2DM. Vitamin D3 binding protein (VDBP) has emerged as a novel target for therapeutic development aimed at treating endocrine nutritional and metabolic disorders, such as obesity and type 2 diabetes mellitus [Mutar, Baraa Abdul-

Kareem, et al, 2023]. VDD is linked to the likelihood of developing T2DM. Hypovitaminosis D is highly widespread among the study participants, with a greater percentage observed in females and patients compared to males and controls, respectively. The findings may have therapeutic significance, as careful vitamin D administration could improve glycemic regulation in type 2 diabetes mellitus [Hadi et al., 2020].

An relationship exists between ABO blood type and dyslipidemia, with metformin employed as the primary treatment for newly diagnosed type 2 diabetes. Blood type A exhibited more significant reductions in total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol relative to other blood types. Blood type B shown more significant decreases in triglycerides (TG) than other blood types. Patients with blood type A had a superior response to metformin treatment, demonstrating decreases in total cholesterol and LDL cholesterol, surpassing the lipid profile enhancements observed in other blood types (B, AB). Individuals with blood type O exhibited no notable impact on lipid profiles following metformin treatment [Mohammed et al., 2017].

1.7.Oxidative Stress

Disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, with regard to its potential role in causing tissue damage in diabetic patients. This paper describes important free radicals, discusses their biological sources, and the main antioxidant defense mechanisms. It provides examples of the potential consequences of free radical damage, with a particular focus on lipid peroxidation. Finally, it discusses the issue of increased oxidative stress in diabetic patients [Azzi, A.,2022].

Oxidative stress is a historical concept from the time when all diseases were considered as being caused by too much free radical production or too little elimination; it was not supposed to be measured but “cured” by antioxidants. However, large-scale intervention trials using antioxidant supplements provided with free radical scavenging capacity have shown no significant advantage in humans.

Consequently, a new hypothesis for oxidative stress in disease had to be formulated: Oxidative stress occurs due to alteration of thiol redox circuits, which normally plays a role in cellular signaling and physiological regulation .

However, both with the definition of oxidative stress as an imbalance between oxidants and antioxidants in favor of the antioxidants and the concept of redox disruption by oxidants, the measurement of the rates of oxidants' production vs. that of oxidants' elimination appears to be necessary. In principle, these measurements are possible, and a number of methods have been developed towards this goal, in the hope of understanding the role of oxidative stress in human disease. Measurement of disease-associated oxidative stress is important, although it would not solve the question whether oxidative stress is a cause or consequence of disease [Azzi, A.,2022].

Figure (1.6) Alleged diseases linked to oxidative stress [Azzi, A.,2022].

1.8. Coenzyme Q10 (Co Q10)

The 2,3-Dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone (CoQ10) is an essential biomolecule that functions in the electron transport chain within the mitochondria in the inner membrane. It is also characterized by being a lipid-soluble molecule due to its non-polar structure, which allows it to move within the inner mitochondrial membrane and facilitates energy transfer in the form of ATP Banun,et al,2021].

Protein nanoparticles for enhanced oral delivery of coenzyme-Q10: in vitro and in silico studies. ACS Biomaterials Science & Engineering, 9(6), 2846-2856.. Its importance also lies in its role as an antioxidant, as it significantly affects the redox state within the cell, in addition to regulating gene expression. Figure (1.7) shows the chemical structure of this important [Samimi F. et al., 2024].

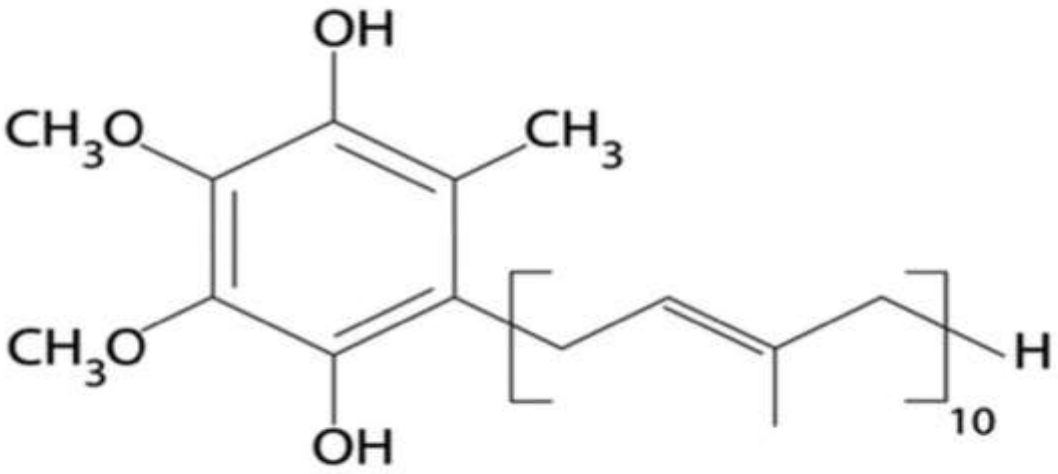


Figure (1.7) *The chemical structure of CoQ10[Samimi F. et al., 2024].*

It is an essential nutritional supplement used in the treatment of many health conditions, including cardiovascular disease, diabetes, neurological disorders, and metabolic disorders such as hyperlipidemia and hypertension, in addition to mitochondrial diseases [Samimi F. et al., 2024].

The molecule is generated through several processes involving mevalonic acid, the formation of which is blocked by hydroxyl-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (Figure 1.8) [Hadi, et al, 2021]. Coenzyme Q10, in its reduced form ubiquinol, has demonstrated the capacity to block the oxidation of proteins, DNA, and lipids. Coenzyme Q10 in serum is predominantly associated with low-density lipoprotein (LDL) cholesterol transport and it is not present in significant concentrations in its unbound form. Coenzyme Q10 treatment elevates, its reduced form in circulating lipoproteins and prevents LDL peroxidation.

The suppression of LDL peroxidation may be crucial to its antiatherogenic properties [Litarru GP, et al., 2007].

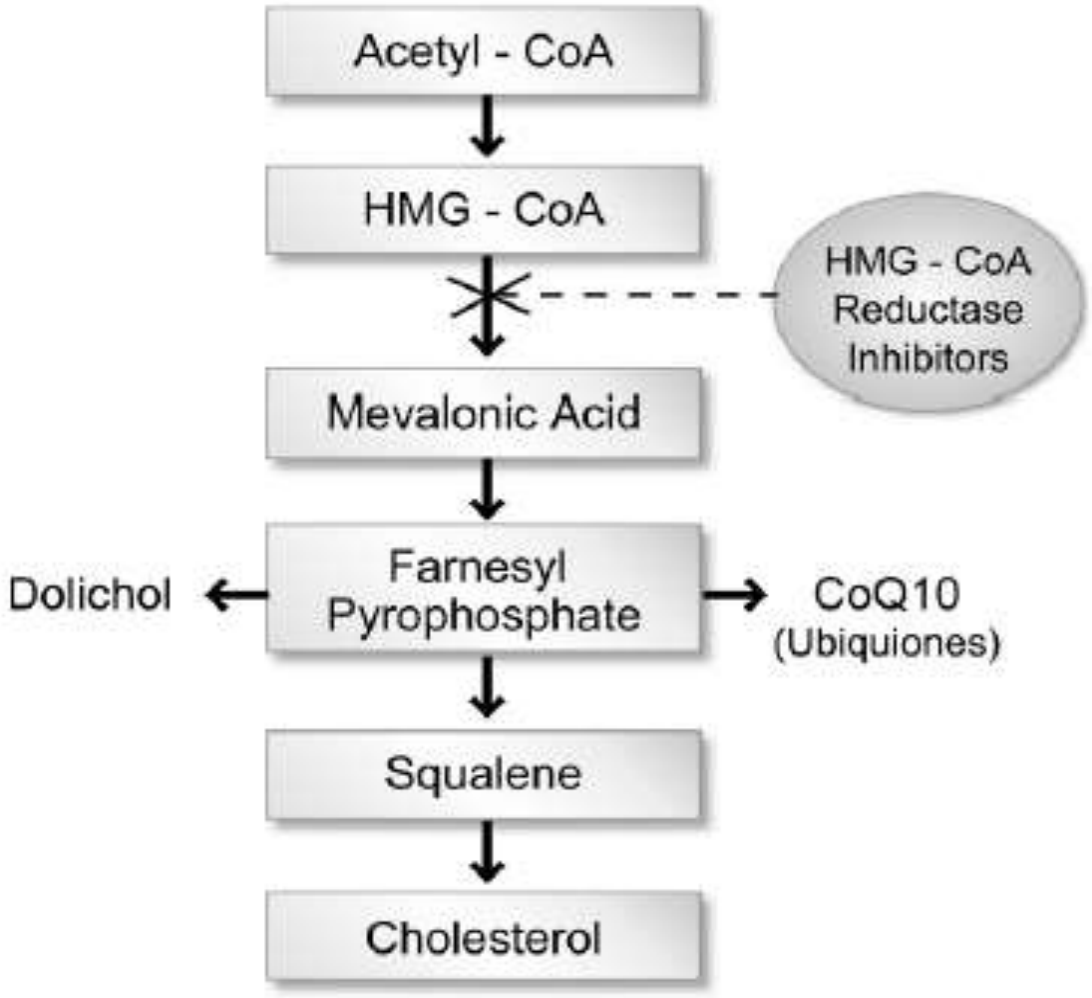


Fig.(1.8) *Role of hydroxyl-methylglutaryl coenzyme A reductase inhibitors on coenzyme Q10 synthesis.*

Coenzyme Q10 (CoQ10) is a vitamin-like compound produced in various tissues throughout the human body. CoQ10 plays a crucial role in cellular energy production through oxidative phosphorylation in mitochondria, facilitating the transfer of electrons from complexes I and II to complex III of the mitochondrial respiratory chain by taking two electrons from NADH to form CoQ10H2. This occurs in complex I, while in complex II, it takes electrons from FADH2 and then donates the electrons received to complex III, which is oxidized to CoQ10 (MRC; Figure 1.9).

In addition, CoQ10 acts as a potent lipid-soluble antioxidant, protecting cell membranes from oxidative damage caused by free radicals [Daei,et al, 2024]. In addition to its function in mitochondrial activity, CoQ10 is found in numerous subcellular organelles, such as lysosomes, peroxisomes, the Golgi apparatus, and the endoplasmic reticulum.

In addition to providing antioxidant protection to organelle membranes from oxidative stress, CoQ10 is involved in regulating intralysosomal pH ,CoQ10 has been shown to directly influence the expression of numerous genes, including those associated with the inflammatory process [Schmelzer, c, et al, 2008]. A small amount of CoQ10, approximately 5 mg per day, is obtained through the typical diet [Heaton et al., 2020].

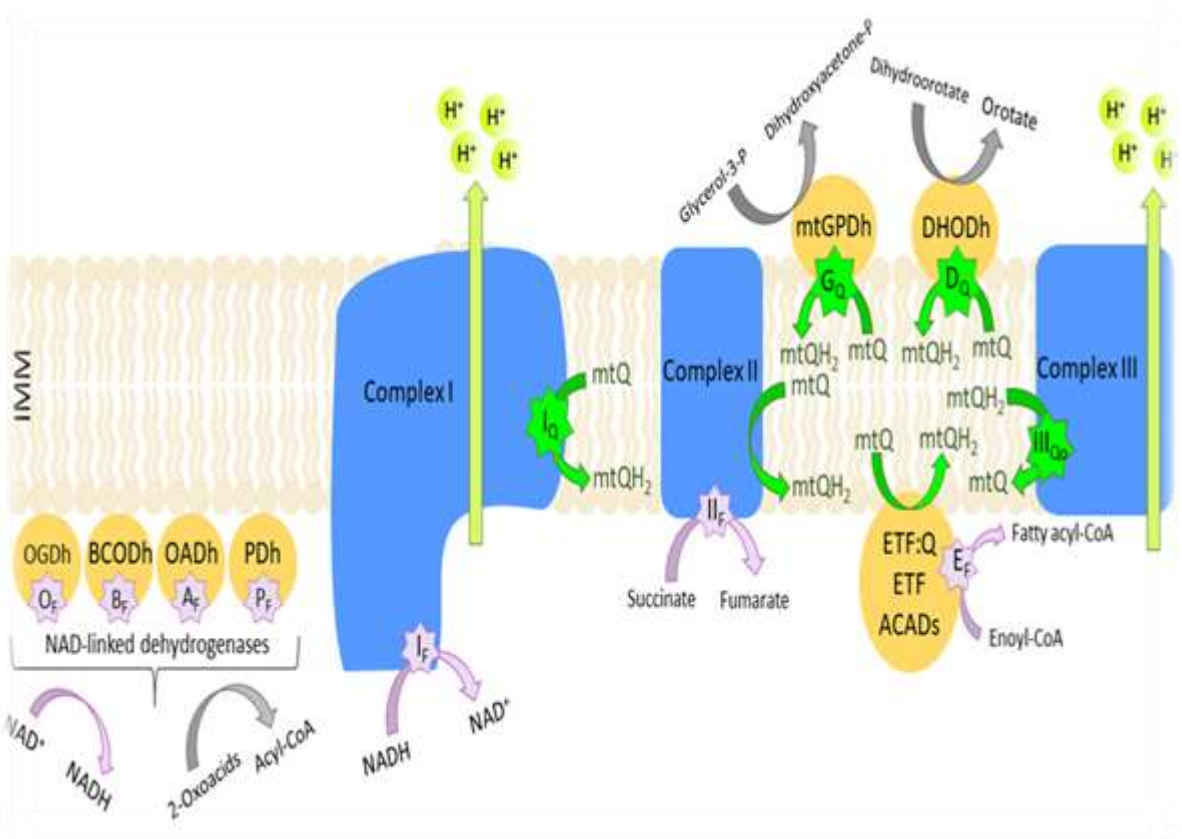


Figure (1.9) Eleven mitochondrial sites of $O2\cdot^{-}$/H2O2 formation [Wong,et al,2017].

1.8.1. Chemical reactions facilitated by CoQ10 in mitochondria

A. Interconversion between oxidized and reduced states:

- Oxidized form: Ubiquinone (Coenzyme Q10)
- Reduced form: Ubiquinol (CoQ10H2)

Basic reaction

$$CoQ10 + 2H^{+} + 2e^{-} \longleftrightarrow CoQ10H_2$$

B. The Function of Complex I in electron transport from NADH:

CoQ10 engages with Complex I (NADH: ubiquinone oxidoreductase), acquiring electrons produced during the oxidation of NADH to NAD^+

$$NADH + H^+ + CoQ10 \longrightarrow NAD^+ + CoQ10H_2$$

C. Its function in Complex II (electron transfer from succinate):

CoQ10 engages with Complex II (succinate: ubiquinone oxidoreductase), accepting electrons produced during the oxidation of succinate to fumarate

$$Succinate + CoQ10 \longrightarrow Fumarate + CoQ10H_2$$

D. Transfer of Electrons to Complex III

CoQ10H2 donates electrons to complex III (Cytochrome bc1 complex), resulting in the oxidation of CoQ10H2 to CoQ10.

$$CoQ10H_2 + 2Cyt\ e(ox) \longrightarrow CoQ10 + 2Cyt\ e\ (red) + 2H^+$$

E. Participation in the Q cycle:

In Complex III, CoQ10 enters a cycle called the "Q cycle," where electrons are exchanged

between CoQ10 and CoQ10H2, forming a semireduced anion called semiquinone

1.8.2. Mechanisms Underlying Diminished CoQ10 Levels

A. Augmented Oxidative Stress:

Elevated glucose concentrations amplify the generation of reactive oxygen species (ROS), resulting in the depletion of CoQ10 [Svensson O.L, 2011].

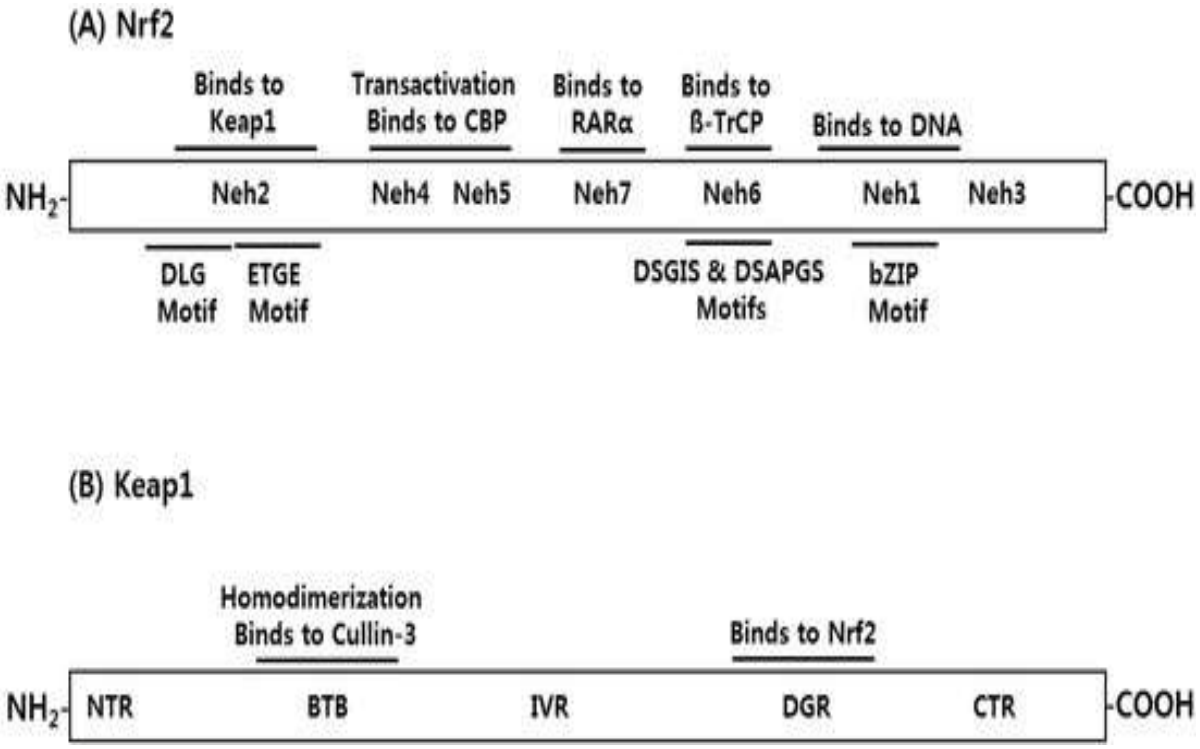
B. Mitochondrial Dysfunction: Impaired mitochondrial function in T2DM may diminish endogenous CoQ10 synthesis [Svensson O.L, 2011].

C. medicines: Common T2DM medicines, including statins, are recognized for

their ability to suppress CoQ10 production [Littarru GP, et al, 2007].

1.9. Nuclear Factor Erythrocyte-Associated Factor 2 (Nrf-2)

Nuclear factor erythrocyte-associated factor 2 (Nrf-2) is located in the nucleus and belongs to the Cap n Collar transcription factor family (Figure1.10). It is an essential transcription factor that plays a key role in mitigating oxidative stress and inflammation in cells. Under normal conditions, Nrf-2 is confined to the cytoplasm due to its association with the repressor protein Keap1 (Kelch-like ECH-associated protein 1), which leads to the ubiquitination of Nrf-2 and its subsequent destruction via the proteasome system, preventing its accumulation in the nucleus. Keap1 consists of several cysteine residues that act as sensors of oxidative stress and act as a negative regulator of Nrf-2 [Bellezza I, et al., 2018]. Altering the cysteine residue in Keap1 releases and facilitates translocation of Nrf-2 to the nucleus, binds to a DNA sequence where it forms a heterodimer with a small Maf protein and binds to the antioxidant response element (ARE), thereby stimulating the expression of heme oxygenase 1 (HO-1), with mRNA levels significantly lower in the diabetic group compared to healthy control group [Sharath Babu G, et al, 2017]. Dysfunction of this crucial antioxidant system is now recognized as a fundamental factor in the etiology of diabetes and its various consequences [Uruno A, et al, 2015; Jiménez-Osorio A. S., et al, 2014].



Fig(1.10) The conserved domains of Nrf2 and Keap1 proteins[Osorio A. S., et al, 2014].

1.9.1. Nrf-2 is Regulated by Oxidative Stress, Inflammation, and Aging

Normally, NRF2 function is repressed though cytosolic binding with Kelch-Like ECH-associated protein 1 (Keap1), which tags NRF2 for ubiquitination and degradation by the proteasome [Itoh, K.;et al,1999; Furukawa,et al,2005] (Figure 1.11). Never the less, in response to certain stimuli, including oxidative stress and inflammation, Keap1 is chemically modified at several cysteine residues which drives a conformational change such that binding to NRF2 and the subsequent ubiquitination is prevented [Eggler, A.L,et al,2005]. Post-translational modifications of NRF2 itself, including acetylation and phosphorylation, can also disrupt its interaction with KEAP1 and increase nuclear translocation [Huang,et al,2002, Joo,et al,2016]. Additionally, NRF2 activity can be controlled at the level of NRF2 transcription [Gounder,et al,2012] and via autoregulation, through binding of ARE-like sequences in its own promoter [Kwak,et al,2002]. The complete pathway is complex and there are undoubtedly other proteins which interact .

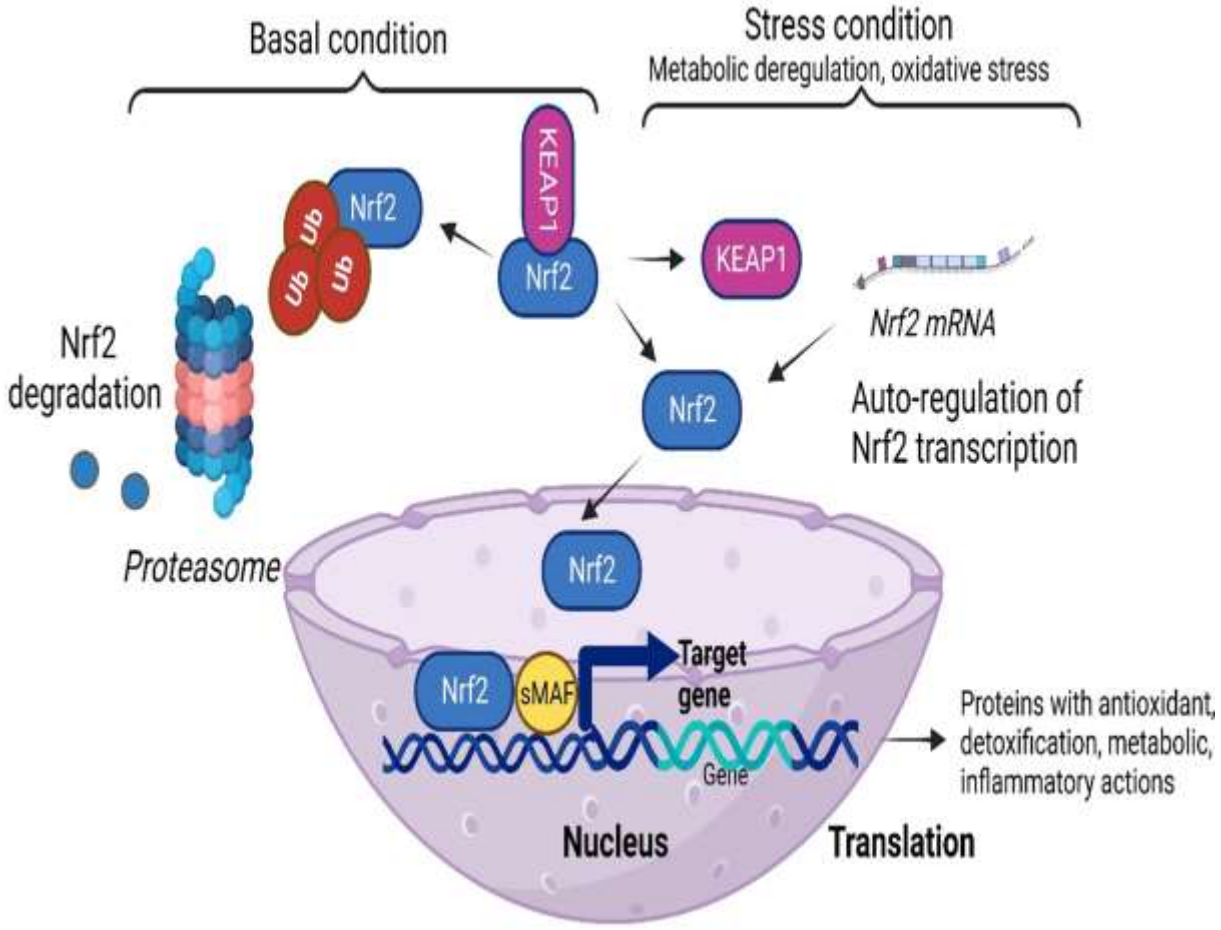


Figure (1.11) Regulation of NRF2. Normally, Keap1 targets NRF2 for ubiquitination and degradation by the proteasome [Uruno A, et al,2013].

with this pathway. One such protein, DJ-1, which is ubiquitously expressed, is known to be deleterious when mutated as it is implicated in early-onset forms of Parkinson's disease. It is known to oxidize itself in response to oxidative stress, thus acting in part as a buffer. It also interrupts the ubiquitination of NRF2 and chaperones it into the nucleus [Uruno A, et al,2013]

1.9.3.NRF2 Increases Antioxidant and Metabolic Gene Expression

The transcription factor Nrf2, a central redox response regulator, is required to program metabolism under oxidative stress. Upon release from Keap1, NRF2 then translocates to the nucleus and binds to target genes at the antioxidant response element (ARE) consensus sequence. NRF2 binding regulates the transcription of antioxidant defense proteins, most notably HMOX1 (HO-1), NQO1, and glutathione-dissolving enzymes such as GCLC and GCLM, as well as the cysteine/glutamate transporter SLC7A11 and glutathione peroxidase-4 (GPX4). NRF2 has also been shown to modulate genes associated with metabolic processes, such as malic enzyme 1 (ME-1), peroxisome proliferator-activated receptors (PPARs), and transaldolases. This includes promoting redox and limiting iron degradation [Mayer et al., 2024; Yang et al., 2025].

NRF2's effects extend beyond antioxidant defense to regulating metabolic pathways; Recent studies in the heart focus on ensuring that it redirects glucose flow towards the pentose phosphate pathway and increases the expression of its key enzymes, including protection against cardiac dysfunction under stress and the "metabolic reprogramming" of NADPH-generating pathways [Zoccarato et al., 2024]. The effectiveness of Nrf2 signaling on mitochondria and their functions has also been demonstrated, and it has been characterized as an anti-fidochondria, supporting bacterial oxidative metabolism [Mayer et al., 2024;Luchkova et al., 2025].

Recent reviews confirm that Nrf2 modification is also regulated by post-translational epigenetic mechanisms (such as phosphorylation and binding with BACH1), which directly influence the activation of its oxidative and metabolic pathways [Yang et al., 2025].

The combined evidence from 2023 suggests that "Nrf2 increases the expression of oxidative and metabolic pathways" through a network of interactions that enhance the biological ability to differentiate between free radicals and reducing agents and redistribute metabolic co-activators in favor of cytoprotection [Meyer et al., 2024; Zuccarato et al., 2024].

1.10. RESEARCH AIMS AND OBJECTIVES

The study aims to:

1.10.1. Investigate the association between serum ubiquinone (CoQ10) levels and Nrf-2 expression in patients with type II DM and prediabetes.

1.10.2.Determine the sensitivity and specifity of serum CoQ10 levels and Nrf-2 toward the severity of diabetic complications.

Chapter Two

Materials and Method

2.1. Patients and Methods

2.1.1.The Study Design and Setting

This case-control study was performed in the Department of Chemistry and Biochemistry at the College of Medicine, University of Kerbala. The subjects were categorized as illustrated in figure (2-1), performed on 88 subjects obtained during December, 2024 to Februar, 2025.

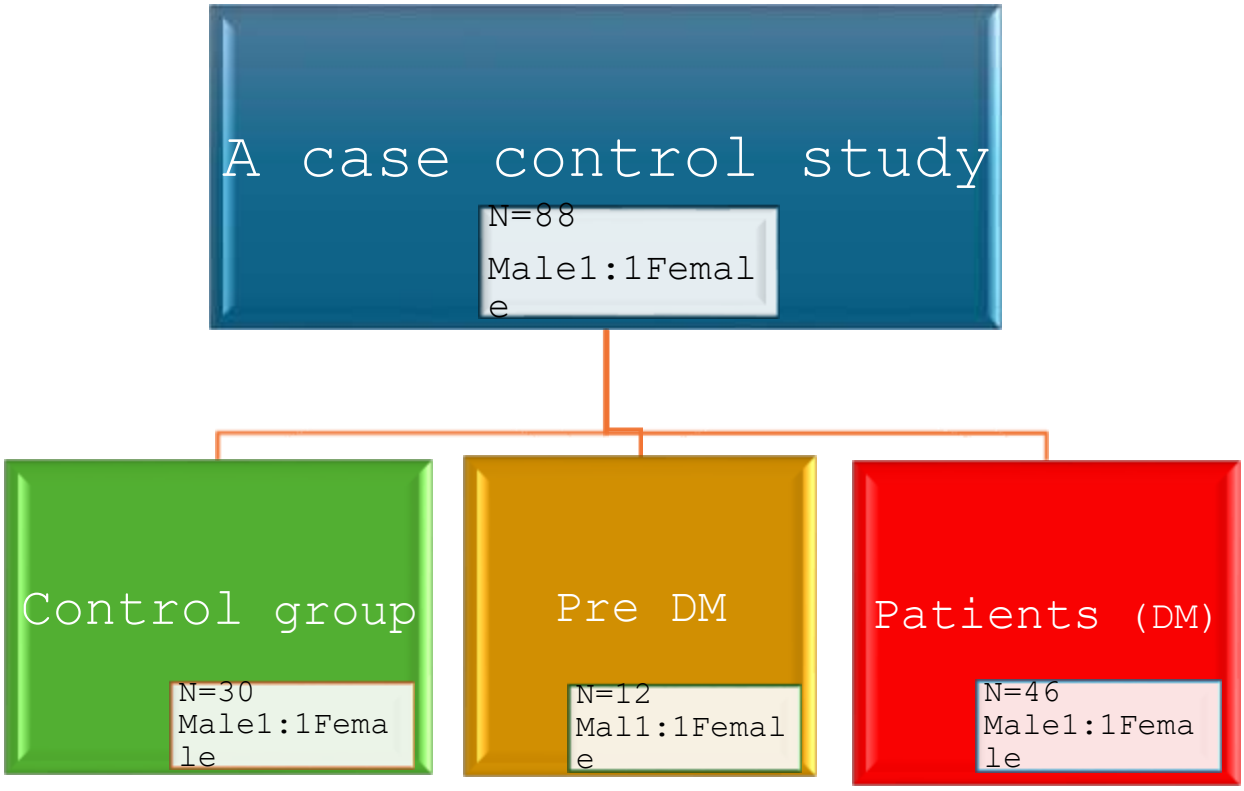


Fig. (2-1) Chart of Study Design

Patients group: Patients ranging in age from 20 to 75 years (Mean =51, R=55) were included in the current study. All of the patients were diagnosed with type 2 diabetes by a consultant physician based on clinical symptoms and laboratory tests (FBS, HbA1c). There were 46 (50% males : 50% females) within the group. Blood samples were taken from the Imam Hassan Center for Endocrinology and Diabetes at the Al-Hassan Teaching Hospital, which is located in the city of Kerbala, Iraq.

Pre Patients Group: This group included 12 volunteers (50% males : 50% females) 20 - 60 years (Mean = 40.7, R=40).

Non Patients Group: This group included 30 volunteers (50% males : 50% females) 20 - 75 years (Mean =42.55, R=55).

2.1.2. Inclusion Criteria:

Type II Diabetes Mellitus, without insulin therapy, across all age groups and both genders, selected controls exhibiting normal fasting blood sugar and haemoglobin A1c (HbA1c) results.

2.1.3. Exclusion criteria:

1. Diabetes Mellitus I (DM I)
2. Gestational Diabetes Mellitus (GDM
3. Chronic Heart diseases
4. Chronic Joints diseases
5. Cancers
6. Any apparently acute inflammation.
7. Auto-immunity patient.
8. Liver and Renal disease
9. Thyroid disease
10. Gout

2.1.4. Collection of The Blood Samples

Following a minimum of 12 hours of fasting, venous blood was obtained via venipuncture using plastic disposable syringes, obtaining up to 5 mL from both the control and patient groups. Each blood sample was subsequently separated into two portions as outlined below:

A. One ml sample in an EDTA tube for the determination of glycated hemoglobin percentage (HbA1c) using the latex turbidity method on the Lifotroni H8 equipment.
B. Comprising 4 ml of blood were placed to gelatin tubes and incubated at room temperature for 10 to 15 minutes. The serum was subsequently centrifuged for(10-15) minutes at (3000 turn) to promote serum separation. The serum was divided into six gelatin tubes and preserved at -20°C until examination for the identification of different biomarkers.
C. 1.5 ml of serum was extracted to quantify Nrf2 levels by ELISA, and 1.5 ml of serum was extracted to assess coenzyme Q10 levels through HPLC in the blood of diabetes individuals.

2.1.5. Ethical Considerations:

In order to obtain the necessary ethical permits, the team of the ethical committee, the faculty of medicine, the university of Karbala, and the Karbala Health Directorates / Karbala-Iraq contributed their support ,provided support through official documents numbered

1. 6638 on 1/12/2024
2. 3967 on 3/12/2024

2.2. The Materials

2.2.1. The Materials and Tools

Table (2.1) shows the materials and tools used in this research, as well as their suppliers.

Table (2.1): The materials & tools use

N	name	Company	Country
1	Pipette(100-1000µl)	Slamed®	Germany
2	Micropipette (10-100 µl)	Slamed®	Germany
3	Multi pipette	Slamed®	Germany
4	Pipette Tips-100µl (yellow)	Kirgen®	China
5	Pipette Tips-1000µl (blue)	Kirgen®	China
8	Gel tubes (6mL)	Nipigon Health LTD	Canada
9	Eppendorf Tubes (1.5 mL)	Kang Gia	China
10	Gloves	MedTech	Malaysia
11	Syringe (5 mL/cc)	Jiangsu kangyou medical instrument co.,LTD	China
12	Tube Rack	Slamed®	Germany
13	Tourniquet	Inzek International Trading	Netherland
15	Plaster	Life Plus Medical	China
17	Plate Sealer	BT Lab	China
18	ELISA Plate (96-well)	BT Lab	China
20	Backer 500 ml	Mheco	China
23	Vial HPLC 1.5 ml		China
24	Filtr Syring HPLC-0.45	Biostar	China
25	Distilled Water & washing bottle		Iraq

2.2.2. The Laboratory Kits

The laboratory kits utilized in the investigation are enumerated in Table (2.2) below, along with their respective suppliers: -

Table (2-2):Diagnostic kits used in the current study

NO	Kit-Diagnostic	Company	Country
1	Fasting glucose-kit	GIESSE	Italy
2	HBA1c-kit	GIESSE	Italy
4	Total cholesterol-kit	GIESSE	Italy
5	Triglyceride-kit	GIESSE	Italy
6	HDL cholesterol-kit	GIESSE	Italy
7	LDL cholesterol-kit	GIESSE	Italy
8	Nrf2-KIT	Bio-assay Technology laboratory	China
9	Standardized CoQ10	Bio-assay Technology laboratory	China

2.2.3. Instruments and Equipments

The instruments and equipments utilized in the current study are detailed below in table (2.3).

Table (2-3): the instruments and equipments used in the current study

NO	Instruments and equipments	Company	country
1	Auto- analyzer (smart-120)	Geon-TEK	Canada
2	ELISA-reader	ELX800	USA
3	DEEP freezer	COOLTECH	USA
5	Centrifuge	Kokusan	Germany
6	Incubator	UKA	Germany
9	Refrigerator	Samsung	Korea

2.3. Methods

2.3.1. Calculation of Body Mass Index

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

$$BMI = \frac{Weight\ (kg)}{Height\ (meters)^2}$$ [WHO,2023].

For adults, the World Health Organization (WHO) classifies the categories as follows as shown in Table (2.4).

Table (2-4): Body mass index**.**

Weight status	BMI range (kg/m^2)
Underweight	less than 18.5
Normal weight	18.5-24.9
Overweight	25-29.9
obesity	≥30

2.3.2. Measurement of Serum Glucose Concentration Principle:

The oxidation of glucose to gluconic acid is catalyzed by glucose oxidase (GOD). A chromogenic oxygen acceptor, phenol-aminophenazone, was used to detect the hydrogen peroxide (H2O2) that was produced when peroxidase (POD) was present.

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

H_2O_2 +Phenol + Aminophenozone ⟶ Quinone +H_2O_2

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

Normal fasting blood glucose (FPG) ≤ 100 mg/dL (7.0 mmol/L) [WHO, 2024; IDF, 2025].

Reagents :

- **Reagent 1 (Buffer):** - Contains 0.75 mmol/L of phenol and 100 mmol/L of phosphate buffer with a pH of 7.5.

- **Enzymes, Reagent 2:** Comprised of 0.25 mmol / 1 of 4-amino-antipyrine ≥1.5 KU/L of proxidase, and ≥15 KU/L of glucose oxidase.
- **Standard Reagent 3:** Comprised of 5.55 mmol/L of glucose or 100 mg/dL.

Preparation of The Reagent A:

In order to create a mixture of reagents 1 and 2, working reagent A was made by mixing the material containing reagent 2 in the vial of enzymes with the vial of reagent 1 (buffer). Reagent A was created by gently mixing the mixture to finish the dissolution of all the constituents.

Procedure:

wavelength: 510 nm (500-520), Light path: 1cm, Temperature: 37 C^{o}, Reading : against blank reagent, Method : Increasing End point, Sample/Reagent : 1/100

After mixing and incubating for five minutes at 37°C, the absorbance of the sample (AX) and the standard (AS) at a wavelength of roughly 510) nm was measured against the blank reagent.

2.3.3. Measurement of (HbA1C %) Principle

Glycated hemoglobin (HbA1c%) is based on assessing the percentage of hemoglobin covalently bound to glucose in red blood cells, reflecting average blood glucose levels over the three months prior to the test due to the age of red blood cells, Several analytical techniques are used to measure HbA1c, including: Chromatographic Methods, Enzymatic Methods and Immunoassays [Nature Medicine ,2023].

Immunoassays

The technique measures the HbA1c percentage in whole blood by using the interaction between the antigen and antibody. Total hemoglobin and HbA1c % have the same unspecific absorption rate to latex particles. The formation of a latex-antibody complex occurs when mouse antihuman Hba1c monoclonal antibody (Reagent B) is introduced. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody . The amount of HbA1c absorbed on the surface of latex particles determines how much agglutination occurs. The amount of agglutination is measured as absorbance. The HbA1c values is obtained from the calibration curve [Yun,et al,2023].

Normal blood HbA1c levels = (4.5 – 6.5) mg/dl [WHO, 2024; IDF, 2025].

Procedure:

Wavelength: 640nm (630-660), Light path: 1cm, Temperature:37 C°, the instrument was adjusted to zero with distilled water, After mixing and incubating for five minutes at 37°C, measure the sample's absorbance. After adding reagent (B) for five minutes at a wavelength of roughly 640 nm

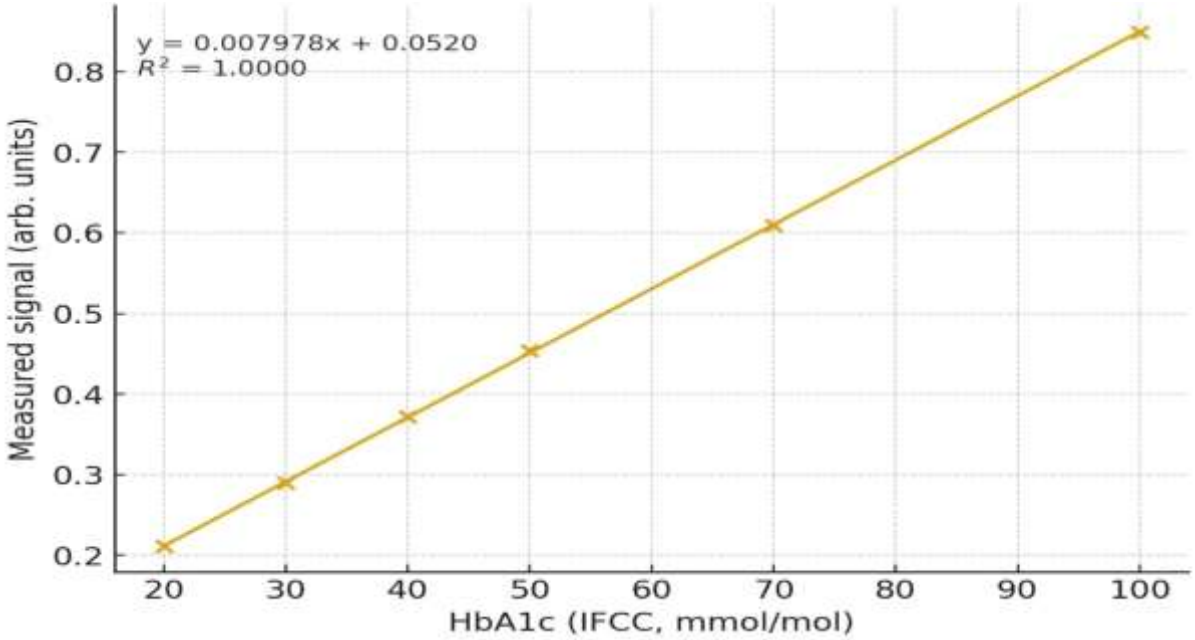


Figure (2-2) Calibration curve for HbA1c %

2.3.4. Measurement of Serum Cholesterol Concentration Principle

Cholesterase (CHE) hydrolyzes esterified cholesterol to produce free cholesterol and fatty acids. Free cholesterol is converted to cholesterol-3-one by cholesterol oxidase (CHOD), which also produces hydrogen peroxide. Hydrogen peroxide (H_2O_2) interacts with phenol and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) to form a colorful complex, the strength of which is directly correlated with the sample's total cholesterol content. [S. Barbosa,et al.2019]. The principle of determination of cholesterol is based on enzymatic hydrolysis according to the following reactions:

Step1 . Cholesterol Esters Hydrolysis :-

The enzyme cholesterol esterase converts cholesterol esters, which make up the majority of blood cholesterol.

$$Colesterol\ ester\ +\ H2O \xrightarrow{Cholesterol\ esterase} Cholesterol\ +\ Fatty\ acids$$

Step2. Oxidation of free cholesterol

by the enzyme cholesterol oxidase to produce hydrogen peroxide,

which is the basis for the subsequent detection reaction $Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholesterol - 3 - one + H_2O_2$

Stepe3. Colorimetric Detection Reaction

The resulting peroxide reacts with two reagents:

1. **4-Aminophenazone**
2. **Phenol**

This reaction is catalyzed by the enzyme peroxidase to produce a quinoneimine dye, which has a red color.

$$H_2O_2 + Phenol + 4 - amino\ antioyrine \xrightarrow{peroxidase} Quinonimine + 2H_2O$$

Normal blood Cholesterol levels = < 200mg/dl) [WHO, 2024; IDF, 2025].

Reagents

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

Procedure:

Wavelength: 510 nm (500-520), Light path:1cm,Temperature:37C^O

Reading against blank reagent, Sample/Reagent(1/100)

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

2.3.5. Measurement of Serum Triglyceride (TG) Concentration Principle:

Lipoproteinlipase (LPL) hydrolyzes triglycerides to provide free fatty acids and glycerol. Glycerol kinase (Gk) and glycerol phosphate oxidase (GPO) are involved in a sequence of connected enzymatic reactions in which the glycerol participates and produces H_2O_2. The hydrogen peroxide reacts with TOOS and 4-AAP to form a colored complex. Whose color intensity is directly proportional to the concentration of triglycerides in the sample [Hearne, ,etal,1981,p.28-31]. The principle of determination of triglyceride is based on enzymatic hydrolysis according to the following reactions:

A. Enzymatic analysis

Triglycerides are analyzed by the enzyme lipase to glycerol and fatty acids

$$Triglyceride \xrightarrow{lipase} Glycerol + Free\ Fatty\ acid$$

B. Glycerol reaction

❖ **Phosphorylation**

Glycerol is converted to glycerol 3-phosphate using the enzyme glycerol kinase and adenosine triphosphate as a phosphate source

$$Glycerol + ATP \xrightleftharpoons{\text{glycerol kinase}} Glycerol-3-Phosphate + ADP$$

❖ **Glycerol-3-phosphate oxidation**

Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by the enzyme glycerol -3 - phosphate oxidase , producing hydrogen peroxide.

$$Glycerol - 3 - Phosphate + O_2 \xrightarrow{glycerol-3-phosphate\ oxidase} Dihydroxyaceto - Phosphate + H_2O_2$$

C. Colorimetric Detection Reaction

The resulting peroxide reacts with the chromogenic substance 4-aminophenazone and phenol in the presence of the enzyme peroxidase to produce a colored substance at a wavelength of 500-550 nm

$$H_2O_2 + Parachlorophenol + 4 - aminoantipyrine \xrightarrow{peroxidase} Quinonimine + 2H_2O + HCL$$

Reagents:

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

Normal blood TG levels = < 150 mg/ dl) [IDF, 2023].

Procedure:

Wavelength 546 nm (546 – 570), Light path 1 cm, Temperature37C^o, Reading against blank reaction, Method Increasing End Point, Sample/reagent (1/100)

After mixing and incubating for five minutes at 37°C, the absorbance of the sample (Ax) and the standard (As) at a wavelength of around 546 nm was measured against a blank reagent.

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

There are two methods for measuring HDL:The homogeneous method, The standard chemical method

2.3.6.1.The homogeneous method involves:-

1.Blocking other lipoproteins (LDL, VLDL) by adding antibodies specific to the Apo B protein found on LDL and VLDL, which prevents their interaction.

2.Through the direct interaction of HDL with cholesterol esterase and Oxidase [Chary,et al,2022].

2.3.6.2.The standard chemical method :-

1.Esterification reaction

2. Cholesterol oxidation

3. Color reaction

Reagents:

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

Normal blood HDL levels = >40 mg/dL in male

>50 mg /dL in female [IDF, 2023].

Procedure:

Wavelength 600 nm, Lightpath 1 cm, Temperature 37C^{o} , Reading against blank reagent, Method Increasing End point

After mixing and incubating for five minutes at 37°C, the absorbance of the calibrator (Ac) and sample (Ax) was measured against a blank reagent at a wavelength of around 600 nm.

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

Reagent (A) attaches itself to LDL and shields it from enzyme reactions when combined with a sample ctions . Chylomicrons, VLDL, and HDL are examples of non-LDL lipoproteins that are reacted with by cholesterol esterase and cholesterol oxidase. When hydrogen peroxide is generated, catalase breaks it down. The protective reagent that is extracted from LDL and catalase inactivated is added when reagent (B) is applied. When oxidase condensation with HDAOS [N-(2-hydroxy-3-sulfopropyl)- 3,5-dimethoxyaniline]

and 4-AAP occurs in the presence of peroxidase, the color complex that results from the enzymatic reactions carried out exclusively on the LDL fraction and the hydrogen peroxide generated is directly proportional to the quantity of LDL cholesterol in the sample.

Reagents:

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

Normal blood LDL values = <100 mg/dl [IDF, 2023].

Procedure:

Wavelength 600 nm, Light path1 cm, Temperature 37C^{o} , Reading against blank.

After mixing and incubating for five minutes at 37°C, the absorbance of the calibrator (Ac) and sample (Ax) was measured against a blank reagent at a wavelength of around 600 nm.

2.4. Measurement of Serum Human CoQ10 Concentration

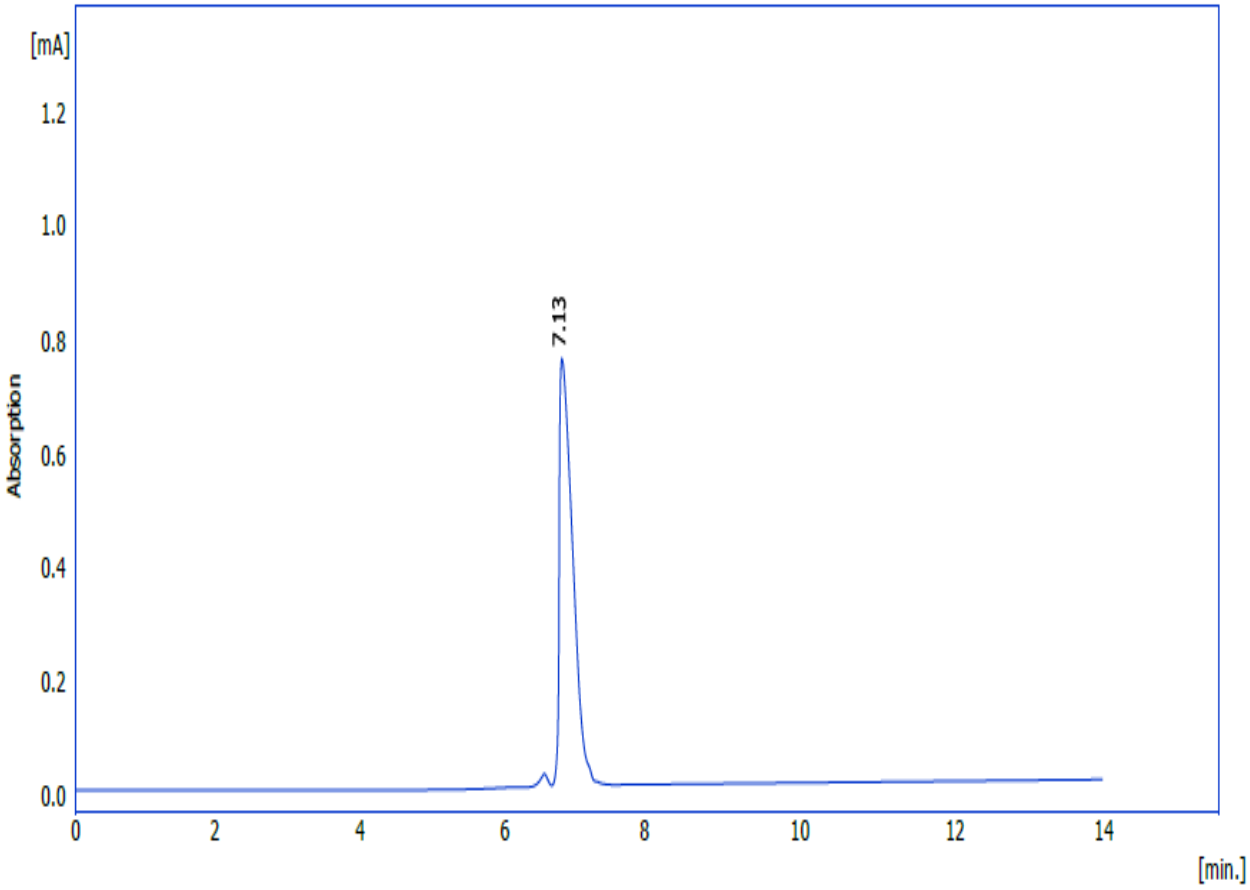
2.4.1. Preparation and measurement of CoQ10 standard

- Stock solutions of CoQ10 at a concentration of 20 ppm were prepared in a methanol-water (98-2) solvent, protected from light, and stored at -20°C.
- Before analysis the accurate concentration of CoQ10 in the working standard solution was obtained by spectrophotometry at 275 nm, e¼14020 L=mol. cm.

The standardized CoQ10 graph was obtained from HPLC as shown in the figure (2-3) and table (2-5).

Sample Info:

Sample ID : CoQ10 (20 ppm)
Sample : CoQ10 (20 ppm)
Inj. Volume [mL] : 0.1
Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
Amount 0
ISTD Amount 0
Dilution 1
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10Sample. per Sec.
Matching : No Change



Figer (2-3) Chromatogram F:\ CoQ10 standard (20 ppm)

Table(2-5) Result chromatography (Uncal - F:\ CoQ10 (20 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	7.13	1250.99	774.14	100.00	100.00	0.25	
	Total	1250.99	774.14	100.00	100.00		

2.4.2.Measurement of Coenzyme Q10 in a Group of Patients and Healthy Individuals

1. Before the analysis, the samples were allowed to thaw at room temperature.
2. 600 uL of plasma were supplemented with 800 uL of cold1-propanol, stirred with vortex for 2 min, and centrifuged at 9000 g during10 min at 4C to spin down the protein precipitate and finally the organic layer was evaporated to dryness under a stream of nitrogen.
3. The dry residue was dissolved in 400 uL of mobile phase and then 100 uL of the treated sample is injected into the equipment.
4. Utilize the HPLC device to determine the absorbance of the sample. The subsequent findings were attained:

2.4.3. HPLC analysis :

A Japan HPLC model, SHIMADZU (Japanany).This model was used to determine and detect CoQ10 in human plasma using a column with a mobile phase of methanol and water at a ratio of 98:2 v/v, a flow rate of 0.7 mL/min, and a column temperature of 30°C. The column used was a C18-ODS-2, measuring 25 cm x 4.6 mm, and the detector was a UV-Vis (at a wavelength of 275 nm) detector for 15 minutes.

See Chapter 6 in A.6.Appendix (6) for schematic diagrams of some samples resulting from absorbance readings in HPLC

2.5. Measurement of Serum Human Nuclear Factor Erythroid 2-related Factor 2 Concentration

USER INSTRUCTION

Cat.No: E3244Hu **Standard Curve Range:** 0.2-60ng/ml

Sensitivity: 0.11ng/ml

Size 48T, 96T

Storag :Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1month. Avoid repeated thaw cycles.

2.5.1.Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision. We measured random samples of Nrf-2 within the same batch/lot to ensure the consistency of the kits' performances.

Intra-assay	*Sample*	*n*	*Mean*	*Standard deviation*	*CV %*
Intra-Assay	1	18	20.7	0.45	2.2
Intra-Assay	2	18	16.3	1.02	6.2
Intra-Assay	3	18	4.5	0.27	6.2

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Inter-Assay: CV< 10%

2.5.2.Assay Principle

The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human NFE2L2 antibody. NFE2L2 present in the sample is added and binds to antibodies coated on the wells. and then biotinylated Human NFE2L2 Antibody is added and binds to NFE2L2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated NFE2L2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human NFE2L2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Precautions

- Before use, the kit and sample were naturally exposed to room temperature for 30 minutes.
- After removing the required number of strips, the bag was immediately resealed to protect the remaining strips from damage Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- The withdrawal of reagents and the rate of addition from one well to the next were arranged. Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material.
- Pipette tips and disc seals were kept clean and disposable to avoid cross-contamination.
- The solution of Substance B is light-sensitive; therefore, it should be stored in a dark place.
- Wear eye, hand, and skin protection when handling solutions containing acid.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Standard:** Reconstitute the 120ul of the standard (80ng/ml) with 120ul of standard diluent to generate a 40ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (40ng/ml) 1:2 with standard diluent to produce 20ng/ml, 10ng/ml,5ng/ml and 2.5ng/ml solutions. Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

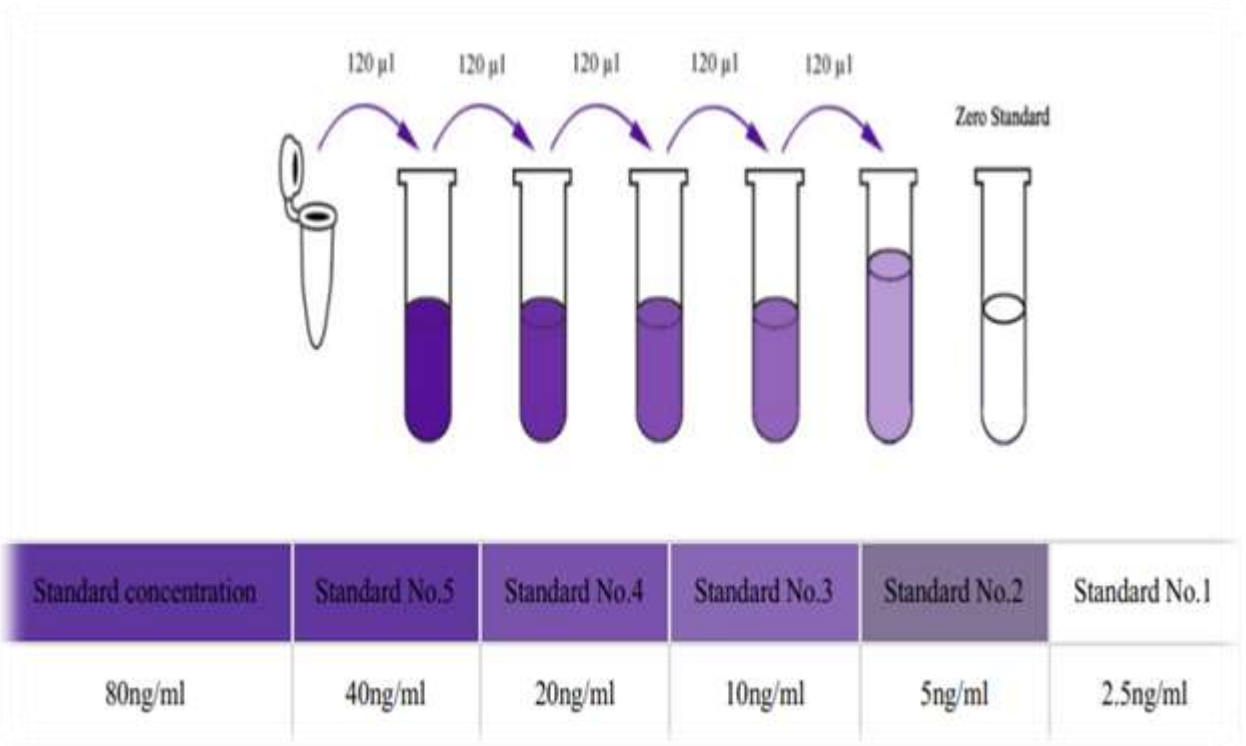
40ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
20ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent

10ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
5ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
2.5ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

Table (2-6) Steps for diluting standard solutions

Figure (2-4) the reagents preparation for (NFE2L2) concentrations

❖ **Wash Buffer**



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml	2.5ng/ml

Twenty milliliters (20ml) of 25x Wash Buffer Concentrate were diluted with deionized or distilled water to final volume 500 ml of 1x Wash Buffer. If crystals were present in the concentrate, the solution was gently mixed until the crystals were completely dissolved.

2.5.6. Assay Procedure

❖ **Preparation of Reagents and Samples**

All reagents, standard solutions, and samples were prepared and brought to room temperature before use. The assay was performed at room temperature. The number of strips required for the experiment was determined and inserted into the designated trays, while unused strips were stored at 2–8 °C.

❖ **Incubation with Standards and Samples**

standard solution (50 µL) was added to the measurement well without antibody, as the solution contained a biotin-labeled antibody. 40 µL of sample was placed in the sample wells, followed by the addition of 10 µL of human antibody NFE2L2, followed by 50 µL of streptavidin-HRP to both sample and standard wells (except for the blank control well). The contents were mixed well, the plate was covered with a tight lid, and then incubated for 60 minutes at 37 °C.

❖ **Washing Procedure**

After incubation, the cover was removed and the plate was washed five times with wash solution. The wells were soaked with 300 µL of wash solution for 30 seconds to 1 minute per wash. In the case of automated washing, the wells were aspirated or emptied and washed five times with wash solution. The plate was then dried on paper towels or suitable absorbent materials.

❖ **Color Development**

substrate solution A (50 µL) was added to each well, followed by 50 µL of substrate solution B. The plate was covered with a new cover and incubated for 10 minutes at 37°C in the dark. 50 µL of stop solution was then added to each well, resulting in a blue-to-yellow color change.

❖ **Measurement**

The optical density (OD) of each well was measured using a microplate reader set at a wavelength of 450 nm, within 10 minutes of adding the stop solution.

2.5.8. Calculation of Result

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

2.5.9. Typical Data

The standard curve of Nrf-2 is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

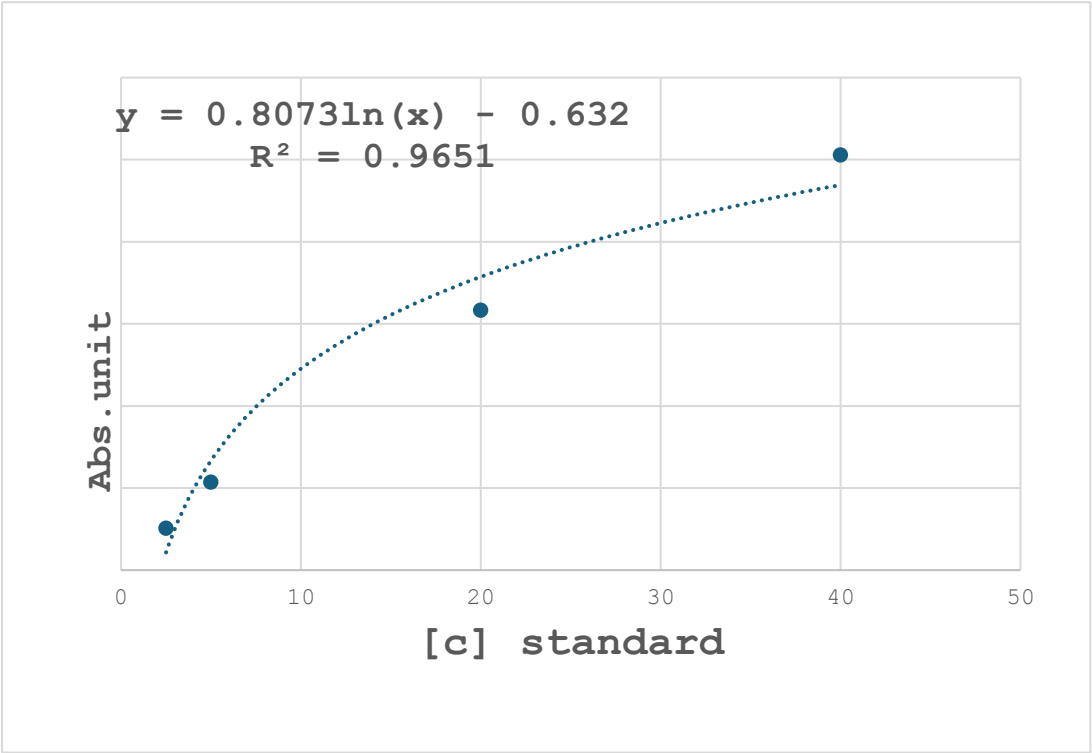


Figure (2-5) the standard curve for (Nrf-2) concentrations

Concentration	*Abs*
40ng/ml	2.53
20ng/ml	1.585
5ng/ml	0.537
2.5ng/ml	0.256

Chapter Three

Results

3.1.Results

3.1.1. Demographic and Clinical Characteristics

The demographic and clinical characteristics of patients with (T2DM) and control groups are reported in Table (3-1).

Table (3-1) Demographic Distribution of Study groups

Characteristics		*Diabetic*	*Pre -Diabetic*	*Control*
Sex	***Male***	50.00 %	50.00 %	50.00 %
	Female	50.00 %	50.00 %	50.00 %
Age	***20-30 years***	6(13.04%)	3(25.00 %)	6(20.0%)
	31-40 years	6(13.04%)	3(25.00 %)	8(26.7%)
	41-50 years	10(21.74%)	3(25.00 %)	7(23.3%)
	51-60 years	10(21.74%)	3(25.00 %)	6(20.00%)
	61-70 years	8(17.4%)	/	3(10.00 %)
	>71years	6(13.04%)	/	/
Age Mean ±2SD (year)		51.00±15	40.7 ±11.34	42.55 ±13.22
BMI	***Normal weight***	12(26.1%)	4(33.3 %)	18(60.00 %)
	Overweight	34(73.9%)	8(66.7 %)	12(40.00 %)
Smoking	***No (%)***	38(76.00%	63.30 %	19(63.30%)
	Yes (%)	12(24.00%)	36.70%	11(36.70%)
Family history	**No**	38 (76.1 %)	7(58.3 %)	26 (86.6%)
	Yes	12 (23.9 %)	5 (41.7 %)	4 (13.33 %)
Hyperlipidemia	**No**	17 (34.8%)	12 (100.0%)	28 (93.3%)
	Yes	33 (65.2%)	0 (0.00%)	2 (6.7%)

3.2. Comparison of Routine Biomarkers in the study Groups

3.2.1. Hemoglobin (HbA1c)

Table (3.2) shows the median and range of serum glycated Hemoglobin (HbA1c) levels for the study participants.

The data demonstrates a clear and expected pattern of increasing HbA1c levels across the three groups, reflecting their glycemic control status. There is a notable and a distinct separation in the median HbA1c values among the groups, with the Healthy Control group showing optimal glycemic control, followed by the Pre-DM group indicating impaired glucose regulation, and finally the DM Patients group reflecting established diabetes. The median HbA1c values align well with standard clinical diagnostic criteria:

The wide range observed in DM Patients (5.2% - 13%) indicates a significant heterogeneity in glycemic control within this group. While some diabetic patients might have relatively better control (HbA1c closer to 5.2%), others show very poor control (up to 13%), highlighting varied disease management or severity among individuals diagnosed with DM.

The tighter ranges for both Healthy Controls and the Pre-DM group (4.7-5.6% and 5.7-6.1% respectively) suggest a more consistent level of glycemic control within these non-diabetic and pre-diabetic populations, respectively.

The HbA1c data effectively differentiates between the three glycemic states, providing a clear biochemical validation of the group classifications and illustrating the progression of glucose dysregulation from healthy individuals to pre-diabetic states and finally to overt diabetes.

Table (3.2) Serum Levels of glycated Hemoglobin among study groups

	DM Patients	***Pre DM group***	***Control***
HbA1c Median (Mini-Max)	7.9 (5.2-13)	5.9 (5.7-6.1)	4.9 (4.7-5.6)

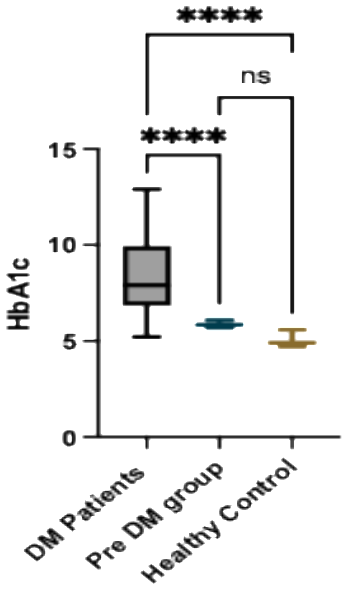


Figure (3.1) Distribution of Serum Levels of HbA1c among study groups (Post Hoc ANOVA test was *: significant at p ≤ 0.05, **: significant at p ≤ 0.01, ***: significant at p ≤ 0.001), *: significant at p ≤ 0.0001***

3.2.2.Lipid Profile

Table (3.3) presents the median and range (Min-Max) for Triglycerides (TG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Total Cholesterol among DM Patients, Pre-DM Group, and Healthy Controls.

The lipid profile data reveals distinct patterns across the three groups, consistent with the progressive metabolic dysregulation from a healthy state to pre-diabetes and overt diabetes.

There is a clear ascending trend in TG levels from Healthy Controls to the Pre-DM group, and further to DM Patients. The median TG is lowest in healthy individuals (99 mg/dL), moderately elevated in pre-diabetes (131 mg/dL), and highest in diabetic patients (162 mg/dL). The DM Patients group shows substantial variability (60-617 mg/dL), indicating that while many diabetic individuals have elevated triglycerides, some may still be within a normal range, while others exhibit severe hypertriglyceridemia.

This trend aligns with the known association between insulin resistance (characteristic of pre-diabetes and DM) and increased hepatic triglyceride production and reduced clearance.
HDL, shown a descending trend across the groups. Healthy Controls have the highest median HDL

(65 mg/dL), followed by the Pre-DM group (62 mg/dL), and significantly lower in DM Patients (48 mg/dL). DM Patients group's median HDL is below the clinically desirable threshold (typically >50 mg/dL for men, >60 mg/dL for women), indicating an increased cardiovascular risk profile. This "diabetic dyslipidemia" pattern, characterized by low HDL, is a common feature of insulin resistance and type 2 diabetes.

LDL, demonstrated an increasing trend from Healthy Controls (median 68 mg/dL) to the Pre-DM group (90 mg/dL) and DM Patients (93 mg/dL). Interestingly, the medians for Pre-DM and DM Patients are quite similar, suggesting that LDL elevation begins early in the glucose dysregulation spectrum. The DM Patients group also exhibits a wide range in LDL (21-150 mg/dL), reflecting varied individual responses and management strategies. The lower median in healthy controls is desirable for cardiovascular health.

Total cholesterol levels also show an ascending trend, with Healthy Controls having the lowest median (164 mg/dL), followed by the Pre-DM group (185 mg/dL), and DM Patients (188 mg/dL). Similar to LDL, the medians for Pre-DM and DM Patients are very close, implying that overall cholesterol levels begin to rise even before a full diabetes diagnosis. The DM Patients group again show the widest range (90-308 mg/dL), demonstrating variable total cholesterol control. The lipid profile data strongly corroborates the glycemic status of the groups. Diabetic patients, and to a lesser extent pre-diabetic individuals, exhibit a pro-atherogenic lipid profile characterized by higher triglycerides, lower HDL, and higher LDL and total cholesterol compared to healthy controls.

Table (3.3) Serum Levels of Lipid profile among study groups

	DM Patients	***Pre DM group***	***Control***
TG Median (Mini-Max)	162 (60-617) mg/dL	131 (100-145) mg/dL	99 (84-153) mg/dL
HDL Median (Mini-Max)	48 (33-68) mg/dL	62 (42-67) mg/dL	65 (39-69) mg/dL
LDL Median (Mini-Max)	93 (21-150) mg/dL	90 (86-94) mg/dL	68 (50-104) mg/dL
CHOLESTROL Median (Mini-Max)	188 (90-308) mg/dL	185 (168-193) mg/dL	164 (124-199) mg/dL

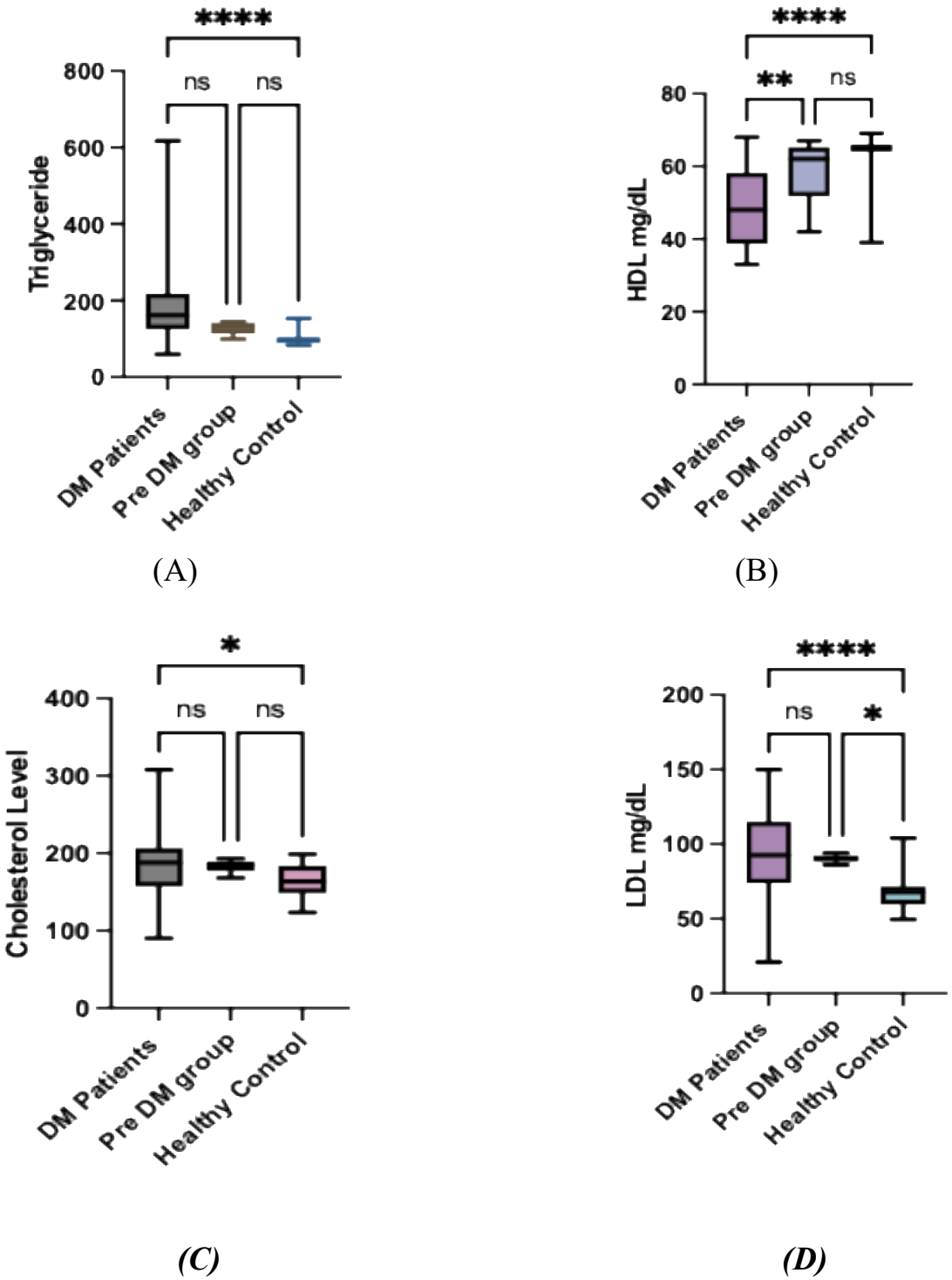


Figure (3.2) Distribution of Serum Levels of Lipid profile among study groups (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$), *: significant at $p \leq 0.0001$***

3.2.3. Liver Enzymes

Table (3.4) presents the median and range (Min-Max) for Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in DM Patients, Pre-DM Group, and Healthy Controls.

There was a descending trend in median ALT levels from Healthy Controls to the Pre-DM group, and further to DM Patients. Healthy Controls have the highest median ALT (31 U/L), followed by the Pre-DM group (29 U/L), and the lowest in DM Patients (20 U/L).

The Control group shows a remarkably narrow range for ALT (29-32 U/L) around its median, except for one outlier, indicated very consistent levels within this group**.** While the medians shown lower ALT in DM patients, the DM Patients group exhibits the widest range (9.5-61 U/L)**.** This indicates significant variability, with some diabetic individuals having relatively low ALT and others having elevated levels that might suggest liver involvement (such as non-alcoholic fatty liver disease, NAFLD, which is common in DM). Generally, elevated ALT is an indicator of liver damage. The observed trend of *lower* median ALT in DM patients compared to healthy controls. Similar to ALT, AST also shows a descending trend in median levels across the groups, with Healthy Controls having the highest median (28 U/L), followed by the Pre-DM group (26 U/L), and DM Patients (23 U/L). The ranges for all groups overlap considerably, indicating that a single AST measurement might not effectively differentiate between these groups.

The Control group has the widest overall range for AST (16-83 U/L)**,** potentially due to the presence of outliers or a broader spectrum of physiological conditions within this group, including one individual with a notably higher AST. While the median values for both ALT and AST show a slightly decreasing trend from healthy controls to DM patients, the broad ranges (particularly for DM patients in ALT and healthy controls in AST) indicated heterogeneity within each group. This might suggest differences in liver health or metabolic compensatory mechanisms among these specific cohorts or a need for more nuanced statistical analysis beyond medians and ranges.

Table (3.4) Serum Levels of Liver function enzymes among study groups

	DM Patients	***Pre DM group***	***Healthy Control***
ALT Median (Mini-Max)	20 (9.5-61	29 (10-36	31 (29-32
AST Median (Mini-Max)	23 (15-42	26 (16-33	28 (16-83

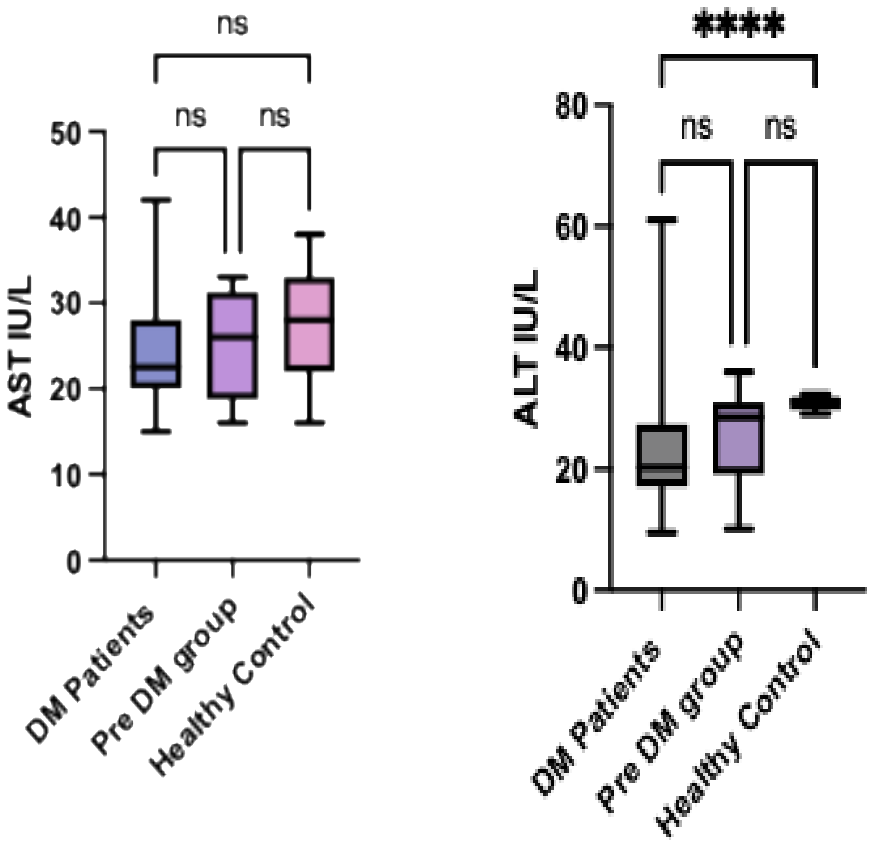


Figure (3.3) Distribution of Serum Levels of Liver function enzymes among study groups (Post Hoc ANOVA test was *: significant at p ≤ 0.05, **: significant at p ≤ 0.01, ***: significant at p ≤ 0.001), *: significant at p ≤ 0.0001***

3.3. Comparison of CoQ10 and Nrf2 Median Values between The study Groups

Table (3.5) illustrates the median and range (Min-Max) for serum Nrf2 and Co Q10 in DM Patients, Pre-DM Group, and Healthy Controls.The median Nrf2 levels are remarkably similar and largely overlapping across all three groups: Healthy Control (26), DM Patients (29), and Pre-DM Group (30).

The ranges for all groups are very wide and show overlap, indicating a high degree of variability within each group and making it difficult to discern a clear pattern or significant difference based on these median values alone.

Nrf2 does not appear to be a strong differentiator among healthy individuals, pre-diabetic, or diabetic patients based on median serum levels. This might suggest that systemic Nrf2 levels in

circulation do not consistently reflect its activity or cellular response within specific tissues relevant to diabetes (e.g., pancreas, liver, muscle) or that its dysregulation in diabetes manifests differently compared to thyroid diseases**.** Regarding the Coenzyme Q10, there was a clear and significant descending trend in serum Co Q10 levels from Healthy Controls to the Pre-DM group, and further to DM Patients. Healthy Controls exhibit the highest median Co Q10 (1.6), with a tight and high range (1.3-1.7), indicative of robust antioxidant status.

The Pre-DM group shows an intermediate median (0.89), with a broader range (0.22-1.6), indicating some individuals may have Co Q10 levels similar to healthy controls, while others are lower. DM Patients have the lowest median Co Q10 (0.38), with a broad range (0.16-1.5), demonstrating a considerable reduction in this vital antioxidant. The lower median and often lower values in the range for diabetic patients are consistent with increased oxidative stress and mitochondrial dysfunction known to occur in diabetes.

This trend strongly suggests that Co Q10 levels are progressively depleted as individuals move from a healthy metabolic state through pre-diabetes to overt diabetes, potentially reflecting increased oxidative burden or impaired Co Q10 synthesis/utilization in these conditions.while serum Nrf2 levels show no significant differences among the groups related to diabetes status, Co Q10 levels clearly demonstrate a progressive decline from healthy individuals to pre-diabetic and diabetic patients**.** This reduction in Co Q10 in pre-diabetes and diabetes highlights its potential as a marker for oxidative stress and metabolic health deterioration, which is a significant finding given Co Q10's crucial role in cellular energy production and antioxidant defense.

Table (3.5) Serum Levels of Nuclear respiratory factor 2 (Nrf2), Co Q10 among study groups

	DM Patients	**Pre DM group**	**Healthy Control**
Nrf2 Median (Mini-Max)	29 (12-69)	30 (12-49)	26 (13- 70)
Co Q10 Median (Mini-Max)	0.38 (0.16-1.5)	0.89 (0.22-1.6)	1.6 (1.3-1.7)

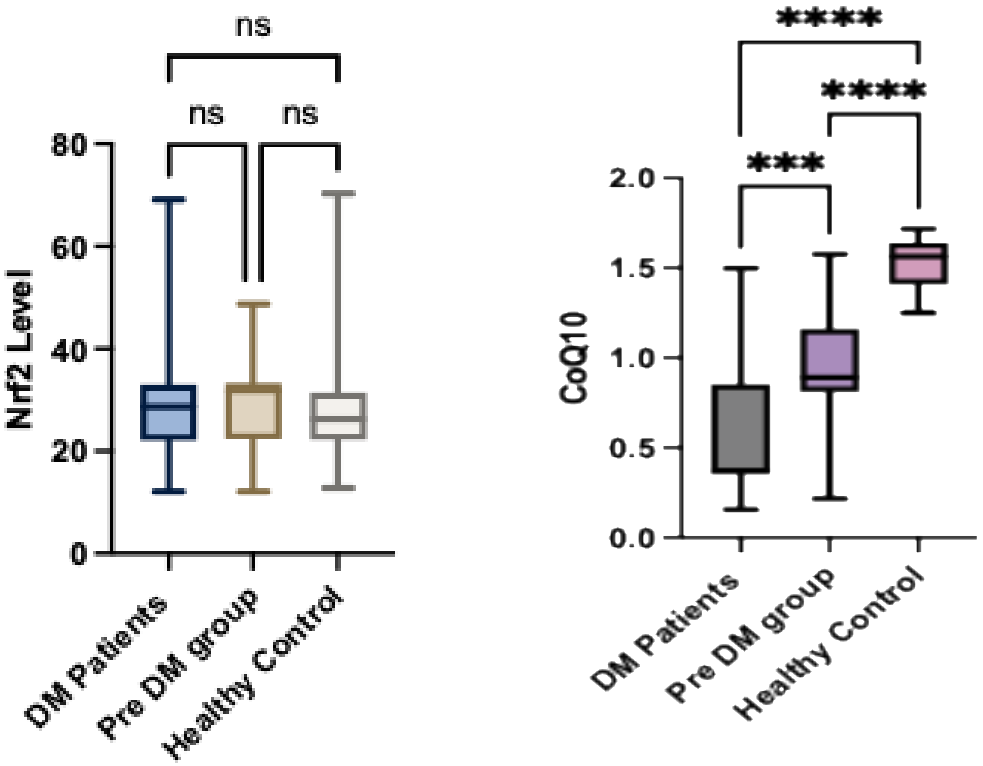


Figure (3.4) Distribution of Serum Levels of Nrf2 & Co Q10 among study groups (Post Hoc ANOVA test was *: significant at p ≤ 0.05, **: significant at p ≤ 0.01, ***: significant at p ≤ 0.001), *: significant at p ≤ 0.0001***

3.4. Comparison of CoQ10 and Nrf2 Median Values According to Study groups

The Table (3.6) presented the Pearson correlation coefficient (r) and the corresponding p-value for various biomarker pairs within the DM patient group. A p-value which is less than 0.05 is considered statistically significant.

There is a positive and a statistically significant correlation between serum CoQ10 and Nrf2 (r = 0.4, p = 0.05). This indicates that in DM patients, as CoQ10 levels tend to decrease, Nrf2 levels also tend to decrease, suggesting a potential interplay or parallel regulation of these two antioxidant-related molecules.

CoQ10 shows a positive weak and a statistically significant correlation with HDL (r = 0.332, p = 0.005). This is an important finding, suggesting that low levels of the antioxidant CoQ10 are associated with low levels of "good" cholesterol (HDL) in diabetic patients.

Cholesterol (Chol), Triglycerides (TG), LDL, HbA1c, ALT, and AST: CoQ10 did not show statistically significant correlations with Cholesterol (r = -0.372, p = 0.172), TG (r = -0.131, p = 0.642), LDL (r = -0.184, p = 0.511), HbA1c (r = -0.154, p = 0.584), ALT (r = 0.094, p = 0.739), or AST (r = -0.179, p = 0.523).

All other biomarkers (Chol, TG, HDL, LDL, HbA1c, ALT, AST): Nrf2 did not show any statistically significant correlations with any of these parameters within the DM group (all p-values > 0.05).

The significant positive correlation between CoQ10 and Nrf2 suggests that in the context of diabetes, these two key players in cellular defense against oxidative stress might be regulated in a somewhat coordinated manner, or that healthier antioxidant status

The significant positive correlation between CoQ10 and HDL is clinically relevant. In diabetes, low HDL is a common feature of dyslipidemia and contributes significantly to increased cardiovascular risk. This result suggests that low CoQ10 levels in diabetic patients might be protective or associated with a less unfavorable lipid profile, specifically concerning HDL.

Table 3.6: The correlation coefficient (r) between Serum Levels of Nuclear respiratory factor 2 (Nrf2), and CoQ10 among DM group

Biomarkers	*CoQ10*	*NRf2/p*

	R	P value	R	P value
CoQ10	1[a]	-	0.4	0.05[S]
NRf2/p	0.4	0.05[S]	1[a]	-
Chol	-0.372	0.172[NS]	0.097	0.504[NS]
TG	-0.131	0.642[NS]	-0.054	0.707[NS]
HDL	0.332	0.005[S]	-0.053	0.714[NS]
LDL	-0.184	0.511[NS]	0.204	0.155[NS]
HbA1c	-0.154	0.584[NS]	-0.003	0.986[NS]
ALT	0.094	0.739[NS]	0.038	0.793[NS]
AST	-0.179	0.523[NS]	-0.061	0.677[NS]

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ,
R:Correlation Coefficient

The Table (3.7) displays the Pearson correlation coefficient (r) and the corresponding p-value for various biomarker pairs within the Pre-DM patient group. As per the definition of the table, p-values less than 0.05 are considered statistically significant.There was a positive and highly statistically significant correlation between serum CoQ10 and Nrf2 (r = 0.65, p = 0.001). This suggests a robust positive association where low levels of one tend to correspond with low levels of the other in individuals with pre-diabetes.

CoQ10 also shows a negative correlation with HbA1c (r = -0.920, p = 0.027). This indicates that within the Pre-DM group, individuals with low CoQ10 levels tend to have higher HbA1c, which is consist finding given CoQ10's antioxidant role and its general depletion in diabetes progression.

CoQ10 shows a weak negative and a statistically significant correlation with AST (r = -0.234, p = 0.05). This suggests that as CoQ10 levels increase, AST levels tend to slightly decrease.

The Pre-DM group exhibits a very strong positive correlation between CoQ10 and Nrf2, highlighting a potential cooperative antioxidant defense. The strong nehative correlation of CoQ10

with HbA1c is an interesting result specific to this group that merits deeper investigation. While liver enzyme correlations suggest some protective association with Nrf2, the overall lack of significant correlations with traditional lipid parameters contrasts with the DM group, indicating dynamic changes in biomarker interactions during the progression of metabolic disease.

Table 3.7: *The correlation coefficient (r) between Serum Levels of Nuclear respiratory factor 2 (Nrf2), and CoQ10 among Pre-DM group*

Biomarkers	***CoQ10***		***NRf2/p***	
	R	***P value***	***R***	***P value***
CoQ10	1[a]	-	0.65	**0.001[S]**
NRf2/p	0.65	**0.001[S]**	1[a]	-
Chol	-0.090	0.885[NS]	-0.163	0.653[NS]
TG	-0.387	0.520[NS]	-0.218	0.545[NS]
HDL	0.856	0.064[NS]	0.094	0.796[NS]
LDL	-0.129	0.836[NS]	-0.028	0.939[NS]
HbA1c	-0.920[*]	**0.027[S]**	-0.032	0.931[NS]
ALT	-0.512	0.378[NS]	-0.128	0.027[NS]
AST	-0.234	**0.05[S]**	-0.527	**0.003[S]**

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant , *R:Correlation Coefficient*

3.5. Association of Serum Levels of (Nrf2), and CoQ10 among Study Groups

This Table (3.8) assesses the ability of Nrf2 and CoQ10 to predict the likelihood of an individual belonging to the DM Patient group or the Pre-DM group, as opposed to the Healthy Control group. An Odds Ratio (OR) of 1 means no association, an OR greater than 1 means increased odds, and an OR less than 1 means decreased odds.

The Odds Ratio (OR) for Nrf2 in DM Patients was 0.994 (95% CI: 0.985-1.004), with a p-value of 0.016, indicating statistical significance. An OR of 0.994 means that for every one-unit increase in serum Nrf2 levels, the odds of being a DM patient (compared to a healthy control) are 0.994 times, or slightly less than 1. This indicates a very weak, almost negligible inverse

association. Although statistically significant, the OR is extremely close to 1, and the confidence interval marginally crosses 1, suggesting that Nrf2's ability to differentiate DM patients from healthy controls based on a linear increase is very limited in practical terms.

The Odds Ratio (OR) for Nrf2 in the Pre-DM group was 0.990 (95% CI: 0.959-1.021), with a p-value of <0.03, indicating a statistical significance. Similarly, an OR of 0.990 implies a very weak inverse association; for every one-unit increase in Nrf2, the odds of being in the Pre-DM group (compared to a healthy control) are 0.990 times. Despite statistical significance, the OR is extremely close to 1, and the confidence interval includes 1, suggesting Nrf2 is not a strong differentiator for pre-diabetes either.

The Odds Ratio (OR) for CoQ10 in DM Patients was 1.097 (95% CI: 1.035-1.174), with a p-value of <0.001, indicating a high statistical significance. An OR of 1.097 suggests that for every one-unit increase in serum CoQ10 levels, the odds of being a DM patient (compared to a healthy control) are 1.097 times higher (approximately 9.7% higher). The confidence interval does not include 1, reinforcing significance.

The Odds Ratio (OR) for CoQ10 in the Pre-DM group was 4.85 (95% CI: 3.01-4.90), with a p-value of <0.001, indicating a high statistical significance.

An OR of 4.85 implies that for every one-unit increase in serum CoQ10 levels, the odds of being in the Pre-DM group (compared to a healthy control) are 4.85 times higher. The very high OR and non-overlapping confidence interval indicate a strong predictive power. Based on the binary logistic regression analysis as presented, Nrf2 does not appear to be a strong discriminator for either DM or Pre-DM status when compared to healthy controls, despite achieving a statistical significance with ORs very close to 1. CoQ10, on the other hand, shows a highly statistically significant Odds Ratios, suggesting a strong predictive relationship.

Table 3.8: The binary logistic regression of Serum Levels of Nuclear respiratory factor 2 (Nrf2), and CoQ10 among study groups

Biomarkers	*Groups*	*OR (Lower-Upper)*	*P-Value*
NRf2/p	**DM Patient**	0.994 (0.985-1.004)	**0.016[S]**
	Pre DM	0.990 (0.959-1.021)	**<0.03[S]**
	Control	1[a]	-
CoQ10			
	DM Patient	1.097 (1.035-1.174)	**<0.001[S]**
	Pre DM	4.85 (3.01-4.90)	**<0.001[S]**
	Control	1[a]	-
p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant 1[a] : reference category is Control			

3.6 Receiver Operating Characteristic (ROC) Curve

This Table (3.9) evaluates the diagnostic performance of serum CoQ10 levels in identifying DM patients and Pre-DM individuals.

The Diagnostic Performance for DM, An AUC of 0.9724 is exceptionally high. This indicates excellent diagnostic accuracy for serum CoQ10 in differentiating DM patients from healthy individuals.

The optimal cut-off, can correctly identify 87.50% of individuals who truly have Diabetes Mellitus (true positives). An individual with a serum CoQ10 level below 1.275 is optimally classified as a DM patient. This aligns perfectly with the descriptive statistics (Table 3.5) which showed DM patients having a median CoQ10 of 0.38 and healthy controls having a median of 1.6, confirming that lower CoQ10 is indicative of DM. While the diagnostic performance in Pre-DM, results were shown that An area under the curve (AUC) of 0.9013 is also very high, indicating an excellent diagnostic accuracy for serum CoQ10 in differentiating Pre-DM individuals from healthy controls.

This means that CoQ10 levels can correctly identify 76.00% of individuals who are in the pre-diabetic stage. Also CoQ10 levels can correctly identify 93.55% of individuals who are ***not*** pre-diabetic (i.e., healthy controls), demonstrating a very good ability to rule out pre-diabetes.

An individual with a serum CoQ10 level below 1.120 is optimally classified as being in the Pre-DM group. This is consistent with the descriptive statistics (Table 3.5) where the Pre-DM group had a median CoQ10 of 0.89 and healthy controls had 1.6, showing lower CoQ10 in pre-diabetes.

Serum CoQ10 levels demonstrate a good diagnostic utility for both Diabetes Mellitus and the Pre-DM state. Its ability to discriminate between healthy individuals and DM patients (AUC = 0.9724) is slightly higher than its ability to discriminate between healthy individuals and those with pre-diabetes (AUC = 0.9013), suggesting a more pronounced difference in CoQ10 levels once overt diabetes has developed.

The cut-off points (< 1.275 for DM and < 1.120 for Pre-DM) are intuitive, indicating that progressively lower levels of CoQ10 are associated with a higher likelihood of being in the diabetic state, which is consistent with the depletion of this antioxidant in metabolic disorders. The high sensitivity and specificity for both classifications suggest that CoQ10 could serve as a valuable non-invasive biomarker for screening and diagnosis in the context of impaired glucose metabolism.

Table 3.9: AUC, optimal threshold, Sensitivity, and specificity of *CoQ10* ***levels among patient groups***

Variable	AUP	Sensitivity %	Specificity %	Cut-off points	P
DM	0.9724	87.50	93.55	< 1.120	<0.0001
Pre-DM	0.9013	76.00	93.55	< 1.275	<0.0001

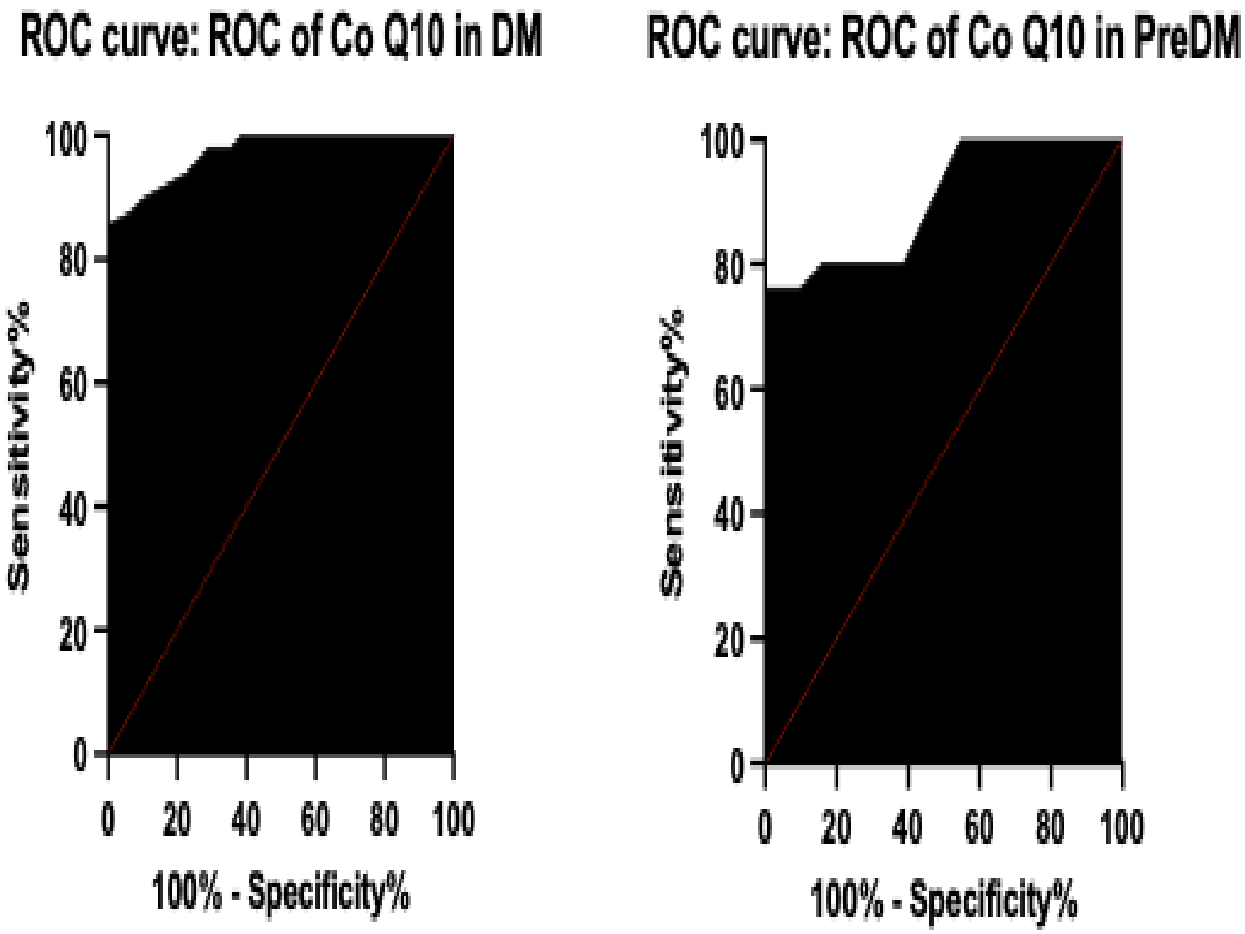


Figure (3.5) Receiver operating characteristic curve of** CoQ10 **levels among patients groups

This Table (3.10) evaluates the diagnostic performance of serum Nrf2 levels in identifying DM patients and Pre-DM individuals.

For DM group An AUC of 0.5257. This indicates that Nrf2 has very poor diagnostic accuracy in differentiating DM patients from healthy individuals. Using the optimal cut-off, Nrf2 can correctly identify only 51.02% of individuals who truly have Diabetes Mellitus.

Nrf2 can correctly identify 65.52% of individuals who do *not* have DM This indicates a significant rate of false positives. An individual with a serum Nrf2 level above 28.58 is classified as a DM patient. This aligns with the descriptive statistics (Table 3.6), which showed DM patients having a slightly higher median Nrf2(29) compared to healthy controls (26). On the other hand, the diagnostic performance in Pre-DM show an AUC of 0.6276 was, but still indicates poor to fair diagnostic accuracy for Nrf2 in differentiating Pre-DM individuals from healthy controls. It is generally considered not clinically useful for discrimination.

Nrf2 levels can correctly identify 68.97% of individuals who are in the pre-diabetic stage. Nrf2 levels can correctly identify 70.00% of individuals who are *not* pre-diabetic. An individual with a serum Nrf2 level below 30.26 is optimally classified as being in the Pre-DM group.

In contrast to CoQ10, serum Nrf2 levels demonstrate poor diagnostic utility for both Diabetes Mellitus and the Pre-DM state. For both conditions, the AUC values are close to 0.5, and critically, the p-values are well above 0.05, indicating that Nrf2's ability to discriminate these patient groups from healthy controls is not statistically significant. The sensitivity and specificity values are relatively low for both classifications. The inconsistency in the cut-off direction for the Pre-DM group further highlights the lack of clear discriminative patterns for Nrf2 in this context.

Table 3.10: AUC, optimal threshold, Sensitivity, and specificity of Nrf2 levels among patient groups

Variable	AUP	Sensitivity %	Specificity %	Cut-off points	P
DM	0.5257	51.02	65.52	> 28.58	0.7059
Pre-DM	0.6276	68.97	70.00	< 30.26	0.2

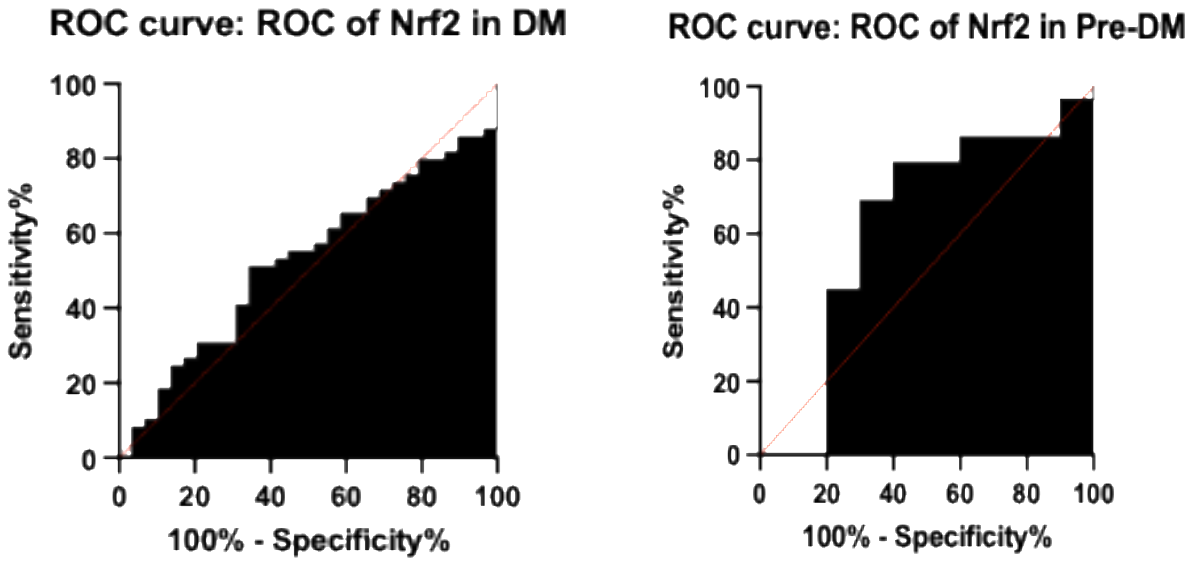


Figure (3.6) Receiver operating characteristic curve of** NRf2/p **levels among patients groups

Chapter Four
Discussion

4.1.Discussion

This study comprehensively investigated a range of demographic, clinical, metabolic, and oxidative stress-related biomarkers across healthy controls, individuals with pre-diabetes (Pre-DM), and patients with Type II Diabetes Mellitus (T2DM). The findings provide crucial insights into the progressive metabolic dysregulation associated with diabetes development and highlight the potential utility of specific biomarkers for screening and diagnosis.

The observed clear and increasing pattern of median HbA1c levels from healthy controls (4.9%) to the pre-DM group (5.9%) and subsequently to DM patients (7.9%) is a fundamental aspect of diabetes pathophysiology. This trend directly reflects the progressive deterioration of glycemic control along the spectrum of glucose dysregulation. HbA1c measures the average blood glucose levels over the preceding 2-3 months and is a widely accepted diagnostic criterion for diabetes and pre-diabetes [American Diabetes Association, 2024; World Health Organization, 2011]. The median values observed in this study (4.9% for healthy, 5.9% for Pre-DM, and 7.9% for DM) are in excellent agreement with the diagnostic thresholds set by major health organizations (e.g., HbA1c <5.7% for non-diabetic, 5.7%-6.4% for pre-diabetes, and ≥6.5% for diabetes)[American Diabetes Association, 2024]. This provides robust biochemical validation for the classification of the study groups.

The distinct separation in median HbA1c values between the groups underscores the utility of this biomarker in identifying individuals at different stages of glucose metabolism. The gradual increase from optimal glycemic control in healthy individuals, through the impaired glucose regulation .

The characteristic of pre-diabetes, to the overt hyperglycemia defining established diabetes, illustrates the natural continuum of the disease [Grundy, et al., 2018]. This highlights the importance of early detection of pre-diabetes, as individuals in this stage are at significantly increased risk of progressing to type 2 diabetes and developing cardiovascular complications [Tabák, et al., 2012].

The wide range of HbA1c observed in DM patients (5.2% - 13%) is also a clinically pertinent finding. This heterogeneity is typical in established diabetes and reflects various factors, including the duration of the disease, the efficacy of treatment regimens, patient adherence to medication and lifestyle interventions, and the presence of diabetes-related complications [Halcox, et al., 2015]. While some diabetic patients might achieve relatively good glycemic control (HbA1c closer to the lower end of the observed range), others exhibit very poor control, which significantly increases their risk for microvascular (e.g., retinopathy, nephropathy, neuropathy) and macrovascular complications (e.g., cardiovascular disease) [UKPDS, 1998; Inzucchi et al., 2015]. This variability underscores the need for individualized management strategies in diabetes care.

Conversely, the tighter ranges for both Healthy Controls (4.7-5.6%) and the Pre-DM group (5.7-6.1%) suggest a more consistent metabolic state within these populations. In healthy individuals, glucose regulation mechanisms are generally robust, leading to stable HbA1c levels. In pre-diabetes, while glucose regulation is impaired, it has not yet deteriorated to the extent seen in overt diabetes, resulting in a narrower band of elevated but not severely high HbA1c values [American Diabetes Association, 2024].

In conclusion, the HbA1c data from this study effectively differentiates the three glycemic states, providing clear biochemical evidence of the classification of the groups. This aligns with the established role of HbA1c as a reliable indicator of long-term glycemic control and a critical tool for the diagnosis, monitoring, and risk stratification of individuals across the spectrum of glucose metabolism disorders [Sherwani et al., 2016].

4.1.1.Lipid Profile

The comprehensive analysis of the lipid profile data from Table (3.3) strongly corroborates the glycemic status of the study groups, revealing distinct patterns consistent with the progressive metabolic dysregulation associated with pre-diabetes and overt diabetes. These findings align well with established understanding of diabetic

dyslipidemia, a critical contributor to the elevated cardiovascular risk in these populations.

The observed lipid profile is a hallmark of the metabolic abnormalities present in impaired glucose tolerance and diabetes. This "diabetic dyslipidemia" is characterized by a constellation of changes, primarily driven by insulin resistance, which is central to the pathophysiology of both pre-diabetes and type 2 diabetes [Taskinen & Borén, 2015; Zimmet et al., 2001].

The clear ascending trend in Triglyceride (TG) levels from healthy controls (median 99 mg/dL) to the Pre-DM group (131 mg/dL) and significantly higher in DM Patients (162 mg/dL) is a classical feature. Elevated triglycerides in diabetic and pre-diabetic states result from increased hepatic very-low-density Lipoprotein (VLDL) production and impaired catabolism of triglyceride-rich lipoproteins due to reduced lipoprotein lipase (LPL) activity, are both consequences of insulin resistance [Lewis et al., 2002; Reaven, 2005]. The substantial variability in TG levels among DM patients (60-617 mg/dL) underscores the heterogeneous nature of the disease and individual responses to metabolic perturbations and/or treatment, with some individuals experiencing severe hypertriglyceridemia, which is an independent risk factor for cardiovascular disease and pancreatitis [Miller et al., 2011].

Conversely, High-Density Lipoprotein (HDL) showed a descending trend across the groups, with the highest median in healthy controls (65 mg/dL), moderately lower in the Pre-DM group (62 mg/dL), and significantly reduced in DM Patients (48 mg/dL). This low HDL, particularly below the clinically desirable threshold (typically >50-60 mg/dL), is another characteristic of diabetic dyslipidemia [American Diabetes Association, 2024]. Insulin resistance leads to increased catabolism of HDL particles and reduced synthesis of apolipoprotein A-I (apoA-I), a major component of HDL, thereby diminishing its anti-atherogenic functions, such as reverse cholesterol transport [Lewis et al., 2002; Chapman et al., 2011].

The increasing trend in Low-Density Lipoprotein (LDL) levels from healthy controls (median 68 mg/dL) to Pre-DM (90 mg/dL) and DM Patients (93 mg/dL) is also a critical observation. While the median total LDL cholesterol might not be drastically

high in all diabetic patients, the dyslipidemia associated with insulin resistance often leads to an increased proportion of small, dense LDL particles. These small, dense LDL particles are highly atherogenic, being more susceptible to oxidation and more prone to entering the arterial wall, even at seemingly "normal" total LDL concentrations [Krauss, 2001; Sniderman et al., 2003]. The similarity in median LDL between the Pre-DM and DM groups suggests that LDL elevation, or at least qualitative changes towards a more atherogenic profile, begins early in the glucose dysregulation process. The wide range in LDL among DM patients again highlights the diverse metabolic profiles within the diabetic population.

Finally, total cholesterol levels also showed an ascending trend, mirroring the changes in LDL and reflecting the overall lipid burden. The close medians for total cholesterol between Pre-DM and DM groups further reinforce that significant lipid disturbances are present even before a full diagnosis of diabetes, placing these individuals at heightened cardiovascular risk. the lipid profile data strongly corroborates the glycemic status of the groups, demonstrating a clear progression towards a pro-atherogenic phenotype. Diabetic patients, and to a lesser extent pre-diabetic individuals, exhibit a characteristic pattern of higher triglycerides, lower HDL, and higher LDL and total cholesterol compared to healthy controls. [Emerging Risk Factors Collaboration, 2011; American Diabetes Association, 2024].

4.1.2.Liver Function

The analysis of serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels, as presented in Table (3.4), reveals intriguing patterns within the study groups. While transaminases are key indicators of live health, the observed trends, particularly the lower median levels in diabetic patients and the wide ranges within groups ALT and AST are common enzymes used as biomarkers for liver function and cellular integrity. Elevated levels typically indicate hepatocellular injury [Pratt & Kaplan, 2000].

The current findings indicate that the wide range in DM patients' ALT, despite a low median, suggests that a substantial subset of diabetic individuals in this study *do* exhibit elevated ALT levels consistent with liver injury, likely due to

NAFLD/NASH, which is estimated to affect 60-70% of type 2 diabetes patients [Targher et al., 2020]. This variability underscores that while the central tendency (median) might be low, a significant proportion of individuals within the group are experiencing liver pathology. While ALT and AST are useful screening tools, their median values alone, particularly when within or near the "normal" range, should not rule out liver pathology in high-risk populations like those with pre-diabetes or diabetes. [Chalasani et al., 2012]. the liver enzyme data, particularly the lower median ALT and AST in diabetic patients, presents a complex picture that might reflect the nuances of NAFLD progression, metabolic adaptations, and individual variability rather than a lack of liver involvement. The wide ranges observed within groups, especially the DM group, emphasize the importance of individualized clinical assessment and the need for more comprehensive diagnostic approaches to evaluate liver health in individuals with glucose dysregulation.

4.1.3. Nuclear factor erythroid 2-related factor 2 (Nrf2) and Coenzyme Q10 (CoQ10) Levels

The analysis of serum Nuclear Respiratory Factor 2 (Nrf2) and Coenzyme Q10 (CoQ10) levels, as presented in Table (3.5), offers insights into the antioxidant status of individuals across different glycemic states. The findings highlight contrasting patterns for these two biomarkers, with CoQ10 emerging as a more distinct indicator of metabolic health deterioration in pre-diabetes and diabetes.

The findings in this study show that median serum Nrf2 levels are remarkably similar and largely overlapping across healthy controls (26), DM patients (29), and the Pre-DM group (32). Furthermore, the wide ranges and substantial overlap across all groups underscore significant individual variability, making it difficult to discern a clear pattern or statistically significant difference based on these median values alone. This suggests that systemic circulating Nrf2 levels, as measured in this study, do not appear to be a strong differentiator among healthy, pre-diabetic, or diabetic individuals.

This lack of a clear differentiating pattern for serum Nrf2 might be attributed to several factors included Complex Regulation and Tissue Specificity. Nrf2 activity is

tightly regulated at multiple levels (transcription, translation, post-translational modifications, nuclear translocation). Serum levels might not accurately reflect the specific Nrf2 activity within relevant target tissues (e.g., pancreatic beta cells, liver, muscle, adipose tissue), where its dysregulation in diabetes is often more pronounced [Lim et al., 2011; Uruno et al., 2019].

Nrf2 activation is often an acute response to stress. In chronic conditions like diabetes, there might be initial compensatory upregulation followed by chronic suppression or functional impairment that is not reflected by total serum levels [Kim et al., 2010].

The Finding of the study's finding of a clear and significant descending trend in serum CoQ10 levels from Healthy Controls (median 1.6) to the Pre-DM group (0.89) and further to DM Patients (0.38) is highly consistent with existing literature. This progressive depletion of CoQ10 in metabolic disorders is well-documented and can be attributed to several mechanisms involved in increased Oxidative Stress**.** Diabetes is characterized by chronic oxidative stress, which consumes CoQ10 as it neutralizes reactive oxygen species, leading to its depletion [Hodges & Salpea, 2014].

Moreover, it might be reflected by mitochondrial dysfunction, since Insulin resistance and hyperglycemia contribute to mitochondrial dysfunction, impairing the de novo synthesis of CoQ10 and its effective utilization [Mootha et al., 2003].While systemic serum Nrf2 levels did not emerge as a significant differentiator among the glycemic groups in this study, CoQ10 levels clearly demonstrate a progressive decline from healthy individuals to pre-diabetic and diabetic patients. This reduction in CoQ10 in individuals with impaired glucose metabolism is a significant finding, underscoring its potential as a robust biomarker for oxidative stress and metabolic health deterioration. Given CoQ10's crucial role in cellular energy production and antioxidant defense, its depletion suggests an increased susceptibility to oxidative damage and

mitochondrial dysfunction, both of which are central to the pathogenesis and complications of diabetes [Gutierrez-Mariscal,et al,2019]. Coenzyme Q10: From bench to clinic in aging diseases, a translational review.

4.1.4.Association Odds Ratio (OR)

The binary logistic regression analysis, as displayed in Table 3.9 (referring to Table 3.8 as per previous context), assesses the independent predictive power of serum Nrf2 and CoQ10 levels in classifying individuals into DM Patient or Pre-DM groups, relative to a Healthy Control group. The logistic regression analysis indicates that Nrf2 is a statistically significant predictor for both DM patients (OR = 0.994, p = 0.016) and the Pre-DM group (OR = 0.990, p < 0.03) when compared to healthy controls.This finding is consistent with earlier descriptive statistics Table (3.5), which showed largely overlapping median Nrf2 levels across all three groups, and the ROC curve analysis Table (3.10), which demonstrated very poor diagnostic accuracy (AUCs close to 0.5 and non-significant p-values) for Nrf2. While Nrf2 is a crucial intracellular regulator of antioxidant defenses and its dysfunction is implicated in diabetes pathophysiology [Ma, 2013; Uruno et al., 2019], these results suggest that routine measurement of its circulating serum levels may not serve as an effective biomarker for the presence of diabetes or pre-diabetes. This could be due to the complex and compartmentalized nature of Nrf2 activity, which is not necessarily reflected in systemic circulation [Kim et al., 2010].

The logistic regression analysis indicates highly statistically significant Odds Ratios for CoQ10 in both DM patients (OR = 1.097, p < 0.001) and the Pre-DM group (OR = 4.85, p < 0.001) when compared to healthy controls.

CoQ10 levels are indeed strong predictors of group. This aligns with the excellent diagnostic performance of CoQ10 shown in the ROC curve analysis (Table 3.9), where lower CoQ10 levels were correctly identified as the optimal cut-off points for classifying DM and Pre-DM. It is probable that the model accurately identified CoQ10 as a strong differentiator. CoQ10 is a known key player in mitochondrial function and antioxidant defense, and its depletion is strongly associated with the pathogenesis and progression of insulin resistance and diabetes [Hodges & Salpea, 2014].

4.1.5.Receiver Operating Characteristic (ROC) curve

The ROC curve analysis presented in Table 3.10 (referring to Table 3.9 as previous context) robustly evaluates the diagnostic performance of serum Coenzyme Q10 (CoQ10) levels in discriminating individuals with Diabetes Mellitus (DM) and Pre-Diabetes (Pre-DM) from healthy controls. The findings collectively establish CoQ10 as an exceptionally promising biomarker for metabolic health assessment.

The diagnostic metrics for CoQ10 demonstrate its high efficacy in identifying distinct glycemic states. Exceptional Diagnostic Accuracy for Diabetes Mellitus shown that The Area Under the Curve (AUC) of 0.9724 which is exceptionally high, indicating outstanding diagnostic accuracy. An AUC value above 0.9 is generally considered excellent, suggesting that CoQ10 can almost perfectly distinguish between individuals with DM and healthy controls [Fawcett, 2006; Swets, 1988].

The distinct cut-off points (< 1.275 for DM and < 1.120 for Pre-DM) are intuitive and reflect the progressive decline of CoQ10 as individuals move from a healthy metabolic state through pre-diabetes to overt diabetes. This decline is attributed to increased oxidative stress and mitochondrial dysfunction that are fundamental to the pathophysiology of these conditions [Hodges & Salpea, 2014; Mootha et al., 2003].

The high sensitivity and specificity for both classifications suggest that CoQ10 could serve as a valuable, non-invasive biomarker for early screening, diagnosis, and risk stratification in the context of impaired glucose metabolism.

The ability of CoQ10 to accurately identify individuals in the pre-diabetic stage is particularly significant, as early detection allows for timely lifestyle interventions or pharmacological strategies to prevent or delay the progression to full-blown type 2 diabetes and its associated complications [Tabák et al., 2012].

Furthermore, given CoQ10's crucial role in cellular energy production and antioxidant defense, its diagnostic utility underscores its central involvement in the metabolic derangements of diabetes, potentially guiding future therapeutic strategies involving CoQ10 supplementation

Chapter Five

Conclusions and Recommendtions

5.1.Conclusions

- A central finding is the progressive and significant depletion of serum Coenzyme Q10 (CoQ10) as individuals transition from a healthy metabolic state through pre-diabetes to overt diabetes. This decline strongly correlates with worsening glycemic control in the pre-diabetic stage and an unfavorable lipid profile in established diabetes, reinforcing CoQ10's critical role in mitigating oxidative stress and supporting mitochondrial function, which are compromised in these conditions. Crucially, CoQ10 demonstrated excellent diagnostic accuracy in differentiating both diabetic (AUC=0.9724) and pre-diabetic (AUC=0.9013) individuals from healthy controls, with clear and clinically relevant cut-off points. This positions CoQ10 as a highly promising, non-invasive biomarker for screening, early detection, and monitoring of impaired glucose metabolism.
- serum Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2) showed no significant differences across the groups and exhibited negligible diagnostic utility. This suggests that systemic Nrf2 levels, as measured in this study, do not consistently reflect its crucial intracellular activity or its dynamic tissue-specific responses to oxidative stress in diabetes.
- While Nrf2 appears to be a poor systemic biomarker for diabetes status, the robust and progressive decline of CoQ10, coupled with its strong associations with key metabolic parameters and superior diagnostic performance, establishes it as a valuable and reliable indicator of metabolic health deterioration. These findings contribute significantly to our understanding of the biochemical landscape of diabetes progression and highlight the potential of CoQ10 as a clinically actionable biomarker for early intervention and personalized management strategies.

5.2. Recommendations

The findings of this study provide a strong foundation for further research into the role of Coenzyme Q10 (CoQ10) and Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2) in metabolic health and diabetes progression. Building upon the current insights, several key avenues for future work emerge:

5.2.1.Longitudinal Studies to Establish Causality and Disease Progression:

- The current study employed a cross-sectional design. Future research should involve longitudinal cohort studies to track changes in CoQ10 and Nrf2 levels over time as individuals progress from health to pre-diabetes and then to overt T2DM. This would help establish a causal relationship between biomarker changes and disease onset, as well as identify critical windows for intervention.
- Investigating the duration of diabetes and medication use as covariates in relation to CoQ10 levels will be crucial, as factors like statin use are known to influence CoQ10 status.

1. Validation of CoQ10 as a Diagnostic and Prognostic Biomarker:

- The excellent diagnostic performance of CoQ10 warrants validation in larger, independent, and diverse cohorts from different geographical regions and ethnicities to confirm its generalizability.
- Further research should explore CoQ10's utility as a prognostic marker, predicting the risk of progression from pre-diabetes to T2DM, or identifying individuals at higher risk of diabetes-related complications.

5.2.2.Investigating CoQ10's Therapeutic Potential:

- Given the clear depletion of CoQ10 in pre-diabetes and T2DM, interventional clinical trials evaluating CoQ10 supplementation are warranted. These trials should assess whether CoQ10 supplementation can prevent or delay the progression of pre-diabetes to T2DM, improve glycemic control, enhance lipid profiles, or reduce oxidative stress and inflammation in diabetic patients.

- Research into optimal CoQ10 dosing, formulation (e.g., ubiquinol vs. ubiquinone), and duration of supplementation for specific patient populations is also needed.

5.2.3.Deeper Exploration of Nrf2 Activity:

Since serum Nrf2 levels did not prove to be a useful biomarker, future studies should focus on intracellular Nrf2 activity within specific metabolically active tissues (e.g., liver, muscle, pancreatic beta cells, adipose tissue). This could involve measuring nuclear Nrf2 translocation, the expression of Nrf2 target genes, or specific Nrf2 pathway components. This approach would provide a more accurate reflection of its functional role in the context of diabetes.

Chapter Six

References

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Appendix

6.1.References

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6.2.Appendix

A.1.Appendix(1)

A.1.1.Patients and controls Questionnaire

1.Personal profile of the study population

Case Number ( )	Sampling Date : 202 / / Diagnosis date :	
Patient Name		**Married:** *Yes* ☐ *No* ☐
Age		**Gender:** *Female* ☐ *Male* ☐
Address		**Mobile Number:**
Hospital Name		
Occupation		

2.Medical information for patient

Risk factors and Diseases	
Diabete ☐	**Duration of DM2:**
kidney failure ☐	
Heart diseases ☐	
Hypertension ☐	
Hyperthyroidism ☐	

3. about the Medications or(and) Supplements you use

Medications	Yes	What year (or age) did you take this?	If you have stopped taking this, why?
glibesyn			
Metformin			
Sulfonylurea			
Insulin			
Thiazolidinediones			
Glycophage			

4.Anthropometric measurement:

Height : **(cm)**	**Weight :** **(kg)**
Body Mass Index :	

5- ABOUT YOUR HABITS

Do you exercise regularly	**Minutes per day :** **Days per week :**
Do you or have you ever smoked	**Packs per day :** **Number of years :**

A.2.Appendix (2)

BMI		P value (sg≤0.05)	
Groups	**M±SD**	**Sensitive**	**Resistance**
Control			
Sensitive			
Resistance			

A.3.Appendix (3)

FBS		P value (sg≤0.05)	
Groups	**M±SD**	**Sensitive**	**Resistance**
Control			
Sensitive			
Resistance			

A.4.Appendix (4)

Measurement of Serum Human Nuclear Factor Erythroid 2-related Factor 2 Concentration

USER INSTRUCTION

Cat.No. E3244Hu

Standard Curve Range 0.2-60ng/ml

Sensitivity 0.11ng/ml

Size 48T, 96T

Storage : Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1month. Avoid repeated thaw cycles.

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision. We measured random samples of E3244Hu within the same batch/lot to ensure the consistency of the kits' performances.

Intra-assay	Sample	n	Mean	Standard deviation	CV %
Intra-Assay	1	18	20.7	0.45	2.2
Intra-Assay	2	18	16.3	1.02	6.2
Intra-Assay	3	18	4.5	0.27	6.2

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100 Inter-Assay: CV< 10%

Intended Use

This Sandwich kit is for the accurate quantitative detection of Human Nuclear Factor Erythroid 2-Related Factor 2 (also known as NFE2L2) in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human NFE2L2 antibody. NFE2L2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human NFE2L2 Antibody is added and binds to NFE2L2 in the sample. Then Streptavidin-HRP is added and binds to the

Biotinylated NFE2L2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human NFE2L2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Provided

Components	Quantity (96T)	Quantity (48T)
Standard solution (80ng/ml)	0.5ml x1	0.5ml x1
Pre-coated ELISA plate	12 × 8 well strips x1	12 * 4 well strips x1
Standard diluent	3ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Stop solution	6ml x1	3ml x1
Substrate solution A	6ml x1	3ml x1
Substrate solution B	6ml x1	3ml x1
Wash buffer Concentrate (25x)	20ml x1	20ml x1
Biotinylated Human NFE2L2 antibody	1ml x1	1ml x1
User instruction	1	1
Plate sealer	2 pics	2 pics

Material Required but Not Supplied

- 37°C±0.5°C incubator Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- This instruction must be strictly followed in the experiment.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.

- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment. **Cell culture supernatant** Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment. **Tissue** Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant. Avoid freeze/thaw cycles. **Urine/Ascites/Cerebrospinal fluid** Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Note

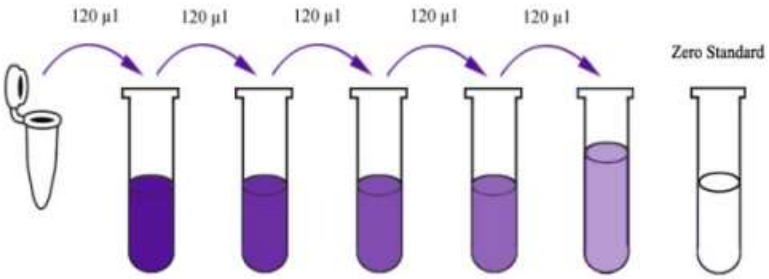
- Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for user's particular experiment.
- Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 3 months. Avoid repeated freeze thaw cycles.

- Samples should be brought to room temperature before starting the assay.
- Centrifuge to collect sample before use.
- Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).
- Collect the supernatants carefully. When sediments occurred during storage,centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

*All reagents should be brought to room temperature before use.

- **Standard** Reconstitute the 120ul of the standard (80ng/ml) with 120ul of standard diluent to generate a 40ng/mlstandard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (40ng/ml) 1:2 with standard diluent to produce 20ng/ml, 10ng/ml,5ng/ml and 2.5ng/ml solutions. Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

40ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
20ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
5ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
2.5ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml	2.5ng/ml

Figure (2-3) the reagents preparation for (NFE2L2) concentrations

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

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50ul standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40ul sample to sample wells and then add 10ul Human NFE2L2 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50ul Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

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

Typical Data

The standard curve of E3244Hu is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

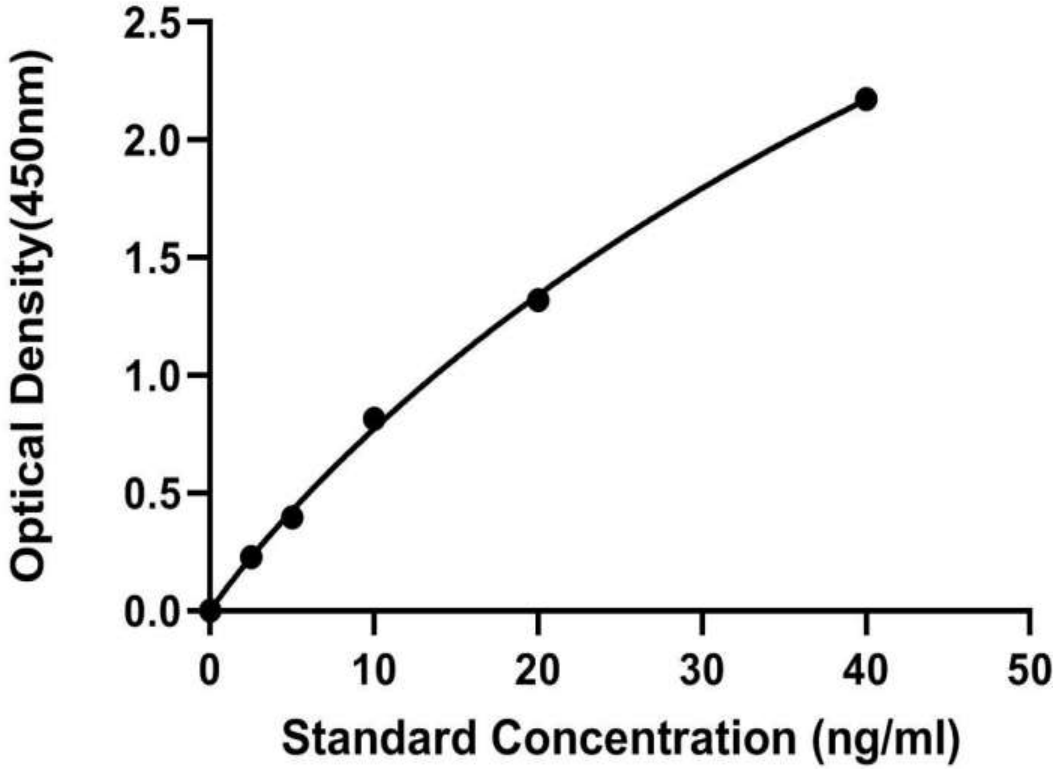


Figure (6-1) *the standard curve for(NFE2L2)concentrations*

Concentration	O.D	Average	Corrected
40ng/ml	2.255 / 2.186	2.22	2.174
20ng/ml	1.3 / 1.431	1.366	1.319
5ng/ml	0.403 / 0.482	0.442	0.396
2.5ng/ml	0.304 / 0.244	0.274	0.228
0ng/ml	0.039 / 0.053	0.046	0

A.5.Appendix (5)

Product Specification

Reference Standards

Cat No.	SC8390
Product Name	Coenzyme Q10
CAS No.	303-98-0
Storage	Store at -20 C°, 2years.(sealed storage,away from light)

Formula	$C_{59}H_{90}O_4$
Molecular Wt	863.34
Appearance	light yellow to yellow pawder
Purity	HPLC ≥ 98%
Solubility	≥ 10 mg/m L in chloroform

Note The substance is liquid pure product, please dissolve directly if necessary (See more information on www.solarbio.com)

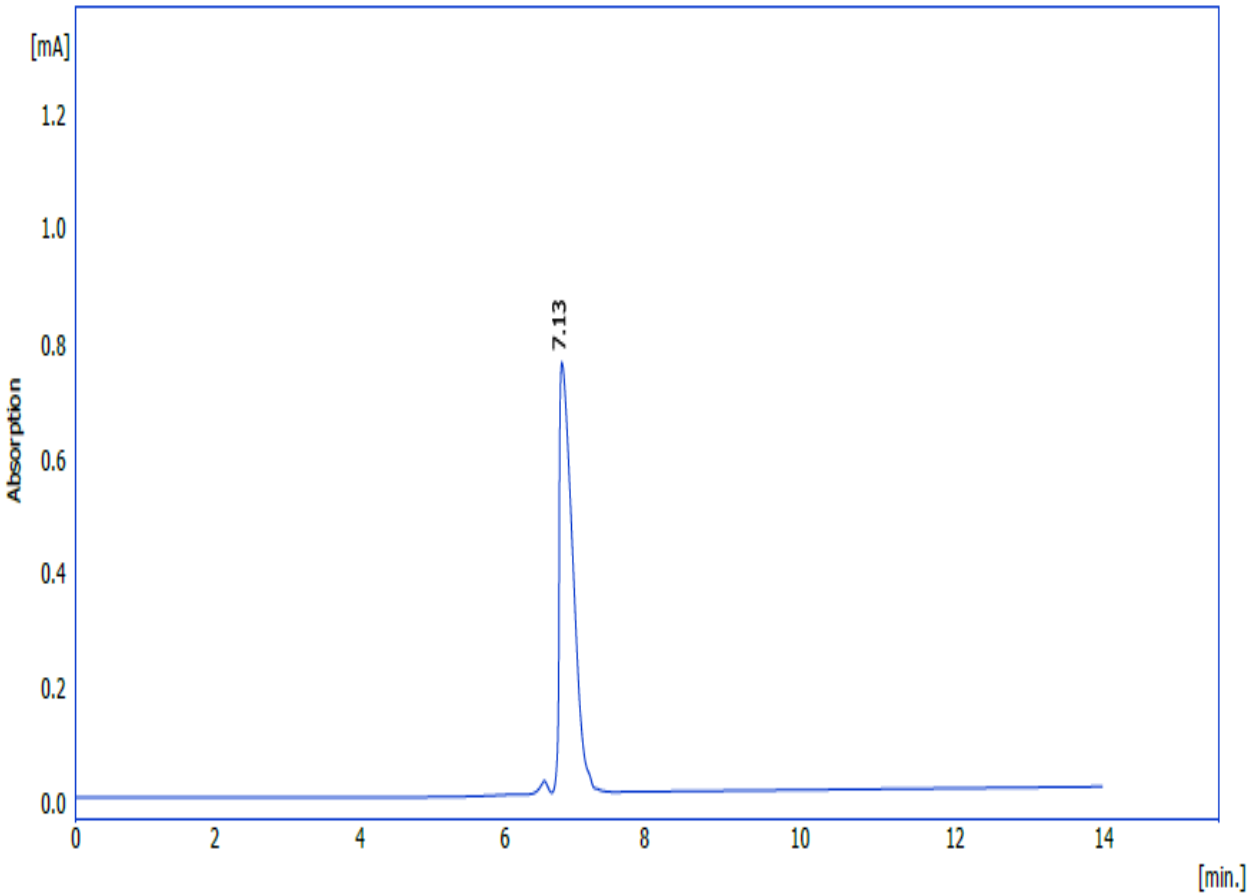
References welcome to follow our company scholarship program http://en.solarbio.com/lw.php

A.6.Appendix (6)

A.6.1. Figure showing the absorbance of the CoQ10 standard obtained by HPLC.

Sample Info:

Sample ID : CoQ10 (20 ppm)	Amount 0
Sample : CoQ10 (20 ppm)	ISTD Amount 0
Inj. Volume [mL] : 0.1	Dilution 1
Autostop : 20.00 min	External Start : Start - Restart, Down
Detector 1 : Detector 3	Range 1 : Bipolar, 2000 mAU, 10Sample. per Sec.
Subtraction Chromatogram : (None)	Matching : No Change



Result chromatography Table (Uncal - F:\ CoQ10 (20 ppm))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.13	1250.99	774.14	100.00	100.00	0.25	
	Total	1250.99	774.14	100.00	100.00		

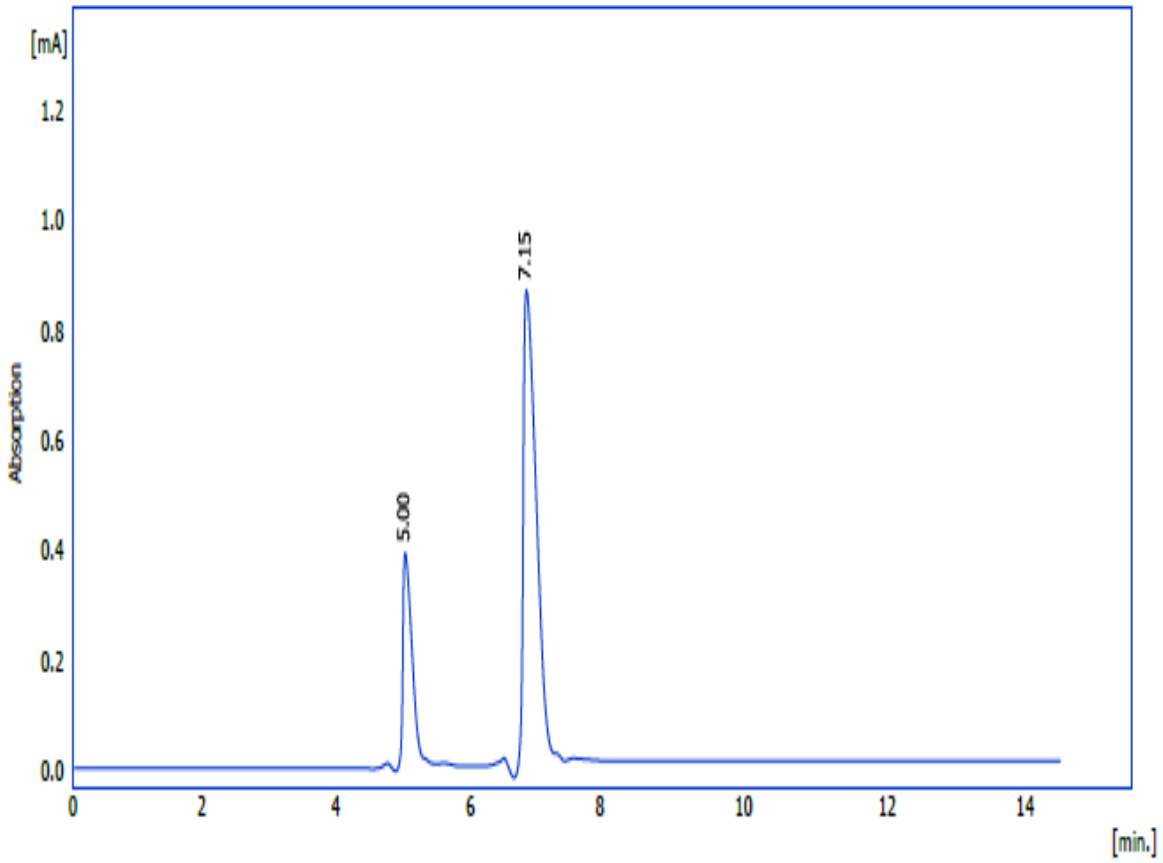
A.6.2. The following figures show the absorbance of CoQ10 obtained by HPLC for some randomly selected samples:

A.6.2.1.Sample control 1

Sample Info:

Sample ID	: con 1	Amount	0
Sample	: con 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ con 1)

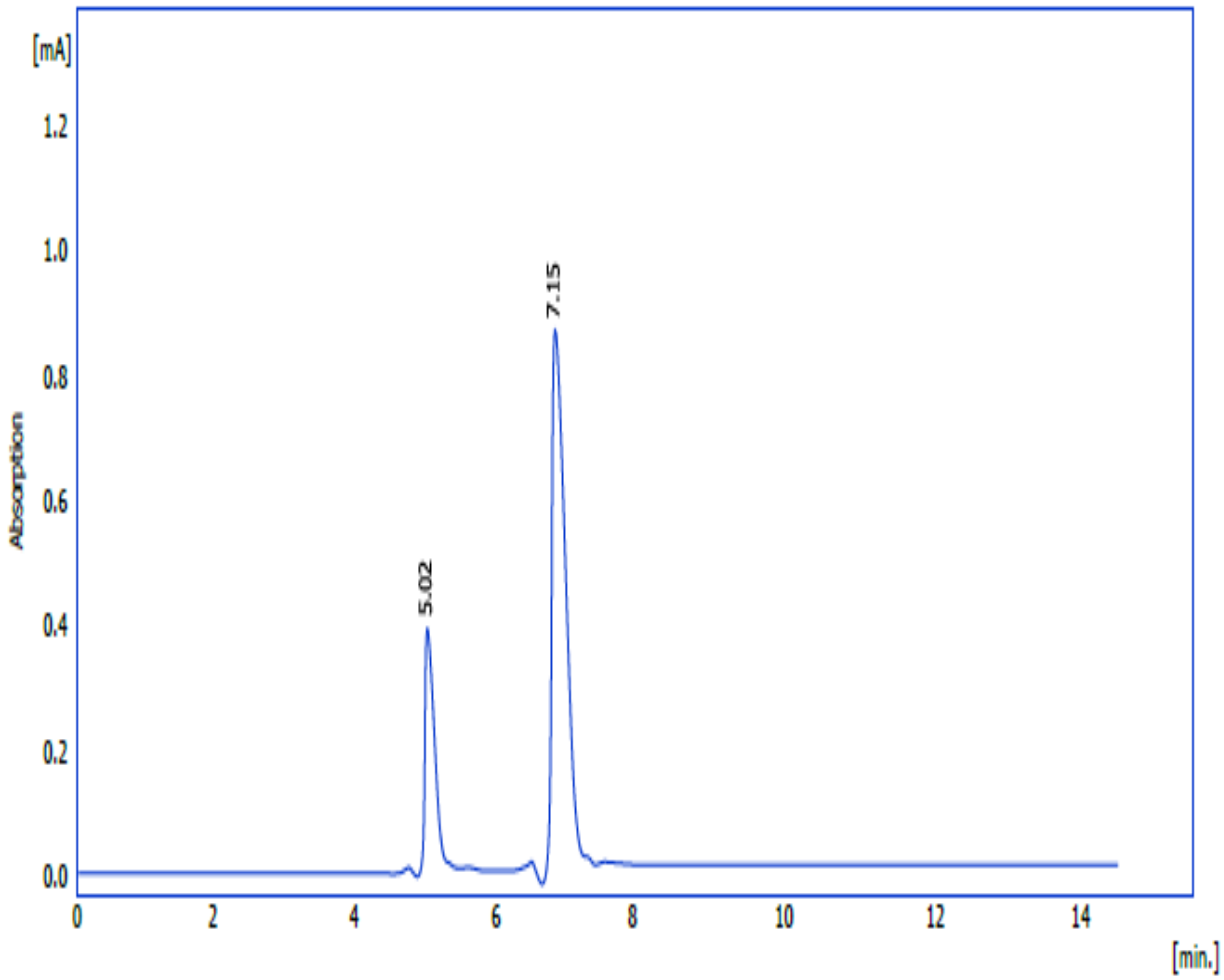
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.00	3256.90	375.80	20.00	20.00	0.05	
2	7.15	25652.09	820.00	80.00	80.00	0.015	
	Total	29809.99	1195.80	100.00	100.00		

A.6.2.2.Sample control 4

Sample Info:

Sample ID	: con 4	Amount	0
Sample	: con 4	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ con 4)

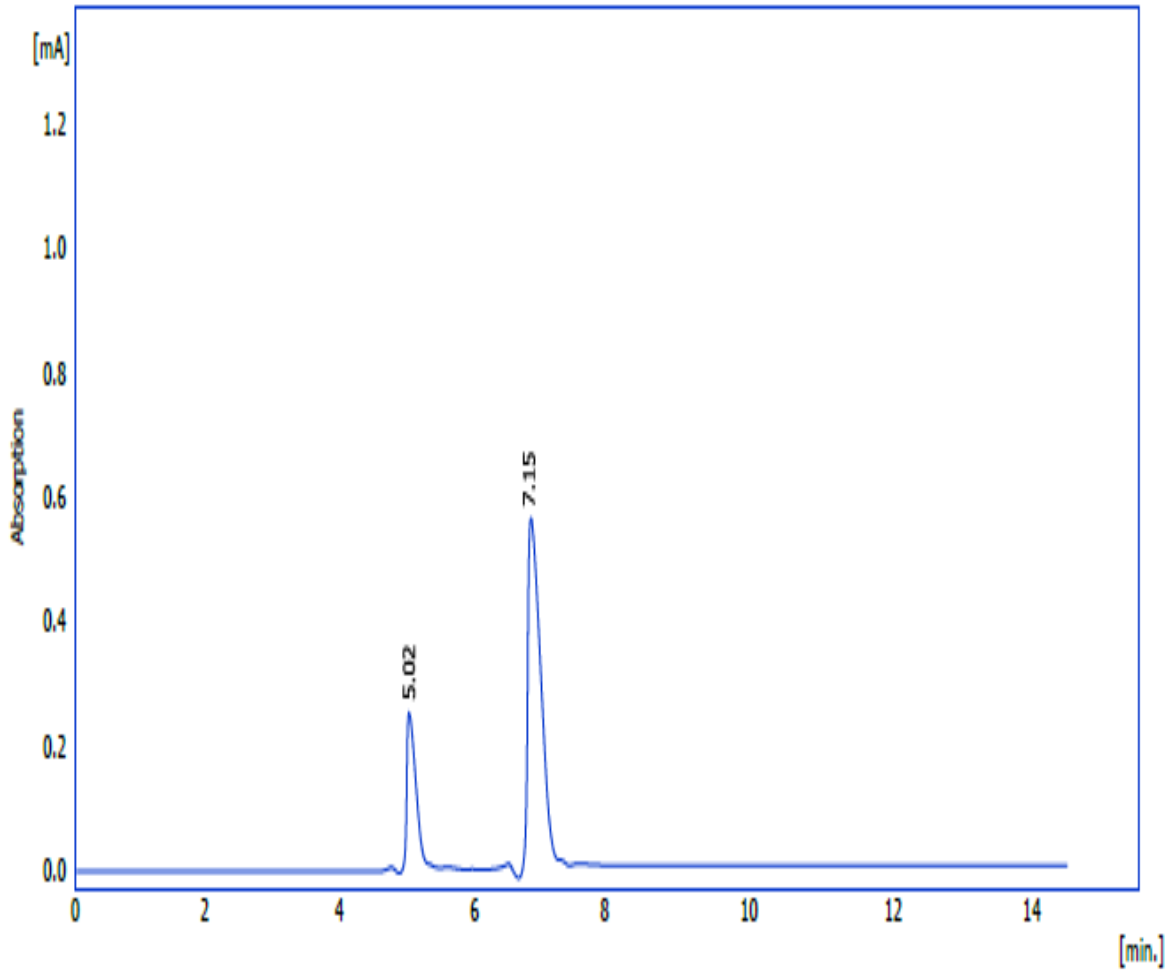
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.02	3365.00	373.11	20.00	20.00	0.05	
2	7.15	24252.01	822.22	80.00	80.00	0.015	
	Total	27617.01	1195.33	100.00	100.00		

A.6.2.3.Sample pre DM 1

Sample Info:

Sample ID	: pre DM 1	Amount	0
Sample	: pre DM 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ pre DM 1)

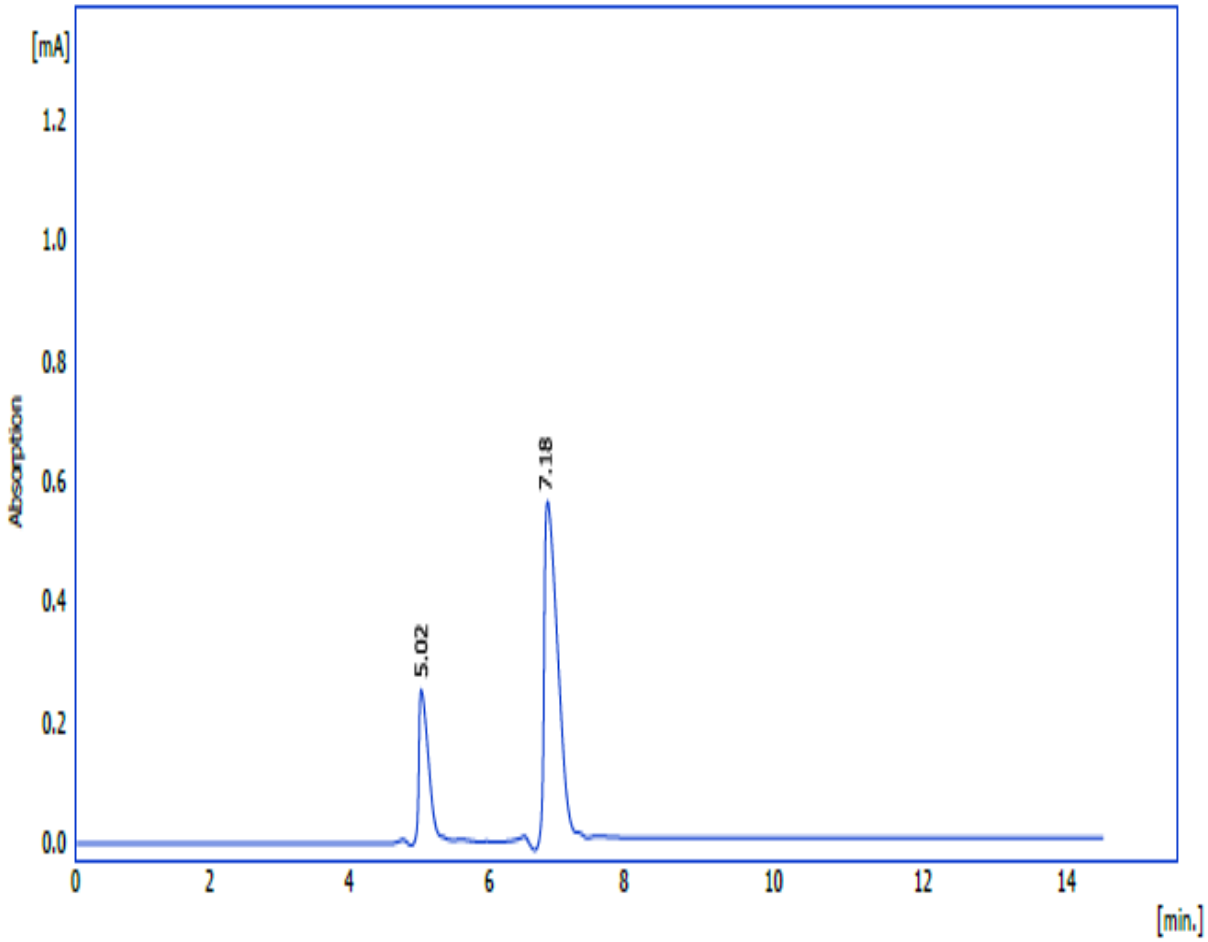
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.02	8521.11	220.12	30.00	30.00	0.05	
2	7.15	17451.23	571.11	70.00	70.00	0.15	
	Total	25792.34	791.23	100.00	100.00		

A.6.2.4.Sample pre DM 3

Sample Info:

Sample ID	: pre DM 3	Amount	0
Sample	: pre DM 3	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ pre DM 3)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.02	8160.11	220.11	30.00	30.00	0.05	
2	7.18	15998.07	573.11	70.00	70.00	0.15	
	Total	24158.18	793.22	100.00	100.00		

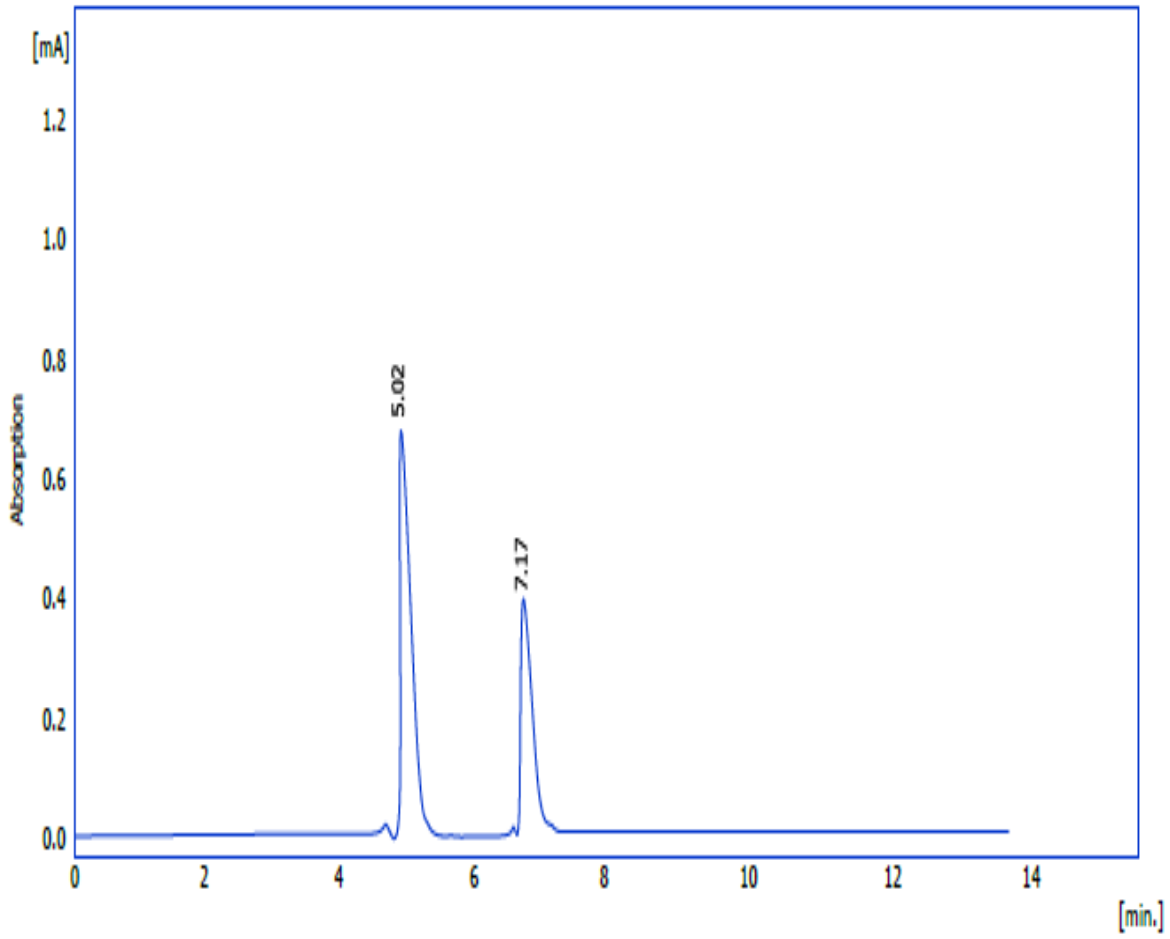
O

A.6.2.5.Sample DM 1

Sample Info:

Sample ID	: patient 1	Amount	0
Sample	: patient 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 1)

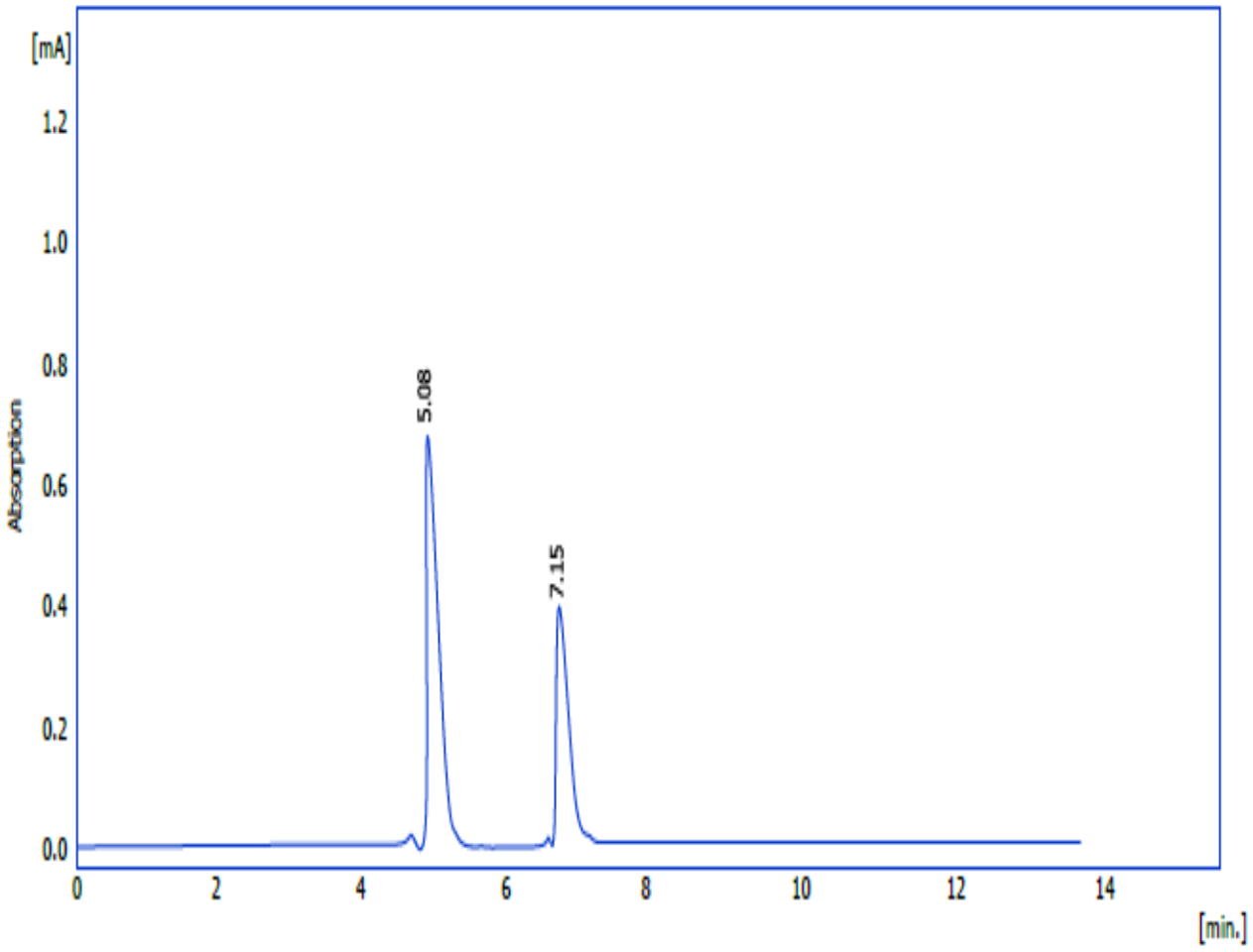
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.02	13250.66	620.11	60.00	60.00	0.15	
2	7.17	12120.14	389.07	40.00	40.00	0.08	
	Total	25370.80	1009.18	100.00	100.00		

A.6.2.6.Sample patient 3

Sample Info:

Sample ID	: patient 3	Amount	0
Sample	: patient 3	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 3)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.08	12652.14	622.12	60.00	60.00	0.15	
2	7.15	12114.08	383.22	40.00	40.00	0.08	
	Total	24766.19	1005.34	100.00	100.00		

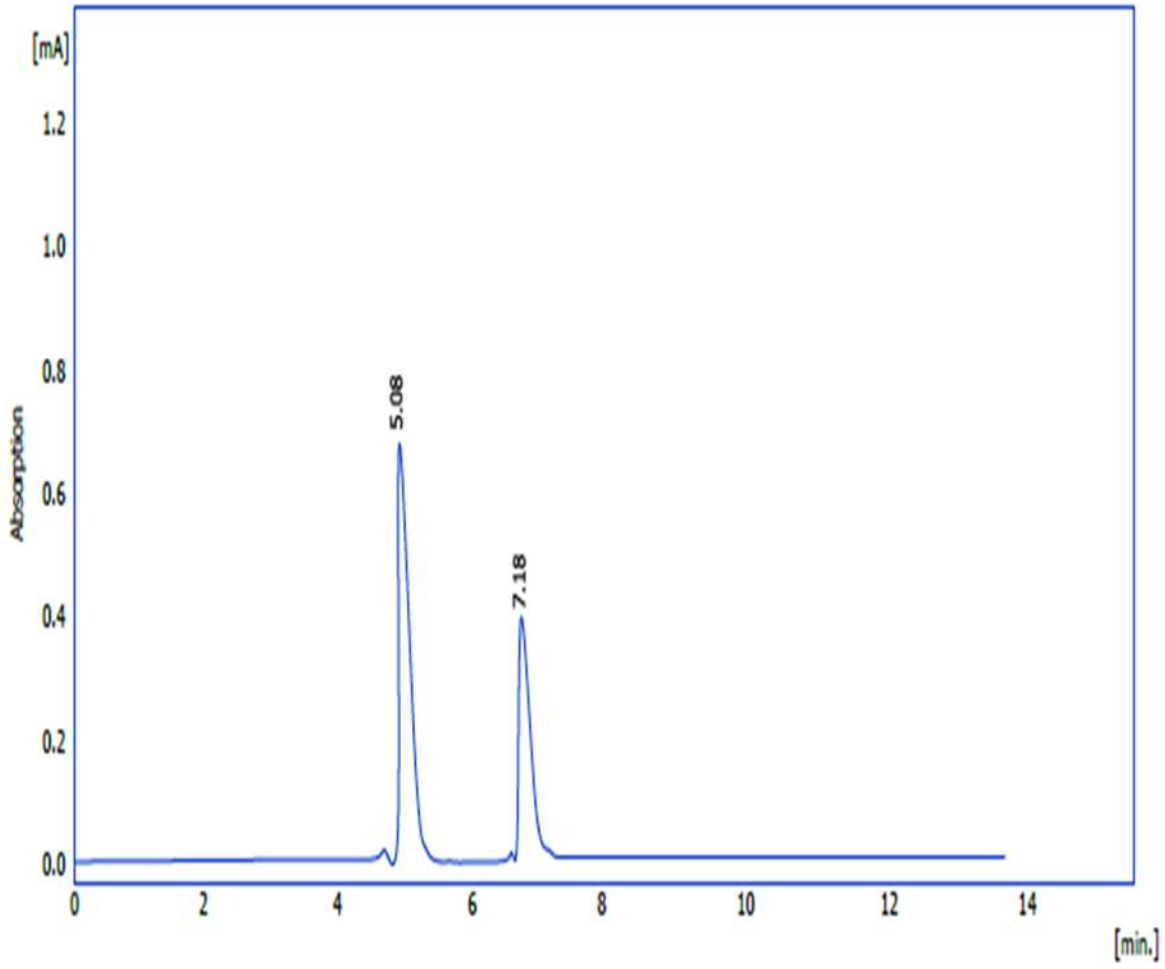
Q

A.6.2.7.Sample patient 6

Sample Info:

Sample ID	: patient 6	Amount	0
Sample	: patient 6	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 6)

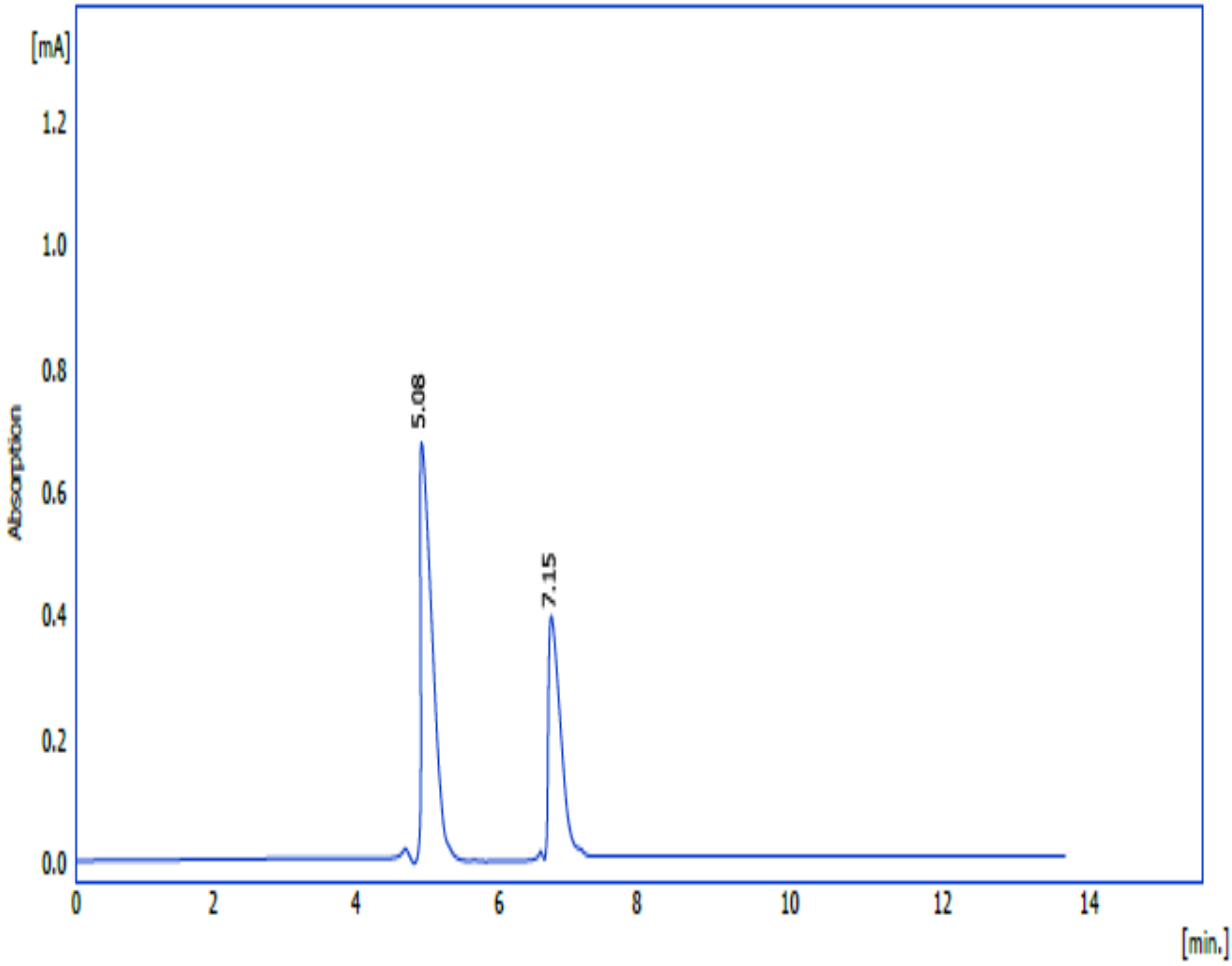
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.08	13265.90	625.14	60.00	60.00	0.15	
2	7.18	9211.41	382.25	40.00	40.00	0.08	
	Total	22477.31	1007.39	100.00	100.00		

A.6.2.8.Sample patient 10

Sample Info:

Sample ID	: patient 10	Amount	0
Sample	: patient 10	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Sample. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 10)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.08	13320.14	624.15	60.00	60.00	0.15	
2	7.15	9112.60	381.11	40.00	40.00	0.08	
	Total	22432.74	1005.26	100.00	100.00		

الخلاصة

يتميز داء السكري من النوع الثاني (T2DM) وسلائفه، ما قبل السكري، بإجهاد تأكسدي مزمن وخلل استقلابي. يُعدّ كلٌ من الإنزيم المساعد Q10 (CoQ10)، وهو مضاد أكسدة حيوي للميتوكوندريا، والعامل النووي المرتبط بالعامل 2 (Nrf2)، وهو منظم رئيسي لدفاعات مضادات الأكسدة، أساسيين في حماية الخلايا. هدفت هذه الدراسة إلى تقدير مستويات CoQ10 وNrf2 في مصل الدم لدى مرضى داء السكري من النوع الثاني، والأفراد المصابين بمقدمات السكري، وضوابط صحية، وتقييم إمكاناتهم التشخيصية. الطريقة: أُجريت دراسة حالة وشاهد في الفترة من سبتمبر 2024 إلى يوليو 2025 في العراق، في مركز الإمام الحسن للغدد الصماء والسكري ومستشفى الإمام الحسن المجتبى التعليمي في مدينة كربلاء. شملت هذه الدراسة 88 فردًا، منهم 50 مريضًا بداء السكري من النوع الثاني و32 متطوعًا كمجموعة ضابطة، مع معايير إدراج شملت مستوى طبيعيًا لسكر الدم أثناء الصيام ونتائج الهيموغلوبين A1c. جُمعت عينات الدم من كلٍ من مجموعتي الشاهد والمرضى بعد 12 ساعة من الصيام. جُمعت البيانات من خلال استبيان مُنظّم يشمل البيانات الاجتماعية والديموغرافية والتاريخ الطبي ونمط الحياة، مُكمّلًا بتقييمات سريرية أجراها أطباء متخصصون. قُيست لوحة الدهون في المصل باستخدام جهاز تحليل كيميائي أوتوماتيكي بالكامل (SMART-120، Geno TEK، الولايات المتحدة الأمريكية). استُخدم نظام إليزا للكشف عن مستوى Nrf2. قُيست مستويات Q10 كميًا باستخدام كروماتوغرافيا السائل عالية الأداء.

النتائج: تم التمييز جيدًا بين مجموعات الدراسة من خلال مستويات الهيموغلوبين السكري (HbA1c) ومستويات الدهون، حيث أظهرت مجموعات مرضى السكري وما قبل السكري أنماطًا نمطية لسكر الدم واضطراب شحميات الدم. أظهرت مستويات CoQ10 في المصل انخفاضًا تدريجيًا واضحًا وهامًا من مجموعة الضوابط الصحية (متوسط 1.6 وحدة/لتر) إلى مجموعة ما قبل السكري (متوسط 0.89 وحدة/لتر) وكانت الأدنى لدى مرضى السكري (متوسط 0.38 وحدة/لتر). في المقابل، كانت مستويات Nrf2 في المصل متشابهة بشكل ملحوظ ومتداخلة إلى حد كبير في جميع المجموعات الثلاث (المتوسطات: 26 مجموعة صحية، 30 مجموعة ما قبل السكري، 29 مجموعة داء السكري)، مما يشير إلى عدم وجود فرق كبير. أظهر تحليل منحنى ROC دقة تشخيصية ممتازة لـ CoQ10: مساحة تحت المنحنى (AUC) تبلغ 0.9724 (قيمة الاحتمال <0.0001) لتمييز مرضى السكري عن مجموعة الضوابط الصحية، مع حد فاصل مثالي يبلغ <1.275 وحدة/لتر. لتحديد الأفراد المصابين بمرحلة ما قبل السكري، أظهر CoQ10 مساحة تحت المنحنى (AUC) قدرها 0.9013 (قيمة الاحتمال <0.0001)، مع حد فاصل مثالي قدره <1.120 وحدة/لتر. مع ذلك، أظهر Nrf2 فائدة تشخيصية ضعيفة (مساحة تحت المنحنى <0.63، قيمة الاحتمال > 0.05). كشف تحليل الارتباط عن وجود ارتباط إيجابي كبير بين CoQ10 وNrf2 في كل من مجموعتي السكري (r=0.4، قيمة الاحتمال = 0.05) وما قبل السكري (r=0.65، قيمة الاحتمال = 0.001). والجدير بالذكر أن CoQ10 ارتبط ارتباطًا سلبيًا قويًا بمستوى الهيموغلوبين السكري (HbA1c) في مجموعة ما قبل السكري (r=-0.920، قيمة الاحتمال = 0.027)، وارتبط ارتباطًا إيجابيًا بمستوى البروتين الدهني عالي الكثافة (HDL) في مجموعة السكري (r=0.332، قيمة الاحتمال = 0.005).

الاستنتاج: تنخفض مستويات CoQ10 في المصل تدريجيًا مع تقدم الاضطراب الأيضي، وتُعدّ مؤشرًا حيويًا دقيقًا للغاية لتشخيص كلٍّ من مرحلة ما قبل السكري وداء السكري من النوع الثاني. يُبرز ارتباطه القوي بضبط نسبة السكر في الدم ومستويات الدهون دوره الأساسي في الصحة الأيضية. في المقابل، لا يبدو أن مستويات Nrf2 في الدم تُمثّل مؤشرًا تشخيصيًا موثوقًا به لهذه الحالات. تُؤكد هذه النتائج على إمكانات CoQ10 كأداة قيّمة غير جراحية للفحص والمراقبة في سياق ضعف استقلاب الجلوكوز

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

تقدير مستويات الانزيم المساعد (Co Q10) والعامل النووي المرتبط بكريات الدم الحمراء 2(Nrf2) في المرضى المصابين بداء السكري من النوع الثاني

رسالة مقدمة الى
جامعة كربلاء - فرع الكيمياء والكيمياء الحياتية - مجلس كلية الطب
كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل
حيدر عبدالحسين طاهر
بكالوريوس علوم كيمياء / جامعة كربلاء /2015

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كلية الطب / جامعة كربلاء

2025 م 1447 هـ