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Estimation of Antioxidant Status and Oxidative Stress in Diabetic Retinopathy patients

This Thesis is Submitted in Partial Fulfillment of the Requirements for
the Master's Degree of Sciences in Chemistry.

By

Yusra Ali Hameed

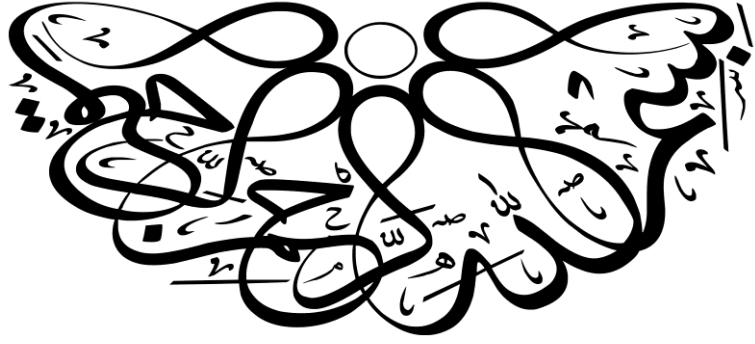
B. Sc. 2006

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

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Dedication

To the ideal in my life and mountain on which it was based..... ***My dear Father***

To who paradise is under her feet and the source of tenderness..... ***My dear Mother***

To the father and mother who supported me ***my Father and my Mother in law***

To Prince of my life and the source of my strength..... ***My dear Husband Mushtaq***

To the pulse of my heart and the light of my eyes ***My dear daughters Fatima and Zainab***

To those who supported me and encouraged me ***My Brothers and Sister***

I give this humble work

Yusra

2020

Acknowledgement

In the name of Allah, the most merciful and most gracious. First of all I thank my God, Allah, for inspiring me with strength, patience and willingness to perform this research.

First, I would like to express my sincere gratitude to my supervisor **Prof. Dr. Jawad Kadhun Muraih**. Was more generous in giving their time, encouragement, support, and advice. I thank Professor Jawad for his incredible patience for always keeping the door to his office open for questions, and for his continual support.

Regards and thanks must be introduced to **Dr. Khawla Kani Jassim** (Head of Chemistry Department). I would like to thank the all staff members of the Department of Chemistry at, College of Science in Al-Muthanna University, for their valuable help and device.

Thanks and gratitude to my dear brother in law **Dr. Mohammed Talib Abed Al-Jubory** /College of Nursing, Babylon University . For all the assistance, advice and guidance provided to me to complete this research.

I would like to thank all the patients who have cooperated with me and also the patients' families. I wish God to write to them healing, health and wellness.

I extend my sincere thanks to the staff of the laboratory division in each of the Diabetes Specialist Center and the biochemistry unit at Al-Hussein Teaching Hospital in Al-Muthanna province.

Special thanks for the dearest persons to me my father, mother, my husband, my brothers and my sister for their support and encouragements. Also, I cannot forget my friends and colleagues, I am very grateful to those who participated and helped me to complete this study.

Yusra

Table of content

Contents		Pages No.
List of tables		vii
List of figures		x
List of abbreviations		xi
Abstract		xvi
Chapter One		
1	Introduction and literature review	1
1.1	Introduction	1
1.2	Literature Review	5
1.2.1.	Diabetes mellitus	5
1.2.1.1	Classification of diabetes mellitus	6
1.2.1.1.1	Type 1 diabetes mellitus	6
1.2.1.1.2	Type 2 diabetes mellitus	6
1.2.1.1.3	Gestational diabetes mellitus	8
1.2.1.1.4	Other types of diabetes mellitus	8
1.2.1.2	Diagnosis of diabetes mellitus	8
1.2.1.3	Global prevalence of diabetes mellitus	9
1.2.1.4	Complications of diabetes mellitus	10
1.2.1.5	Pathogenesis type 2 diabetes mellitus	10
1.2.1.6	Aetiological factors underlying diabetes mellitus	11
1.2.1.7	Most significant symptoms of diabetes mellitus	12
1.2.2	Diabetic retinopathy	12
1.2.2.1	Diabetic retinopathy stages	12

1.2.2.2	Risk factor for diabetic retinopathy	13
1.2.2.3	Pathogenesis of diabetic retinopathy	14
1.2.3	Type 2 diabetes and oxidative stress:	15
1.2.3.1	Oxidative stress	15
1.2.3.2	Oxidative stress in type 2 diabetes mellitus	15
1.2.3.3	Free radicals	17
1.2.3.4	Reactive oxygen species (ROS)	18
1.2.3.4.1	Mechanisms of production of ROS	19
1.2.3.4.2	Mechanisms of generation of ROS in type 2 diabetes mellitus	20
1.2.4	Antioxidants	22
1.2.4.1	Classification of antioxidants	23
1.2.5	Lipid peroxidation	24
1.2.6	Superoxide dismutase (SOD)	26
1.2.7	Total antioxidant capacity	27
1.2.8	Glycated hemoglobin (HbA1c)	28
1.2.9	Dyslipidemia or hyperlipidemia	29
1.2.10	Uric acid in diabetes mellitus	30
1.3	Aims of the study	31
Chapter Two		
2.0	Materials and Method	33
2.1	Subjects	33

2.2	Inclusion criteria	33
2.3	Exclusion criteria	33
2.4	Instruments and tools	34
2.5	Materials	34
2.6	Methods and procedures	35
2.6.1	Measurement of BMI	35
2.6.2	Measurement of blood pressure	36
2.6.3	Blood samples collection	36
2.7	Metabolic biochemical marker for investigation	36
2.7.1	Estimation of plasma sugar	36
2.7.2	Determination of HbA1c	37
2.7.3	Measurement of total cholesterol	38
2.7.4	Measurement of Triglycerides	40
2.7.5	Measurement of HDLc	41
2.7.6	Very Low density lipoprotein (VLDLc) estimation	43
2.7.7	Low density lipoprotein (LDLc) estimation	43
2.7.8	Measurement of uric acid	43
2.8	Measurement oxidative stress markers for investigation	45
2.8.1	Measurement of malondialdehyde	45
2.8.2	Measurement of superoxide dismutase	47

2.8.3	Measurement of total antioxidant capacity	50
2.9	Statistical analysis	52
Chapter Three		
3.0	Results and Discussion	54
3.1.	Anthropometric parameters	54
3.1.1	Some anthropometric parameters	54
3.2.	Biochemical parameters	56
3.2.1.	FBS and HbA1c	56
3.2.2.	Lipid profile	57
3.2.3	Oxidative stress parameters	60
3.3	Distribution studied groups according to age	63
3.3.1	Comparison of study groups among age groups for biochemical parameters	63
3.3.1.1	FPG and HbA1c means among age groups	63
3.3.1.2	Lipid parameters means among age groups	64
3.3.1.3	Oxidative stress parameters means among age groups	65
3.4	Distribution studied groups according to BMI	66
3.4.1	Comparison of biochemical parameters among BMI groups	67
3.4.1.1	FPG and HbA1c means among BMI groups	67
3.4.1.2	Lipid parameters means among BMI groups	68
3.4.1.3	Oxidative stress parameters means among BMI groups	69
3.5	Study of diabetic groups based on the diabetic duration	70

3.5.1.	Comparison of biochemical parameters among diabetic duration	70
3.5.1.1	FPG and HbA1c means among diabetic duration	70
3.5.1.2	Lipid parameters means among diabetic duration	71
3.5.1.3	Oxidative stress parameters among diabetic duration	72
3.6	Distribution of the studied groups according to smoking	73
3.6.1	Effect of smoking on biochemical parameters	73
3.6.1.1	Effect of smoking on sugar parameters	73
3.6.1.2	Effect of smoking on lipid parameters	74
3.6.1.3	Effect of smoking on oxidative stress parameters	75
3.7	Distribution studied groups according to location	76
3.7.1	Effect of residence of patient on biochemical parameters	76
3.7.1.1	Effect of residence of patient on FPG and HbA1c	76
3.7.1.2	Effect of residence of patient on lipid parameters	77
3.7.1.3	Effect of residence on oxidative stress parameters	78
3.8	Distribution according to blood pressure	79
3.8.1	Effect of blood pressure biochemical parameters	79
3.8.1.1	Effect of blood pressure on FPG and HbA1c	79
3.8.1.2	Effect of blood pressure on lipid profile	80
3.8.1.3	Effect of blood pressure on oxidative stress parameters	81
3.9	Distribution based on the gender	82
3.9.1	Comparison of biochemical parameters between male	83

	and female	
3.9.1.1	Sugar parameters means between male and female	83
3.9.1.2	Lipid parameters means between male and female	84
3.9.1.3	Oxidative stress parameters between male and female	85
Chapter four		
4.0	Conclusion and Recommendation	87
4.1	Conclusion	87
4.2	Recommendation	87
Reference		
	Reference	89
Appendix		
Appendix 1	Questionnaire of the study	110
Appendix 2	Normal values for lab tests	112

List of Tables

Table	Title of table	Page No.
1.1	Main reactive oxygen species (ROS)	17
1.2	Main reactive nitrogen species (RNS)	18
2.1	Tools that were used in the current study	34
2.2	Instruments that were used in the current study	34
2.3	Materials that were used in the current study	35
2.4	Classification of body mass index	35
2.5	Kit components of plasma sugar.	37
2.6	Assay procedure of plasma sugar	37
2.7	Kit Components of total cholesterol	39
2.8	Assay procedure of total cholesterol	39
2.9	Kit Components of Triglycerides	41
2.10	Assay procedure of Triglycerides	41
2.11	Kit Components of HDL-cholesterol	42
2.12	Assay procedure of HDL-cholesterol	43
2.13	Kit Components of uric acid	44
2.14	Assay procedure of uric acid	44
2.15	Stander prepare of Malondialdehyde	46
2.16	Standard dilution of SOD	48
2.17	Work reagent of SOD	49
2.18	Standard prepare of TAC	50
2.19	Working reagent of TAC	51

3.1	Comparison of anthropometric parameters means among all study groups	55
3.2	Comparison of FPG and HbA1c means	56
3.3	Comparison of lipid profile parameters means	58
3.4	Comparison of oxidative stress parameters means	60
3.5	Comparison of FPG and HbA1c means among age	64
3.6	Comparison of lipid parameters means among age	65
3.7	Comparison of oxidative stress parameters means among age groups	66
3.8	Comparison of FPG and HbA1c means among BMI groups	67
3.9	Comparison of lipid parameters means among BMI groups	68
3.10	Comparison of oxidative stress parameters means among BMI groups	69
3.11	Comparison of sugar parameters means among diabetic duration groups	70
3.12	Comparison of lipid parameters means among diabetic duration	71
3.13	Comparison of oxidative stress parameters means among diabetic duration	72
3.14	Effect of smoking on FPG and HbA1c means	73
3.15	Effect of smoking on lipid parameters means	74
3.16	Effect of smoking on oxidative stress parameters	75
3.17	Effect of residenc of patient on FPG and HbA1c	77

3.18	Effect of residence of patient on lipid parameter	78
3.19	Effect of residence of patients on oxidative stress parameters	79
3.20	Effect of blood pressure of patient on FPG and HbA1c	80
3.21	Effect of blood pressure of patient on lipid parameters	81
3.22	Effect of blood pressure of patients on oxidative stress parameters	82
3.23	Comparison of FPG and HbA1c means between male and female	83
3.24	Comparison of lipid parameter means between male and female patients	84
3.25	Comparison of an oxidative stress according to gender	85

List of Figures

Figure	Title of figure	Pages No.
1.1	Chemical reaction involved in glycation of hemoglobin	29
2.1	Reaction malondialdehyde with thiobarbituric acid	45
2.2	The slope of the standard curve of MDA	47
2.3	Reaction between superoxide with WST-1 dye	48
2.4	Standard curve of superoxide dismutase	49
2.5	The slope of the standard curve of TAC	51
3.1	Comparison of FBS means among study groups	57
3.2	Comparison of lipid parameters means among study groups	59
3.3	Comparison of oxidative stress parameters means among study groups	62

List of Abbreviations

Symbols	Meaning
3DG	3-deoxyglucosone
4-AAP	4-aminoantipyrine
ADA	American Diabetes Association
ADP	Adenosine 5'-diphosphate
AGE _s	Advanced glycation end-products
APO	Apolipoproteins
AR	Aldose reductase
ATP	Adenosine-5- triphosphate
BMI	Body mass index
CHD	Coronary heart disease
CRD	Completely randomized design
DAG	Diacylglycerol
DAP	Dihydroxyacetone phosphate
DBP	Diastolic blood pressure
DCCT	Diabetes Control and Complications Trial
dH ₂ O	Distal water
DM	Diabetes mellitus
DMC	Diabetes mellitus completion
DME	Diabetic macular edema
DNA	Deoxyribonucleic acid
DR	Diabetic Retinopathy

EDTA	Ethylene diamine tetra acetic acid
ERK	Extracellular-signal-regulated kinase
ESTP	Ethyl-sulphopropyl toluidine
F(1,6)-P	Fructose-1,6-di
F-6-P	Fructose-6-phosphate
FA	Fatty acid
FADH	Flavin adenine dinucleotide (semiquinone form)
FADH ₂	Flavin adenine dinucleotide (hydroquinone form)
FFA	Free Fatty acid
FPG	Fasting plasma glucose
G-1-P	Glycerol-1-phosphate
G-3-P	Glycerol-3-phosphate
G-6-P	Glycerol-6-phosphate
GDM	Gestational diabetes mellitus
GFA	Glutamine Fructose-6-Phosphate amidotransferase
GK	Glycerol kinase
GO	Glucose Oxidase
GPO	Glycerophosphate oxidase
GSH	Glutathione
GST	Glutathione S-transferases
H ₂ O ₂	Hydrogen peroxide
Hb _{A1c}	Glycated hemoglobin
HBP	Hexosamine biosynthesis pathway
HDL _c	High density lipoprotein cholesterol

IEC	International Expert Committee
IFCC	International Federation of Clinical Chemistry
LADA	Latent autoimmune diabetes in adult
LDL _c	Low density lipoprotein cholesterol
LPL	Lipoprotein lipase
MAPK	Mitogen activated protein kinase
MDA	Malondialdehyde
MG	Methylglyoxal
MODY	Maturity-onset diabetes of the young
NAD ⁺	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP ⁺	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Reduced Nicotinamide adenine dinucleotide phosphate
NEG	Non-enzymatic glycation
NGSP	National Glycohemoglobin Standardization Program
NO	Nitric Oxide
NOX	NADPH oxidases
OGTT	Oral glucose tolerance test
OS	Oxidative stress
PKC	Protein Kinase C
POD	Peroxidase
PPP	Pentose phosphate pathway
PUFA	Poly unsaturated fatty acids
RAGE	Receptor of advanced-glycation end-products

RBC _s	Red blood cells
RCS	Reactive chlorine species
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ROS / RNS	Reactive oxygen / Nitrogen species
SBP	Systolic blood pressure
SEM	Standard error of mean
SI units	International system of units
SOD	Superoxide dismutase
SPSS	Statistical program social science
SUA	Serum uric acid
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TC	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglycerides
TOPS	N-Ethyl-N(Sulphopropyl)-3-Methyl Aniline
UDP-GLcNAc	Uridine diphosphate N-acetyl-glucosamine
VLDL _c	Very low density lipoprotein cholesterol
WHO	World Health Organization

WST-1	Water-Soluble Tetrazolium salt
XO	Xanthine oxidase

ABSTRACT

The area of the present research is the relationship between oxidative stress and type 2 diabetes mellitus with/without diabetic retinopathy. Oxidative stress is the imbalance between reactive oxygen species production and the antioxidant scavenging that may play a role in the etiology of type 2 diabetes mellitus and occurrence of diabetic retinopathy resulting from increased activity of free radicals and accumulation of lipid peroxidation products. Subjects is 232 divided into 96 control, 78 subjects have type 2 diabetes mellitus without diabetic retinopathy, and 58 subjects have diabetic retinopathy. The age range of all subjects is more than 21 years. They were recruited from urban and rural areas of Al-Muthanna governorate. Sociodemographic data was collected from the subjects. Anthropometric measurements including systolic and diastolic blood pressure, weight, and height. Blood samples were collected after 12 hours of overnight fasting for the analysis of glucose, HbA1c, lipids profile, oxidative stress indices and other biochemical parameters. In this study, it was found that plasma antioxidant levels were higher in the diabetic subjects than the patients with retinopathy while malondialdehyde levels were found to be higher in the retinopathy subjects as compared to the diabetic subjects. Smoking, BMI, blood pressure, and age play important impact on oxidative stress levels of patients and its complications. Oxidative parameters values are not affected by residence. There is a strong correlation between the duration of the disease and disease progression to diabetic retinopathy incidence, where it was in diabetic retinopathy higher than diabetic patients without retinopathy. The present study recommend periodic medical checkup and suitable life style change, weight loss also to control dyslipidemia and reduce type 2 diabetic complications.

Treatment with antioxidants such as vitamins which might support endogenous antioxidant capacity and reduce peroxidation rates to prevent complications.

Chapter one

Introduction

&

Literature Review

1. Introduction and Literature Review**1.1 Introduction**

Diabetes mellitus (DM) describes a heterogeneous metabolic diseases of multiple a etiology characterized by chronic hyperglycemia resulting from flaws in insulin secretion, insulin action, or both [1- 3]. The chronic hyperglycemia of diabetes mellitus is associated with long-term damage, dysfunction and insufficiency of different organs, especially the kidneys, eyes, heart, blood vessels and nerves [2]. It may be present with characteristics symptoms include polydipsia, polyuria, polyphagia, blurred vision and weight loss. DM is one of the common non communicable diseases worldwide. DM was classified into following types: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM) [2, 3].

T1DM refers to a condition in which there is chronic progressive destruction of beta cell. T1DM is a chronic autoimmune disease that characterized increased blood glucose levels, due to the insulin deficiency that occurs as the result of the loss of β -cells islet [1, 4]. Type 1 is one of the most common endocrine and metabolic disorders occurring in children and adolescents. The loss of β -cells is the result T1DM related autoimmunity. This type has a strong genetic component [4, 5]. The most common type of diabetes mellitus is type 2 diabetes mellitus (non-insulin dependent diabetes). T2DM and obesity are associated with insulin resistance. T2DM is caused by weak biological response to insulin. In the early stages, β - cells respond to the insulin resistance by secreting increased amounts of insulin to maintaining normal levels of blood sugar. But gradually the β -cells start to fail and decrease the levels of insulin, which leads to rising of blood sugar levels [1, 6]. GDM there is any degree of glucose intolerance with the onset during pregnancy

especially. Its diagnosis in the second or third trimester of pregnancy, which increases worldwide [3, 7].

The global number of individuals with diabetes in 2000 was estimated to be 171 million (2.8% of the world's population). The number of people with DM worldwide is projected to increase to 366 million by 2030, 298 million of whom will live in developing countries [3, 8]. There is a relationship between chronic hyperglycemia and long-term complications in diabetes. The long-term effects of diabetes include progressive evolution of micro-vascular complications, particularly in the eye and the kidney and an increased frequency of macro-vascular disease such as peripheral vascular and coronary heart disease [3, 9]. Complications of micro-vascular and macro-vascular are the leading cause of morbidity and mortality in diabetics. However, diabetic patients often die from macro-vascular disease and main mortality is the coronary heart disease (CHD). Long-term complications of DM include retinopathy with potential vision loss [3, 9, 10].

Diabetic retinopathy (DR) is the most common disease that caused by micro-vascular complication of diabetes, occurs in both T1,T2 DM and is secondary to prolonged uncontrolled hyperglycemia and other risk factors. In adolescents and young adults with type 1 diabetes, over 80% have some form of DR when the duration of diabetes is over 15 years. It is estimated that 90% of the blindness due to DR is preventable [4, 11]. Long term of high blood glucose levels in uncontrolled diabetes can be the cause of glucose auto-oxidation, nonenzymatic protein glycation and activation of the polyol pathway with increase oxidative stress.

Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant ratio in favor of the former, potentially leading to form macromolecules and dysfunction in the cell [12]. Improved oxidative stress contributes to the damage of pancreatic β -

cell progressively due to glucose toxicity, which leads to severe weakness of glucose-stimulated insulin secretion [13].

In normal physiological conditions, there is a balance in the generation of reactive oxygen and nitrogen species (ROS, RNS) and antioxidant defense system to protect organisms against ROS, RNS toxicity. In diabetes, imbalance in the pro-oxidant/antioxidant can damage cellular macromolecules, leading to protein modification and lipid peroxidation [14]. Lipid peroxidation is an autocatalytic free radical mediated destructive process where poly unsaturated fatty acids in cell membranes degraded to form lipid hydro peroxides. Through lipid peroxide products such as conjugated dienes and malondialdehyde (MDA) are increased in patients with T2DM. MDA is produced as a relatively stable end product from the oxidative degradation of polyunsaturated fatty acids (PUFA), and this free radical-driven lipid peroxidation has been causatively implicated in the aging process atherosclerosis, Alzheimer's disease and cancer [2, 15]. Serum MDA used as a bio-marker of lipid peroxidation and served as an indicator of free radical damage [16]. Hyperlipidemia has also been reported as one of the factors causing increased lipid peroxidation in diabetes mellitus. The lipid peroxidation pathways promote tissue degeneration and cause many complex syndromes in diabetics such as cataracts, renal failure, nerve damage and atherosclerosis [2, 12]. Although the mechanisms underlying the pathogenesis of T2DM still under study, oxidative stress has been shown to be responsible, at least in part, for the development of T2DM and its complications [17]. This supported by increased oxidative and weak antioxidants in these patients, as well as the use of some antioxidant nutrients as aid or supplements in the managing T2DM. Apart from lipid peroxidation used as a marker for oxidative

stress, there are variety of natural antioxidants to scavenge ROS and prevent oxidative damage to biological membranes [18].

An antioxidants defined as any substances, exogenous or endogenous delaying or preventing oxidative damage to a target molecule and protecting biologically important molecules such as DNA, proteins and lipids from oxidative damage and thus reduce the risk of many chronic diseases [19]. Hyperglycemia impairs the endogenous antioxidant defense mechanism in several ways during diabetes mellitus [2, 20]. People with diabetes do not have enough antioxidants defenses, but, in contrast, too much of the free radical induced damaged. One group of these antioxidants is intracellular enzymatic action , which include superoxide dismutase (SOD), antioxidant enzymes in blood cited as markers for vascular injury in T2DM [21]. Total antioxidant capacity (TAC) is a biomarker often used to investigate oxidative stress in several pathological conditions. (TAC) defined as the moles of oxidants neutralized by one liter of solution, is a biomarker measuring the antioxidant potential of body fluids [22].

The study of the oxidative stress status may be the knowledge base for understanding of the pathogenic mechanisms of complications in diabetes and may have important implications for antioxidant supplements in order to slow progress, choose optimal treatments, prevent complications and their consequences.

1.2 Literature Review**1.2.1 Diabetes Mellitus**

DM is the most common metabolic disorder , etiopathology being multi-factorial, is characterized by chronic hyperglycemia that results from defects in insulin secretion, insulin action or both along with decreased glucose utilization and increased glucose production [1, 23, 24]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [2].

Several pathogenic processes are involved in the development of diabetes [24]. These range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action [25]. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action [26]. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia [2, 23, 27].

Acute, life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non- ketotic hyperosmolar syndrome. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [28].

1.2.1.1 Classification of Diabetes Mellitus

DM has been classified based on diagnostic criteria into four types [8, 23, 30].

- a) Type 1 Diabetes Mellitus (T1DM) ($\leq 10\%$),
- b) Type 2 Diabetes Mellitus (T2DM) (90-95%)
- c) Gestational Diabetes Mellitus (GDM) and
- d) Other types of Diabetes Mellitus ($< 10\%$)

1.2.1.1.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is one of the most common endocrine and metabolic conditions among children [8]. It is an autoimmune disease characterized by islet cell destruction and caused primarily by immune effector cells reacting against endogenous β -cell antigens. Pancreatic β -cell destruction ultimately causes absolute insulin deficiency. Genetic and environmental factors as well as disorder of immune mechanism are thought to be the cause of type 1 diabetes. [4, 24, 30]. Usually mediated by immune mechanisms LADA (latent autoimmune diabetes in adults) is classified as T1DM. T1DM includes 5-10% of diabetic patients. It classically occurs in juveniles and affected patients are dependent on insulin injection in their lifetime and are very prone to ketosis [1, 5, 25, 32].

1.2.1.1.2 Type 2 Diabetes Mellitus

This form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non–insulin dependent diabetes or adult onset diabetes [8]. It is mainly due to insulin resistance or relative insulin deficiency rather than the absolute insulin deficiency seen in T1DM [1,6,32]. T2DM can range from predominant insulin resistance with relative insulin deficiency to prevailing defective secretion with insulin resistance.

It's frequently associated with other problems of the so-called metabolic syndrome [6, 33]. Patients with T2DM may be asymptomatic for-long period of time. Vascular complications such as nephropathy, neuropathy, retinopathy, cardiovascular disease and diabetic foot ulcer may develop in these patients [3, 34]. Encompasses individuals who have insulin resistance, usually have relative (rather than absolute) insulin deficiency at least initially, and often throughout their lifetime, those individuals do not need insulin treatment to survive.

There are probably many different causes for this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β -cells does not occur [24, 35]. T2DM and obesity are associated with insulin resistance. Most obese individuals, despite being insulin resistant, do not develop hyperglycemia. Pancreatic β -cells of the islet of Langerhans release adequate amounts of insulin that are sufficient to overcome insulin level reductions under normal circumstances, thus maintaining normal glucose tolerance [1, 36], whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β -cells function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance [6, 37].

The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. Type-2 diabetes may developed most often in middle age and older people who are also increased body weight or obese [3, 38]. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia.

1.2.1.1.3 Gestational Diabetes Mellitus

GDM defined as any degree of glucose intolerance with onset during pregnancy especially diagnosed in the second or third trimester of pregnancy, is increasing worldwide [7, 8]. The prevalence may range from (1 to 14)% in all pregnancies [7, 39]. GDM occurs when insulin receptors are not able to respond adequately to control blood sugar levels due to hormones produced in pregnancy, such as human placental lactogen, which impacts susceptible insulin receptors. Several risk factors are known for GDM; advanced maternal age, obesity, physical inactivity, parity, ethnicity, family history of T2DM, history of macrosomic babies and a previous history of GDM. GDM is associated with adverse maternal and fetal outcomes during pregnancy and long term health [3, 40].

1.2.1.1.4 Other Types of Diabetes Mellitus

American Diabetes Association (ADA) had recognized more than 56 other specific types of diabetes. Some of these are quite rare, whereas others are much more common. This type of diabetes is associated with insulin deficiency related to non-immune mediated injury to the β -cells or to the entire pancreas. Some of these disorders include cystic fibrosis, acute or chronic pancreatitis, trauma, partial or complete pancreatic resection, hemochromatosis. These disorders and several monogenetic defects in β -cell function collectively known as maturity-onset diabetes of the young (MODY) have impaired insulin secretion with relatively negligible or no defect in insulin action [1, 41].

1.2.1.2 Diagnosis of Diabetes Mellitus

The diagnostic criteria of hyperglycemia for diabetes mellitus have recently been revised by comprehensive reviews of the world's research findings by the expert committees of the ADA and the WHO. The reviews have led to a lowering of

the fasting glucose diagnostic criteria from ≥ 7.8 mmol/l to ≥ 7.0 mmol/l. Criteria for the diagnosis of diabetes FPG ≥ 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h. A random plasma glucose ≥ 200 mg/dL (11.1 mmol/L). The 2-h PG criterion ≥ 200 mg/dL (≥ 11.1 mmol/L) during oral glucose tolerance tests (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water [7]. HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol). level between 6.0 – 6.5% can be considered as high risk of developing diabetes mellitus [42].

1.2.1.3 Global Prevalence of Diabetes Mellitus

The WHO estimated that there were 135 million diabetics in 1995, and the global number of individuals with diabetes in 2000 was estimated to be 171 million (2.8% of the world's population), while in 2010 there were 285 million people with diabetes mellitus. An expected incidence of DM is 300 million by the year 2025. The number of people with DM worldwide is projected to increase to 366 million (4.4% of the world's population). by 2030, 298 million of whom will live in developing countries [3, 8].

In 2007, 23.6 million people (7.8%) in United States had T2DM. T2DM reached epidemic proportions with explosive increase in incidence worldwide over the past few decades [43]. Although T2DM is more prevalent in developed countries, the increase in incidence seems to be more pronounced especially in populations that are experiencing rapid westernization. Apart from microvascular complications, cardiovascular disease, with its attendant morbidity and mortality, is on the rise in the developing countries. Current evidence suggests that environmental factors are major determinants of the increasing rates of diabetes. Overweight and obesity are increasing dramatically and contribute to the burden of diabetes mellitus and other

chronic health conditions. Indeed, the modern environment and sedentary lifestyles promotes the risk factors that cause diabetes [3, 43].

1.2.1.4 Complications of Diabetes Mellitus

The disruption in the vascular system of the body is due to impaired metabolism of carbohydrates, fats, proteins and electrolytes associated with the hyperglycemic state. Under this state, many endothelial cells are damaged, including the retina, glomerulus, central and peripheral nerves due to excessive glucose accumulation in these cells. Impaired lipid metabolism, increased production of reactive oxygen species (ROS) and reduced protective antioxidant system leads to insulin resistance and more damage to beta cells in the pancreas [44]. Hyperglycemia is a major risk causative in the development of several complications in patients with diabetes. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, Charcot joints, autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction [2, 3, 8, 45].

1.2.1.5 Pathogenesis Type 2 Diabetes Mellitus

Under normal physiological conditions, the plasma glucose concentrations are maintained within a narrow range, despite the wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion [1, 46]. The ingestion of carbohydrates causes a momentary increase in blood glucose concentration, resulting in a rapid release of insulin by the β -cells within the islets of Langerhans. Insulin then binds to specific receptors in target peripheral tissues to produce its effects. Under normal conditions, insulin promotes the transport of

glucose into the skeletal muscle and adipose tissue. In the liver, insulin acts by suppressing glycogenolysis and gluconeogenesis and in adipose tissue, it inhibits lipolysis. Through the mechanism of decreasing hepatic and adipose glucose production and by accelerating the uptake of glucose into peripheral tissues. The net effect of insulin's action is to lower blood glucose concentration [2, 46, 47].

People with T2DM, however there is a gradual change in glucose homeostasis manifested as glucose intolerance and inefficient uptake of glucose from the blood by the peripheral tissues. The glucose intolerance is caused, in part, by an attenuated biological response to normal concentrations of insulin, a condition known as insulin resistance. In addition, T2DM is often associated with a progressive decrease in the sensitivity of the pancreatic β -cells to glucose stimulation, with a subsequent decrease in insulin secretion. In time, there may be an increased demand for insulin due to worsening of the insulin resistance. Eventually, the combined effects of increased insulin resistance and inadequate insulin secretion in response to a glucose challenge will result in hyperglycaemia, which is a significant and prolonged increase in blood glucose concentration [3, 48].

1.2.1.6 Etiological Factors Underlying Diabetes Mellitus

T2DM is a heterogeneous syndrome that results from an interaction between a genetic predisposition (non-modifiable) and environmental (modifiable) factors. These interactions increase the risk for insulin resistance, β -cells dysfunction, obesity and ultimately lead to the development of diabetes mellitus. In genetically predisposed individuals, the development and progression of T2DM appears to be facilitated by factors such as obesity, lack of physical activity, cigarette smoking, high intake of calorie-rich diets and low intake of fruits and vegetables [46, 49].

1.2.1.7 Most Significant Symptoms of Diabetes Mellitus

The clinical diagnosis of diabetes frequently driven by unexplained weight loss and high level of glycosuria are usually present along with following symptoms; polyuria (frequent urination), nocturia (urination at night), polydipsia (increased thirst and excessive fluid intake) and dry mouth [2, 47, 50]. Hyperphagia (excessive eating or overeating). Other symptoms include tiredness, fatigue, weight loss and blurring of vision. As far as T1DM is concerned, the affected people start developing symptoms very quickly within weeks or even days, but in the case of T2DM, the symptoms develop in a very slow manner, usually over a period of months or even years. Another complication of this disease is diabetic ketoacidosis (DKA), a condition, wherein the person experiences polyuria, nausea, vomiting and abdominal pain [51].

1.2.2 Diabetic Retinopathy

DR is a serious microvascular complication of diabetes that involves blood–retinal barrier alteration, inflammation and neuronal dysfunction. DR a leading cause of blindness among working adults worldwide. The prevalence of DR increases with the duration of DM, and nearly all T1DP and more than 60% T2DP suffer from some degree of retinopathy in the 20 years after the onset of DM [3, 45, 52].

1.2.2.1 Diabetic Retinopathy Stages

DR has four stages; [53]

1. Mild Non-proliferative Retinopathy: the earliest stage of diabetic retinopathy, at this earliest stage, microvascular aneurysms occur. Early retinal non-perfusion in people with diabetes may cause ischemia and impaired oxygenation of retinal neurons. This microvascular changes are typically asymptomatic, even at advanced stages.

2. Moderate non-proliferative retinopathy: As the disease progresses, some blood vessels that nourish the retina are blocked [45].

3. Severe non-proliferative retinopathy. Many more blood vessels are blocked, depriving several areas of the retina of their blood supply. These areas of the retina send signals to the body to grow new blood vessels for nourishment. The people with non-proliferative retinopathy are at high risk of progressing to sight-threatening diabetic retinopathy remains a challenge.

4. Proliferative retinopathy. At this advanced stage, the signals sent by the retina for nourishment trigger the growth of new blood vessels. This condition is called proliferative retinopathy. These new blood vessels are abnormal and fragile. They grow along the retina and along the surface of the clear, vitreous gel that fills the inside of the eye [44].

Macular edema is the major complication of diabetic retinopathy due to the leaking of fluid from blood vessels in the retina. The macula is swollen vision loss may be mild to severe [53].

1.2.2.2 Risk Factor for Diabetic Retinopathy

DR represents the most common diabetes mellitus complication (DMC). There is a worldwide epidemic of diabetes, which has led to an outbreak of diabetic retinopathy [3, 45, 54]. The assessment of risk factors, as well as of other complications of diabetes, may help in both primary and secondary prevention of DR. According to several studies, the main risk factors associated both with an earlier onset and a more rapid progression of the disease are diabetes duration, high glycated hemoglobin (HbA1c) and hypertension, and hyperlipidemia in patients with T2DM [45,55]. In particular, an optimized glycaemic control demonstrated effective in reducing both new-onset DR and its progression. As established by the most

recent literature of the last two decades, there is a correlation between DR and both the microangiopathic complications of DM (e.g., diabetic nephropathy and neuropathy) and diabetic macroangiopathies (e.g., the cardiovascular risk) [56]. Other factors, genetic and/or environmental, may explain 89% of the variation in retinopathy risk. Together, these observations suggest that genetic factors may play a role in the development and progression of DR [3, 4, 55].

1.2.2.3 Pathogenesis of Diabetic Retinopathy

The increased level of glucose in retinal cells lead to increase the risk of DR and accompanying blindness. In the pathogenesis of DR, oxidative stress is increased in the retina and its vasculature, mitochondria are damaged and have impaired homeostasis, gene transcription associated with oxidative stress are altered, and apoptosis of capillary cells is accelerated [45, 57]. Oxidative stress is considered as one of the crucial contributors in the pathogenesis of DR, but oxidative stress appears to be highly interrelated with other biochemical imbalances that lead to structural and functional changes and accelerated loss of capillary cells in the retinal microvasculature [58].

The oxidative stress response is capable of participating in various secondary pathological responses like apoptosis and inflammation. The resulting pathological products including MDA and nitric oxide (NO) may lead to damage of tissue cells and are deemed to be involved in the pathological responses of DR [44, 56]. The earliest responses of the retinal blood vessels to hyperglycemia are dilatation of blood vessels and blood flow changes. These changes are considered to be a metabolic auto regulation to increase retinal metabolism in diabetic subjects. Pericyte loss is another hallmark of the early events of DR [45].

1.2.3 Type 2 Diabetes and Oxidative Stress:**1.2.3.1 Oxidative Stress**

Oxidative stress was defined as the lack of balance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and the organism's capacity to counteract their action by the anti-oxidative protection systems. Its severity is associated with several pathologies like cardiovascular, cancer and aging [59]. Oxidative stress represents an increased level of production of free radicals or ROS. Free radical-induced damage in oxidative stress has been confirmed as a contributor to the pathogenesis and pathophysiology [60]. Oxidative stress can be broadly divided into exogenous and endogenous mechanisms. Although very different in origin, excessive oxidative stress may lead to damage to lipids, proteins and DNA. Conversely, ROS and RNS are essential for all aerobic organisms where free radicals and ROS are used for advantageous biological effects. The regulation of free radicals in maintaining redox stability is therefore essential for the physiological health of organisms [61].

1.2.3.2 Oxidative Stress in Type 2 Diabetes Mellitus

Oxidative stress plays critical role in the pathogenesis of various diseases. In the diabetic condition, oxidative stress impairs glucose uptake in muscle and fat. Decreases insulin secretion from pancreatic β cells. Increased oxidative stress also underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular wall cells [62]. Several accumulating evidence indicates that oxidative stress may play a role in the a etiology of T2DM, oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy. Oxidative stress occurs due to a delicate imbalance between pro-

oxidant and antioxidant forces in our system. It is associated with many morbidities but its association with obesity and insulin resistance is still controversial. It is evident that with the fast progressing westernization of our society that is associated with a high intake of calorie-dense foods coupled with a low physical activity pattern many subjects in high-risk groups may be predisposed to a defective antioxidant status and increased oxidative stress that may lead to the development of T2DM and its complications [63]. Several mechanisms seem to be involved in the generation of oxidative stress in the presence of elevated glucose concentrations. They include glucose auto-oxidation, enhanced glucose flux through the polyol pathway, and non-enzymatic and progressive glycation of proteins with subsequently increased formation of glucose derived advanced glycosylation end products [64]. Glucose metabolism prominently takes place via two pathways: glycolysis and citric acid cycle. In both pathways, carbon dioxide and reduced forms of nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FADH), such as NADH and FADH_2 , respectively, are formed as metabolic by-products. These reduced forms (NADH and FADH_2) play a vital electron-donating role in the electron transport chain reaction, resulting in the generation of adenosine triphosphate, along with the reduction of oxygen to superoxide radical. The formation of free radicals during this reduction reaction occurs physiologically, every day. Nevertheless, they are produced in quite low amounts and are efficiently removed via various radical-scavenging mechanisms and antioxidants. With increasing glucose concentration, a hallmark of diabetes mellitus, excess amounts of NADH and FADH_2 are produced that, upon entering the electron transport chain, generate a surfeit of superoxide radicals, hence unbalancing the free radical homeostasis, ultimately resulting in oxidative stress [63, 65].

1.2.3.3 Free Radicals

Free radicals and other reactive species are continuously produced in the body and have important functions for the immune system, as our white blood cells produce oxidants such as hydrogen peroxide and superoxide to kill bacteria [66]. ROS and RNS have critical biological functions essential for normal physiology. Overproduction or deficiency of ROS and RNS may result in impaired homeostasis and associated pathology. In healthy organisms, oxidants are balanced by reductants (antioxidants). According to recent reports it is believed that almost all pathogenetic mechanisms involve a disequilibrium in the redox balance as "final common pathway" [66, 67]. Free radical-induced damage in oxidative stress has been confirmed as a contributor to the pathogenesis and pathophysiology [62], when free radicals of an oxygen species attack a cell, they damage its membrane, its proteins, and even its DNA. The cell is then oxidized: it cannot function properly and dies. When its DNA is affected, the disrupted cell can even become a "mutant" cell, therefore potentially a cancerous cell [60, 65, 68].

Free radicals can be classified into following three types [68, 69]:

1. Reactive oxygen species (ROS).
2. Reactive Nitrogen species (RNS)
3. Reactive chlorine species (RCS)

Table 1.1 : Main reactive oxygen species (ROS) [70]

Free radicals	Non-radicals
Superoxide $O_2^{\cdot -}$	Hydrogen peroxide H_2O_2
Hydroxyl $\cdot OH$	Singlet oxygen $^1O_2$

Hydroperoxyl HO_2	Ozone O_3
Peroxyl $\text{ROO}\cdot$	Hypochlorous acid HOCl
Alkoxy $\text{RO}\cdot$	

Table 1.2 : Main reactive nitrogen species (RNS) [70].

Free radicals	Non-radicals
Nitric oxide $\text{NO}\cdot$	Nitrous acid HNO_2
Nitric dioxide $\text{NO}_2\cdot$	Nitrosonium cation NO^+
Nitrate radical $\text{NO}_3\cdot$	Nitroxyl anion NO^-
	Peroxynitrite ONOO^-
	Dinitrogen tetroxide N_2O_4
	Dinitrogen trioxide N_2O_3

The production of reactive oxygen species (ROS) is an unavoidable consequence of aerobic life. ROS is a collective term that includes both oxygen radicals, like superoxide ($\text{O}_2^{\cdot-}$), hydroxyl ($\cdot\text{OH}$), peroxyl ($\text{ROO}\cdot$), etc. and other non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$) and ozone (O_3) Table 1.1 . Reactive nitrogen species (RNS) is a similar collective term that includes radicals like nitric oxide ($\text{NO}\cdot$) and nitric dioxide ($\text{NO}_2\cdot$), as well as non-radicals such as nitrous acid (HNO_2) and dinitrogen tetroxide (N_2O_4) among to Table 1.2 [62, 22, 70].

1.2.3.4 Reactive Oxygen Species (ROS)

ROS are continuously formed in small amounts by normal metabolic processes. The addition of one electron to O_2 produces the $\text{O}_2^{\cdot-}$ whereas addition of two electrons results in formation of H_2O_2 which can react with $\text{O}_2^{\cdot-}$ and ferrous to produce the highly reactive $\text{HO}\cdot$ [62]. High ROS concentration, formed under

pathological conditions, can overwhelm cellular defenses leading to cellular damage.

ROS can act as both oxidizing and reducing agents. Although the initial free radical produce only local effects, secondary radicals, and degradation products can have biological effects at distant sites [60, 71].

Oxidants such as $O_2^{\bullet-}$, H_2O_2 , $HO^{\bullet}$ and lipid peroxides play important roles in biological processes such as phagocytosis, aging, inflammation, tissue repair and intracellular messenger pathways [68, 70].

1.2.3.4.1 Mechanisms of Production of ROS

ROS can be generated by both exogenous and endogenous means [61, 66, 72].

Endogenous production of ROS: *in vivo*, ROS are produced both enzymatically and non-enzymatically. One of the main ROS sources under physiologic conditions is mitochondria. In the inner membrane, they contain an electron chain transfer system for ATP generation, permanently generating $O_2^{\bullet-}$. It is estimated that some 1-2% or even 4% of O_2 consumption undergoes transformation to $O_2^{\bullet-}$. The outer mitochondrial membrane carries monoamine oxidase, producing large amounts of H_2O_2 in the mitochondrial matrix as well as in the cytosol directly [66, 73]. Enzymatic sources include NADPH oxidases located on the cell membrane of polymorphonuclear cells, macrophages and endothelial cells. The enzyme NADPH oxidase catalyzes the one electron reduction of O_2 to generate $O_2^{\bullet-}$, using NADPH as the source of electrons (NADPH oxidase inhibitor reduced ROS production) [62, 73]. The proteolytic conversion of xanthine dehydrogenase to xanthine oxidase provides another enzymatic source of $O_2^{\bullet-}$ and H_2O_2 (therefore constitute a source of $OH^{\bullet}$) has been proposed to mediate deleterious processes *in vivo* [68, 74]. The non-enzymatic production of $O_2^{\bullet-}$ occurs when a single electron is directly transferred to oxygen by

reduced coenzymes or prosthetic groups (for example, flavins or iron sulfur clusters) or by xenobiotics previously reduced by certain enzymes (for example, the anticancer agent adriamycin or the herbicide paraquat) [75]. The mitochondrial electron transport chain contains several redox centers that may leak electrons to oxygen, constituting the primary source of $O_2^{\bullet-}$ in most tissues [71, 75].

Exogenous production of ROS: in addition to being generated in the physiological processes, ROS can be produced as primary products under a variety of stress conditions [77]. For example; ROS is generated by UV irradiation, pollutants, toxins, etc. ROS generally causes DNA and protein oxidation that damages these macromolecules, resulting in cell death. Cigarette smoke contains many radicals [59, 76]. In addition numerous toxic environmental chemicals such as xenobiotics, pesticides, herbicides, fungicides, heavy or transition metals ions (Cd, Hg, Pb, Fe, As) and radiation cause their harmful environmental effects via the generation of ROS [65, 66, 77].

1.2.3.4.2 Mechanisms of Generation of ROS in T2DM

Chronic hyperglycemia can activate several well-characterized biochemical pathways that play a significant role in the development of diabetic complications. Activation of these pathways appears to be linked to a hyperglycemia-mediated rise in ROS production and a consequent increase in oxidative stress. Hyperglycemia induces activation of classical pathways like advanced glycation end products, polyol pathways [64], hexosamine, protein kinase C activation and increased flux via the hexosamine biosynthesis pathway and generation of the end product uridine diphosphate N-acetyl-glucosamine, a substrate used for protein glycosylation to mediate cellular damage [62, 78].

AGE/RAGE pathway: advanced glycated end-products (AGEs) describe a heterogeneous group of combined carbohydrates, proteins, lipids and nucleic acids that are formed non-enzymatically [44]. The aldo or keto groups of reducing sugars react non-enzymatically with the free amino groups of proteins, lipids and nucleic acids leading to the formation of AGEs. Reducing sugars react non-enzymatically with free amino groups of proteins to form a Schiff's base, which undergoes a series of irreversible reactions to form an Amadori product and finally yield AGEs [65]. Formation of AGEs occurs with normal aging however, their production accelerates during hyperglycemia. General mechanisms through which AGEs contribute to diabetic complications include the apoptotic effects of AGEs were shown to be mediated via mitochondrial electron transport chain inhibition as well as the NADPH oxidase mediated increase in ROS [65, 79].

Polyol pathway: the polyol pathway consists of two reactions catalyzed by two respective enzymes. The first reaction is the reduction of glucose to sorbitol, which is catalyzed by aldose reductase (AR) [80]. In this pathway also converts NADPH to NADP^+ . The second reaction converts sorbitol to fructose and is catalyzed by sorbitol dehydrogenase, which makes NADH from NAD^+ . So the overall products of the polyol pathway is sorbitol, fructose and NADH [65, 66]. NADH production results from the consumption of NADPH. Because nearly 30% of blood glucose can flux through the polyol pathway in diabetes. Increase NADH/ NAD^+ ratio leading to ROS production and oxidative stress in diabetes [44].

Hexosamine biosynthetic pathway: under normoglycemic conditions, most of the glucose is metabolized through the glycolysis pathway and only 2–5% enters the hexosamine pathway and a small amount of fructose-6-P is produced [66]. The HBP begins with the conversion of fructose-6-phosphate (F-6-P) and glutamine to Glc-6-P

by glutamine:fructose-6-P amidotransferase (GFA). It has been noted that Glc-6-P can inhibit glucose-6-P-dehydrogenase, the enzyme that promotes glucose metabolism via the pentose phosphate pathway (PPP) [78]. One important function of the PPP is the generation of NADPH, necessary for the regeneration of reduced glutathione (GSH), a critical and major cellular antioxidant. Thus, in the presence of elevated Glc-6-P and consequent PPP inhibition caused by exposure to Glc, there occurs an associated oxidative stress [81].

The protein kinase C pathway: PKC is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol that have different functions and tissue distributions[82]. Excess glucose in the intracellular medium results in the accumulation of an intermediate of the glycolytic pathway, dihydroxyacetone phosphate [81]. This leads to the formation of glycerol-3-phosphate which upon conjugation with fatty acids yields diacylglycerol (DAG). DAG is the most important activator of PKC although AGE-RAGE interaction has also been shown to activate it [78]. High blood glucose levels in diabetes activate DAG formation, a physiological activator of PKC [65]. This phosphorylates the subunits of NADPH-oxidase, leading to the production of ROS. The activation of PKC also induces the overproduction of ROS and AGEs by the NADPH oxidase system causing deleterious effects to the cell [83].

1.2.4 Antioxidants

Antioxidants are described as any substance that, when present at low concentrations to compete with those of an oxidizable substrate, significantly delays or prevents the oxidation of that substrate. An oxidizable substrate might be a lipid, DNA, protein or any molecule found *in vivo* [63, 84]. The antioxidant can also be defined as any substance that delays, prevents, or removes oxidative damage to a

target molecule. However, defined antioxidant as any substance that directly scavenges ROS or indirectly acts to up-regulate antioxidant defenses or inhibit ROS production. An antioxidant may have an ability to convert free radicals to a new stable radical through intramolecular hydrogen bonding on further oxidation [59, 85]. Antioxidant functions imply lowering oxidative stress, DNA mutations, malignant transformations, as well as other parameters of cell damage. Epidemiological studies proved antioxidants' ability to contain the effects of reactive oxygen species activity and diminish the incidence of cancer and other degenerative diseases [86]. Antioxidants (either endogenously generated or externally supplied) are capable of scavenging ROS and reducing the oxidation of cellular molecules, thus alleviating oxidative stress [68, 84]. The possible mechanisms by which antioxidants may project against ROS toxicity are:

1. Prevention of ROS formation
2. Interception of ROS attacks by scavenging the reactive metabolites and converting them to less reaction molecules and/or by enhancing the resistivity of sensitive biological targets to ROS attacks.
3. Facilitating the repair of damage caused by ROS.
4. Providing (e.g. a cofactor or by acting to maintain a suitable redox status) a favourable environment for the effective functioning of other antioxidants [66, 87].

1.2.4.1 Classification of Antioxidants

The antioxidant defense systems of the human body are complex and various classification systems exist. An antioxidant defense can be parted in two groups: the enzymatic and non enzymatic the first one include SOD, peroxidases such as catalase and glutathione peroxidase, glutaredoxins, thioredoxins and glutathione S-transferases (GST) [61,66,68,88]. The second group typically consist of small

molecules which are soluble in an aqueous environment or in a lipid environment, which include glutathione, ascorbate (vitamin C), tocopherols (vitamin E), phytochelatins, polyamines, metallothioneins and other products [61, 89].

Antioxidants can be categorized by their mode of function [90]:

Preventive antioxidants: preventive antioxidants act by binding to and sequestering oxidation-enhancers and transition metal ions, such as iron and copper, which contain unpaired electrons and strongly accelerate free radical formation such as superoxide dismutase enzymes (1,2 and 3), catalase, glutathione peroxidase and DNA repair enzymes.

Scavenging antioxidants: scavenging or chain-breaking antioxidants. These antioxidants act by presenting themselves for oxidation at an early stage in the free radical chain reaction, producing low-energy molecules that are unable to propagate the chain further. Scavenging antioxidants are included ascorbate, carotenoids, uric acid, α -tocopherol, polyphenols, bilirubin, albumin, ubiquinone, reduced glutathione and other thiols.

And antioxidants can be categorized according to their origin:

Exogenous antioxidants: carotenoids, ascorbic acid, tocopherols (a,b,c,d), polyphenols, folic acid cysteine [66, 91].

Endogenous antioxidants: catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, ceruloplasmin, transferrin, ferritin, glycosylases [66, 91].

1.2.5 Lipid Peroxidation

In biological systems, the free radical oxidation of polyunsaturated fatty acid (PUFA) is known as lipid peroxidation. PUFA contains two or more double bonds

and the presence of an increasing number of double bonds in fatty acids makes it more susceptible to oxidative damage by free radicals and peroxidation [62, 92].

It is used as an indicator of oxidative stress in cells and tissues. Lipid peroxide is unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is MDA [59, 66, 93]. MDA which is a highly reactive bifunctional molecule. Therefore, measurement of MDA is widely used as an end product and indicator of lipid peroxidation, which is a highly toxic by-product formed in part by lipid oxidation derived free radicals. its concentration is increased considerably in diabetes mellitus. At room temperature, MDA or 1,3- propanedial (OHC-CH₂-CHO); C₃H₄O₂, MW; 72.06 g/mol. MDA measurement in biological samples and attempts to evaluate the significance of MDA as a biomarker of lipid peroxidation in health, disease and lifestyle in modern times [62, 63, 94].

Every lipid peroxidation reaction is a complex process that consists of mainly three-step processes, namely initiation, propagation and termination [95].

Initiation: in a peroxide free lipid system, the initiation of a peroxidation sequence refers to the attack of a ROS, Hydroxyl radical HO[•] is one of the most efficient initiators with sufficient reactivity to substitute an allylic hydrogen atom from a reactive methylene group (-CH₂) of PUFA, these hydrogens have very high mobility. This attack easily generates free radicals from PUFA. The presence of a double bond in the fatty acid weakens the (-CH) bonds in the carbon atom adjacent to the double bond and makes the 'H' removal easier. The (R[•]) radical level is stabilized by a molecular rearrangement to form conjugated diene. Under the aerobic condition, conjugated diene combines with oxygen to give peroxy radicals (ROO[•]) [61, 62, 96].

Propagation: $\text{ROO}^\bullet$ abstracts 'H' from another lipid molecule (adjacent fatty acid), especially in the presence of metals such as copper or iron and cause an autocatalytic chain reaction. $\text{ROO}^\bullet$ combines with 'H' to give a lipid hydroperoxide (ROOH) and a new alkyl radical ($\text{R}^\bullet$), ensuring the propagation of the reaction. This reaction characterizes the propagation stage [62, 95].

Termination: termination (formation of a hydroperoxide) is most often achieved by reaction of $\text{ROO}^\bullet$ with α -tocopherol, which is the main lipophilic “chain-breaking molecule” in the cell membranes. Furthermore, any kind of alkyl radicals can react with a lipid peroxide $\text{LOO}^\bullet$ to give non-initiating and non-propagating species such as the relatively stable dimers LOOL or two peroxide molecules combining to form hydroxylated derivatives (LOH).

During the termination stage $\text{ROO}^\bullet$ react with each other and cause self-destruction by forming non-radical products [97].

In DM abnormal increased levels of lipid, lipoprotein and lipid peroxides in plasma may be due to abnormal lipid metabolism [98].

1.2.6 Superoxide Dismutase (SOD)

SOD is the first detoxification enzyme and most powerful antioxidant in the cell [61, 84]. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against ROS as a major defense system against oxidative stress. It catalyzes the conversion or dismutation of two molecules of superoxide anion ($\text{O}_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), consequently rendering the potentially harmful superoxide anion less hazardous. SOD is a metal-enzyme and hence, requires a metal cofactor for its activity [68, 71, 99]. On the basis of the type of metal ion required as a cofactor by SOD, various

forms of the enzyme exist. The metal ions which are normally bound by SOD are iron (Fe), zinc (Zn) copper (Cu) and manganese (Mn) [100].

Association between SOD deficiency and a number of pathologies has been observed in both animals and humans. Hence, the enzyme is indispensable to cellular health, protecting body cells from excessive oxygen radicals, free radicals and other harmful agents that promote aging or cell death. The levels of SODs decline with age, whereas free radical formation increases. It has been suggested that proper daily SOD supplementation will protect the immune system and significantly reduce diseases and ultimately slow down the aging process [100].

1.2.7 Total Antioxidant Capacity

The concept of TAC originated from chemistry was applied to biology, medicine, further to nutrition and epidemiology [101].

Biological fluids contain numerous compounds with chain breaking antioxidant activity, including urate, ascorbate, bilirubin and thiols in the aqueous phase. Also α -tocopherol, carotenoids and flavonoids in the lipid phase. A comprehensive assessment of oxidative stress would include the measurement of all of these antioxidants, although this would be time consuming, expensive. In addition, numerous other compounds are not normally measured as antioxidants have some antioxidant activity. The combined activity of all of these will be assessed in a TAC assay. In general, TAC is decreased in conditions associated with oxidative stress and the administration of chain breaking antioxidants increases antioxidant capacity [102]. TAC is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a given disease [103].

1.2.8 Glycated Hemoglobin (HbA1c)

Glucose reacts non-enzymatically with intracellular and extracellular proteins to form covalently modified proteins (also referred to as glycated proteins). This process termed non-enzymatic glycation (NEG), is a significant feature of diabetes mellitus, in which persistent hyperglycemia leads to the accumulation of AGE. NEG is associated with pathophysiological outcomes, as well as age-related chronic diseases such as micro-angiopathy, retinopathy and nephropathy [104, 105].

Measurement of glycated hemoglobin is effective in monitoring long glucose control in people with DM. it is correlate nicely with mean plasma glucose values. HbA1c has been established as an index of long-term blood glucose concentrations as a measure of the risk of the development of micro and macrovascular complications in patients with diabetes mellitus and these are free of day to day glucose fluctuations and unaffected by exercise or recent food ingestion. This period of retrospective glucose estimation coincides with the lifespan of healthy red blood cells(RBCs), which is usually between two to three months [105].

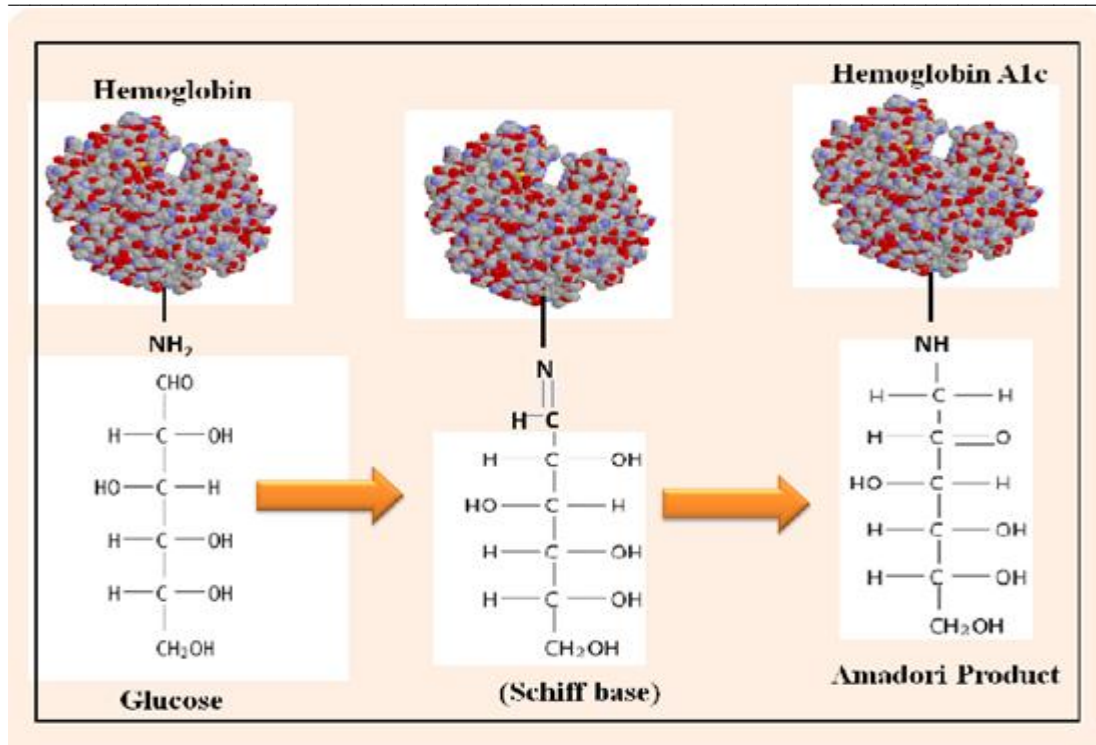


Figure 1.1: Glycation of hemoglobin [106].

Hemoglobin molecule consists of protein globin and heme as a prosthetic group. Globin part is made of four polypeptide chains. The hemoglobin tetramer, referred to as hemoglobin A (HbA), is best described as a pair of identical $\alpha\beta$ dimers ($\alpha_1\beta_1$) that associated to form the tetramer. Chromatographic analysis of HbA identifies several minor hemoglobin, namely HbA1a, HbA1b, HbA1c.

The glycation occurs by the nonenzymatic reaction between the glucose and the N-terminal valine residue of the β -chain, which forms an unstable Schiff base, aldimine or pre- HbA1c. During the rearrangement, the Schiff base is converted into form a more stable ketamine. Amadori products, of which the best known is HbA1c [104, 106].

1.2.9 Dyslipidemia or Hyperlipidemia

Lipids are classified into two main forms. Simple lipids include cholesterol and fatty acids (FA), while complex lipids include triacylglycerol (TG) and cholesterol esters. Cholesterol ester and TG are nonpolar hydrophobic molecules that

are transported in the plasma complexed with proteins. They are transported bound to special proteins forming lipoprotein complexes, while FA is transported bound to albumin [107].

Lipoproteins vary according to their density, lipid content and APO composition. The main clinically important lipids include the TG, TC, low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) [108]. Apo lipoproteins have four major functions including; 1) serving a structural role, 2) acting as ligands for lipoprotein receptors, 3) guiding the formation of lipoproteins, and 4) serving as activators or inhibitors of enzymes involved in the metabolism of lipoproteins [109]. LDLc carries up to 70% of serum total cholesterol clinical trials have shown that LDL lowering reduces the development and progression of macrovascular complications of DM. By contrast, HDLc, also called "good" cholesterol accounts for roughly 30% of the total serum cholesterol [110].

Patients with DM have a significant increase in the risk of developing dyslipidemia. People with diabetes have increased FFA release from insulin-resistant fat cells. FFA stimulates TG production and causes the secretion of apoB and VLDLc. Also, insulin-resistance reduces HDLc levels, increases lipoproteins (particularly LDLc and VLDLc) glycosylation and oxidation, and decreases vascular compliance [111].

1.2.10 Uric Acid in Diabetes Mellitus

Uric acid is the final breakdown product of the metabolism of endogenous purine nucleotides (typically DNA, RNA and ATP) through xanthine oxido reductase and exogenous food-derived purines. In the final phase of purine metabolism, the enzyme xanthine oxidase (XO) catalyzes formation of uric acid, firstly from hypoxanthine and then xanthine. The main function of uric acid to be either anti-

oxidant or pro-oxidant depends on variety of factors. The prooxidant effect of UA is associated with metabolic syndrome, cardiovascular disease and renal disease. glucose intake may affect both the production and excretion of uric acid by several mechanisms. An increased flux of glucose is thought to increase purine generation, while increased anaerobic glycolysis generates increased circulating lactate which leads to a reduction in renal excretion of uric acid [112].. Serum uric acid is the product of endogenous and exogenous purine metabolism and its derivatives. In the purine metabolic pathway, adenosine has vasoactive properties important to retinal blood flow. Adenosine produces superoxide nitric oxide, which affects retinal circulation by causing capillary obstruction, apoptosis and basement membrane thickening [113].

High level of uric acid is reported to be associated with diabetic complications. Uric acid has some physiologic functions including activation of the rennin angiotensin system and direct actions on endothelial cells and vascular smooth muscle cells. These functions are all related to the occurrence and development of diabetic complications, uric acid was usually considered as a marker rather than a risk factor for the progression of disease. There is always a controversy that plasma uric acid concentration is a cause or a result of microvascular complications [113].

1.3 Aims of the Study: The present study aimed to :

- To Evaluate the most important biochemical parameters glycosylated hemoglobin and plasma glucose concentration (FBS,HbA1c) and its association with diabetic retinopathy.
- To investigate lipid profile (TC, TG, HDLc, LDLc, VLDLc) and determine their relationship to diabetes complications.

- To measure lipid peroxidation by the determination of plasma malondialdehyde (MDA) and serum uric acid (SUA).
- To Evaluate antioxidant status total antioxidant capacity (TAC) and superoxide dismutase (SOD) in diabetic retinopathy, type 2 diabetic patients and healthy subjects.
- To investigate any association between glycaemic control, lipid peroxidation and antioxidant status.

Chapter Two

Materials

and

Method

2.0 Materials and Method**2.1 Subjects**

The current study was case - control study to comprised of 232 subjects into three groups, 58 patients (24 male and 34 female) have diabetic retinopathy, 78 patients (26 male and 52 female) have type 2 diabetes mellitus without retinopathy cases and 96 subjects (52 male and 44 female) as healthy control groups. For the purpose of this study, 232 subjects and age and gender-matched control subjects were recruited. The study was managed throughout the period since from first December 2019 to 18th August 2020. The sample collected from Center for Endocrinology in the Al-Hussein Teaching Hospital and the parameters was done worked in the laboratory of biochemistry unit in the Al-Hussein Teaching Hospital in Al-Muthanna province.

2.2 Inclusion Criteria

1. Patient willing and able to give informed consent for participation in the study.
2. Type 2 diabetes mellitus with/without diabetic retinopathy.
3. Male or female, aged 21 years or above.

2.3 Exclusion Criteria

1. Type 1 diabetes mellitus.
2. Pregnancy
3. History of any other chronic conditions such as chronic hepatitis, neurodegenerative disease, cancer, thyroid disorders, liver, and kidney diseases.
4. Regular antioxidant supplementation (vitamins and folic acid) for at least one month before starting of the study.

-
5. Patients were had TG > 400 mg/dl.

2.4 Instruments and Tools

The instruments used in the current study were listed and detailed in Table 2.1 and Table 2.2, respectively.

Table 2.1: Tools that were used in the current study.

NO.	Tools	Company	Origin
1	Medical disposable syringe	Rawabi	Chinese
2	EDTA tubes	ALS	Chinese
3	Vacuum gel tubes	Plastiab	Lebanese
4	Plain tubes	ALS	Chinese
5	Micropipette (5-100 μ L)	Slammed	German
6	Micropipette (100-1000 μ L)	Slammed	German

Table 2.2: Instruments that were used in the current study.

NO.	Instruments	Company	Origin
1	Afinion 2 analyser	Abbott	UK
2	Vortex	Cypress Diagnostics	Belgium
3	Centrifuge	KOKUSAN	Japan
4	Incubator	Binder	German
5	UV/visibe Spectrophotometer	Optima	Italy
6	Water bath	Memmert	German

2.5 Materials

All materials used in the current study were purchased from commercially available sources as detailed in Table 2.3.

Table 2.3: Materials that were used in the current study.

NO.	Solution and kits	Company	Origin
1	Blood glucose kit	Agappe	Switzerland
2	Glycosylated hemoglobin kit	Abbott	UK
3	Total cholesterol kit	Agappe	Switzerland
4	Triglyceride kit	Agappe	Switzerland
5	High density lipoprotein cholesterol kit	Agappe	Switzerland
6	Uric acid kit	Agappe	Switzerland
7	Superoxide dismutase assay kit	Bioassay systems	U.S.A
8	Total antioxidant capacity kit	Bioassay systems	U.S.A
9	TBARS Assay Kit	Bioassay systems	U.S.A

2.6 Methods and Procedures

2.6.1 Measurement of BMI

Body Mass Index is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2) [114].

Table 2.4: Classification of body mass index [62].

Classification	Additional cut of points (kg/m^2)
Normal range	19.5 – 25
Overweight	25 – 30
Obese	>30

2.6.2 Measurement of Blood Pressure

Blood pressure was measured by qualified nurses using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after at least 5 minutes rest in a sitting position. Duplicate measurements were taken with a 5 minutes rest interval between measurements and the mean value was recorded in mmHg [115].

2.6.3 Blood Samples Collection

5-8 ml of blood samples were collected by the vein puncture after 10-12 hours of overnight fast. Rubber tourniquet was applied for less than one minute and the site to be punctured cleaned with methylated spirit. Blood (two milliliters) was dispensed into ethylene diamine tetra acetic acid (EDTA) tubes while the rest for analyzing HbA1c test. The remaining blood placed in gel tube. It was left fifteen minutes at room temperature for coagulation. Blood was centrifuged for 15 minutes at 3000 rpm to obtain the serum which kept into white plane tubes and stored at -80 °C until the analyses [113].

2.7 Biochemical Markers

2.7.1 Estimation of Fasting Plasma Glucose

Method: by glucose oxidase/peroxidase method Trinder's method (Trinder, 1969) [113, 116].

Principle: enzymatic colorimetric determination of glucose according to the following reactions :

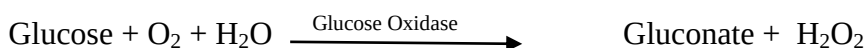


Table 2.5: Kit components of plasma sugar.

Reagent/ Component	Description
Glucose reagent R1	TRIS Buffer (pH 7.40) 92 mmol/L
	Phenol 0.3 mmol/L
	GO 15000 U/L
	4- AAP 2.6 mmol/L
Glucose standard	Glucose standard concentration 100 mg/dL

Table 2.6: Assay procedure of plasma sugar

	Blank	Calibrator	Sample
Glucose reagent	1000 µL	1000 µL	1000 µL
Standard	-	10 µL	-
Sample	-	-	10 µL

The amount of (1000 µL) of the glucose reagent was pipetted into a plain tube, and 10 µL of the plasma was added. The content was well mixed and incubated for ten minutes at 37°C. Finally, (read the absorbance of standard and sample against reagent blank) color intensity was measured at 505 nm using a spectrophotometer.

Calculation

$$\text{Glucose concentration (mmol/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration (5.5 mmol/ L)}$$

2.7.2 Determination of HbA1c

Afinion² HbA1c analyser is a fully automated instrument for determination of HbA1c in human whole blood. The Afinion² HbA1c test cartridge contains all the reagents necessary for the determination of the HbA1c percentage. The blood sample put on the test cartridge that placed in the Alere AfinionTM Analyzer. Then the blood sample automatically diluted and mixed with a liquid that releases hemoglobin from the erythrocytes and hemoglobin precipitates. This sample mixture was transferred to blue boronic acid conjugate, which binds to the cis-diols of glycated hemoglobin.

This reaction mixture is soaked through a filter membrane and all precipitated hemoglobin, conjugate-bound and unbound (glycated and non glycated hemoglobin) remains on the membrane. Any excess of conjugate is removed with a washing reagent.

The Afinion² Analyzer evaluates the precipitate on the membrane. By measuring the reflectance, the blue (glycated hemoglobin) and the red (total hemoglobin) colour intensities are evaluated. The ratio between them being proportional to the percentage of HbA1c in the sample. In this study the HbA1C category was determined based on the American Diabetes Association, namely $\geq 6.5\%$: diabetes and $< 6.5\%$: not diabetes [49].

HbA1c measuring units [117].

mmol/mol: the HbA1c values were aligned to the International Federation of Clinical Chemistry (IFCC) reference method for measurements of HbA1c.

Percentage (%): the HbA1c values were aligned to the assay used in the Diabetes Control and Complications Trial (DCCT) study, also known as National Glycohemoglobin Standardization Program (NGSP-HbA1c).

NGSP-HbA1c (%) = $0.092 \times \text{IFCC-HbA1c (mmol/mol)} + 2.15$

2.8.3 Measurement of Total Cholesterol

Method: cholesterol esterase peroxidase or CHOD-POD Method [118].

Principle: enzymatic colorimetric determination of total cholesterol. The method of analysis was first described by Trinder (1969). Cholesterol esters are broken down to cholesterol and fatty acids. The cholesterol is then oxidized to chole 4-en-3-one and

hydrogen peroxide. The hydrogen peroxide is then hydrolyzed by a peroxidase to a red dye (quinoneimine). According to the following reactions:

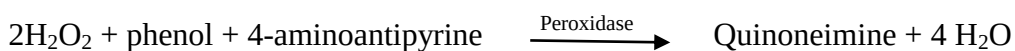
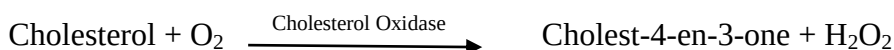
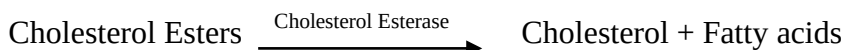


Table 2.7: Kit components of total cholesterol

Reagent/ Component	Description
Cholesterol Reagent	PIPES Buffer Reagent (pH 6.70) 50 mmol/L Phenol 24 mmol/L Sodium Cholate 0.5 mmol/L 4-AAP 0.5 mmol/L Cholesterol Esterase >180 U/L Cholesterol Oxidase >200 U/L Peroxidase >1000 U/
Cholesterol standard	Cholesterol standard conc. 200 mg/dL

Table 2.8: Assay procedure of total cholesterol

	Blank	Standard	Sample
Working reagent	1000 μL	1000 μL	1000 μL
Standard	-	10 μL	-
Sample	-	-	10 μL

The amount of (1000 μL) of the reagent was pipetted into a plain tube, and 10 μL of the serum was added. The content was well mixed and incubated for 5 minutes at 37°C. Finally, (read the absorbance of standard and sample against reagent blank) color intensity was measured at 505 nm using a spectrophotometer.

Calculation

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} * \text{standard concentration (200 mg/dl)}$$

2.7.4 Measurement of Triglycerides

Method: lipoprotein lipase, Glycerol phosphate oxidase and Peroxidase. The method for the analysis was a modification of that of Nagaraju (2013).

Principle: triglycerides in the sample were reacted on by lipoprotein lipase (LPL) to glycerol and fatty acids. The glycerol was then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G -3-P) and adenosine-5diphosphate in a reaction catalyzed by glycerol kinase (GK). Glycerol-3-phosphate was then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacted with 4-aminoantipyrine (4-AAP) and N-Ethyl-N(Sulphopropyl)-3-Methyl Aniline (TOPS) in a reaction catalyzed by peroxidase (POD) to yield a violet coloured complex [118]. According the following reactions :

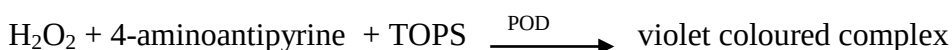
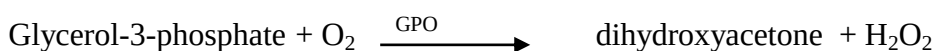
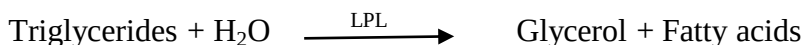


Table 2.9: Kit Components of Triglycerides.

Reagent/ Component	Description
Triglycerides	PIPES–buffer (pH7.00) 5 mmol/L
	TOPS 5.3 mmol/L
	Potassium ferrocyanate 10 mmol/L
	Magnesium Salt 17 mmol/L
	4-Aminoantipyrine 0.9 mmol/L
	ATP 3.15mmol/L
	Lipoprotein Lipase > 1800 U/L

	Glycerol Kinase > 450 U/L Glycerol-3- phosphate oxidase > 3500 U/L Peroxidase > 450
Triglycerides standard	Triglycerides standard concentration 200 mg/dL

Table 2.10: Assay procedure of Triglycerides

	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	-	10 µL	-
Sample	-	-	10 µL

The amount of (1000 µL) of the reagent was pipetted into a plain tube, and 10 µL of the serum was added. The content was well mixed and incubated for 5 minutes at 37°C. Finally, (reading the absorbance of standard and sample against reagent blank) color intensity was measured at 546 nm using a spectrophotometer.

Calculation

$$\text{Triglycerides concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration (200 mg/dl)}$$

2.7.5 Measurement of HDLc

The method is based on phosphotungstate, a precipitating method. LDLc, VLDLc and chylomicrons are precipitated using phosphotungstic acid and magnesium ions. After centrifugation, HDLc are in the supernatant. HDLc content of supernatant is measured by an enzymatic method of measurement for cholesterol. [119].

Table 2.11: Kit components of HDLc

Reagent/ Component	Description
HDLc reagent	Phosphotungstic acid 14 mmol/L
	Magnesium Chloride 1 mmol/L
HDLc standard	HDLc standard concentration 50 mg/dL

Sample 300 μ L

HDLc reagent 300 μ L

Mix well, allow to stand for 10 min. at room temperature, mix again and centrifuge for 10 min, at 4000 rpm. After centrifugation, separate the clear supernatant from the precipitate within 1 hour and determine the HDLc concentration using the cholesterol reagent.

Table 2.12: Assay procedure of HDLc

	Blank	Standard	Sample
Cholesterol reagent	1000 μ L	1000 μ L	1000 μ L
Standard	-	50 μ L	-
Sample	-	-	50 μ L

The amount of (1000 μ L) of the reagent was pipetted into a plain tube, and 50 μ L of the serum was added. The content was well mixed and incubated for 5 minutes at 37°C. Finally, (reading the absorbance of standard and sample against reagent blank) color intensity was measured at 505 nm using a spectrophotometer.

Calculation

$$\text{HDLc (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration} \times 2$$

(50 mg/dl)

2.7.6 Very Low Density Lipoprotein (VLDLc) Estimation

The value of VLDLc can be calculated as follows. If the value of triglycerides is known, VLDLc can be calculated based on Friedwald's equation [119].

$$\text{VLDLc (mg/dl)} = \text{Triglycerides} / 5$$

$$\text{VLDLc (mmol/l)} = \text{Triglycerides} / 2.19 .$$

2.7.7 Low Density Lipoprotein (LDLc) Estimation

After estimating total cholesterol, triglycerides and HDLc, LDLc were calculated using Friedwald's equation as given below. Results were expressed as mg/dl [119].

$$\text{LDL cholesterol} = [\text{Total cholesterol} - \text{HDLc}] - \text{Triglycerides} / 5$$

$$(\text{mg/dl})$$

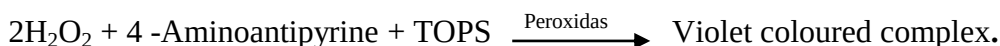
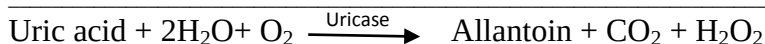
$$\text{LDL cholesterol} = [\text{Total cholesterol} - \text{HDLc}] - \text{Triglycerides} / 2.19$$

$$(\text{mmol/l})$$

2.7.8 Measurement of Uric Acid

Method: uricase / TOPS method [113,119]

Principle: uricase transforms uric acid into allantoin, with formation of hydrogen peroxide. In the presence of peroxidase (POD) it reacts with ethyl-sulphopropyl toluidine (ESTP) and 4-aminophenazone to produce a colored complex color intensity is directly proportional to the uric acid concentration in the sample. Enzymatic determination of uric acid according to the following reactions:

**Table 2.13:** Kit Components of uric acid

Reagent/ Component	Description
Uric acid Reagent	Phosphate Buffer (pH 7.0) 180 mmol/L
	TOPS 1.10mmol/L
	4- Aminoantipyrine 0.25 mmol/L
	Uricase > 50 U/L
	Peroxidase > 100 U/L
Uric acid Standard	Uric acid standard concentration 8 mg/dL

Table 2.14: Assay procedure of uric acid

	Blank	Calibrator	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	-	25 µL	-
Sample	-	-	25 µL

The amount of (1000 µL) of the working reagent was pipetted into a plain tube, and 25 µL of the serum was added. The content was well mixed and incubated for 5 minutes at 37°C. Finally colour intensity was measured at 546 nm using a spectrophotometer.

Calculation

$$\text{Uric acid conc. (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration (8 mg/dl)}$$

2.8 Measurement Oxidative Stress Markers for Investigation

2.8.1 Measurement of Malondialdehyde

Method: MDA is determined as Thiobarbituric acid reactive substances (TBARS) [62, 116, 120].

Principle: Auto-oxidation of unsaturated fatty acids involve the formation of semi stable peroxides, which then undergo a series of reactions to form short chain aldehydes like MDA. One molecule of MDA reacts with two molecules of thiobarbituric acid with the elimination of two molecules of water to yield pink crystalline pigment with absorbance maximum at 535 nm.

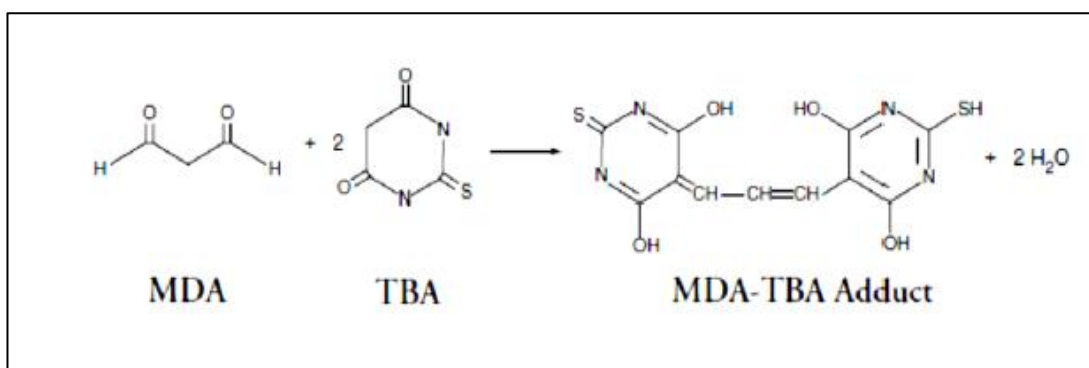


Figure 2.1 : Reaction malondialdehyde with thiobarbituric acid

Kit components

- 1) 10% Trichloroacetic acid (TCA) : 25ml
- 2) TBA reagent: 25ml
- 3) Standard : 50 μ L 6 M MDA

Assay procedure

Sample preparation: The amount of 200 μ L of ice cold 10% TCA was added to the 100 μ L of each sample. The mix was incubated for 5 minutes on ice, than it was centrifuged for 5 min at 1400 rpm than was transferred 200 μ L of each clear supernatant into a new labeled tube. Dilution factor for these pretreated samples is n = 3.

Standard: standard was formed from mix 4 μL of the 6 M MDA with 2396 μL dH_2O (final 10 mM MDA). Next, it was prepared 30 μM MDA by mixing 3 μL of the 10 mM MDA with 997 μL dH_2O . Dilute standards as shown in the Table 2.15.

Table 2.15: Standard prepare of Malondialdehyde

NO.	30 μM MDA	dH_2O	Vol (μL)	MDA (μM)
Standard 1	300 μL	0 μL	300 μL	30
Standard 2	180 μL	120 μL	300 μL	18
Standard 3	90 μL	210 μL	300 μL	9
Standard 4	0 μL	300 μL	300 μL	0

Each standard was put 200 μL into separate tube and 200 μL of each sample into separate tubes. Each of the standards and samples, TBA reagent was added 200 μL and the tube was incubated at 100°C for 60 min. Tubes were cool down to room temperature and the tube was vortexed and briefly centrifuged at 1400 rpm for 2 minutes. And the optical density was read at 535 nm.

Calculation

MDA μM was calculated that equivalents the TBARS concentration of sample.

$$\text{TBARS} = \frac{\text{A Sample} - \text{A Blank}}{\text{Slope } (\mu\text{M}^{-1})} \times n$$

A sample and A blank are the OD at 535nm.

Blank is H_2O (#4).

If calculated TBARS concentration is higher than 30 dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor (n).

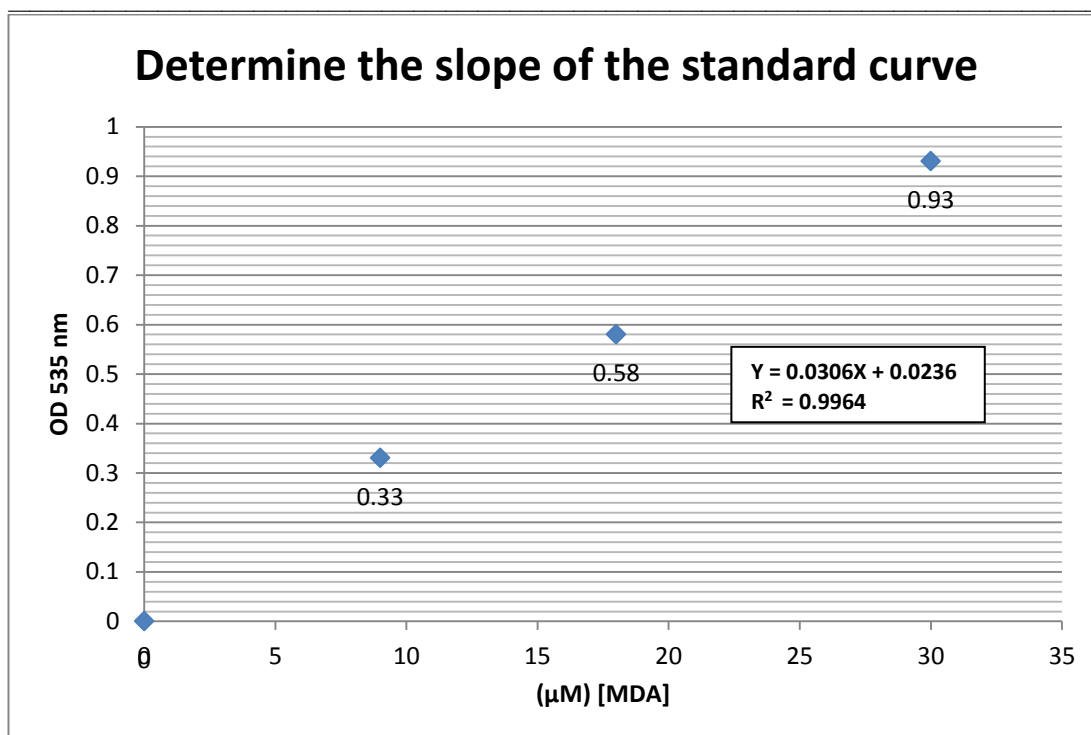


Figure 2.2: The slope of the standard curve of MDA

2.8.2 Measurement of Superoxide Dismutase

Superoxide dismutase was measured by the method of Kuthan H, *et al.*, (1986). SOD assay provides a convenient colorimetric means for the quantitative determination of SOD enzyme activity in biological samples. In the assay, superoxide (O_2^-) is provided by xanthine oxidase (XO) catalyzed reaction. O_2^- reacts with a WST-1 dye to form a colored product. Superoxide dismutase SOD scavenges the the superoxide O_2^- thus less the superoxide O_2^- is available for the chromogenic reaction. The color intensity (440 nm) is used to determine the superoxide dismutase SOD activity in a sample.

Kit components

Assay Buffer:	20 mL
Diluent:	20 mL
SOD Enzyme:	120 μL
XO Enzyme:	120 μL

Xanthine: 600 μ L

WST-1: 600 μ L

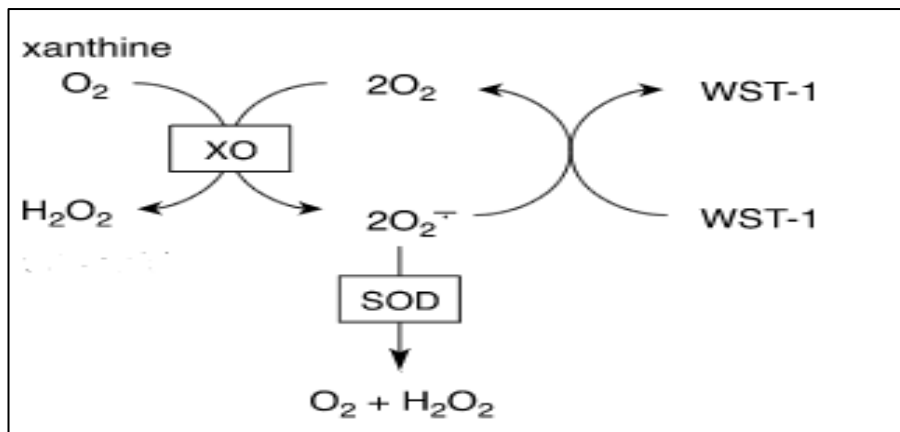


Figure 2.3: Reaction between superoxide with WST-1 dye.

Assay procedure

- Standards: standard prepare from mix 8 μ L SOD Enzyme with 392 μ L diluent to give 3 U/mL SOD standard. Standards were diluted as Table 2.16.

Table 2.16: Standard dilution of SOD

NO.	3U/mL SOD	Diluent	Vol (μ L)	Standard (U/mL)
Standard 1	100 μ L	0 μ L	100 μ L	3.0
Standard 2	80 μ L	20 μ L	100 μ L	2.4
Standard 3	60 μ L	40 μ L	100 μ L	1.8
Standard 4	40 μ L	60 μ L	100 μ L	1.2
Standard 5	18 μ L	μ L 82	100 μ L	0.54
Standard 6	μ L 8	μ L 92	100 μ L	0.24
Standard 7	μ L4	μ L 96	100 μ L	0.12
Standard 8	μ L0	μ L 100	100 μ L	0

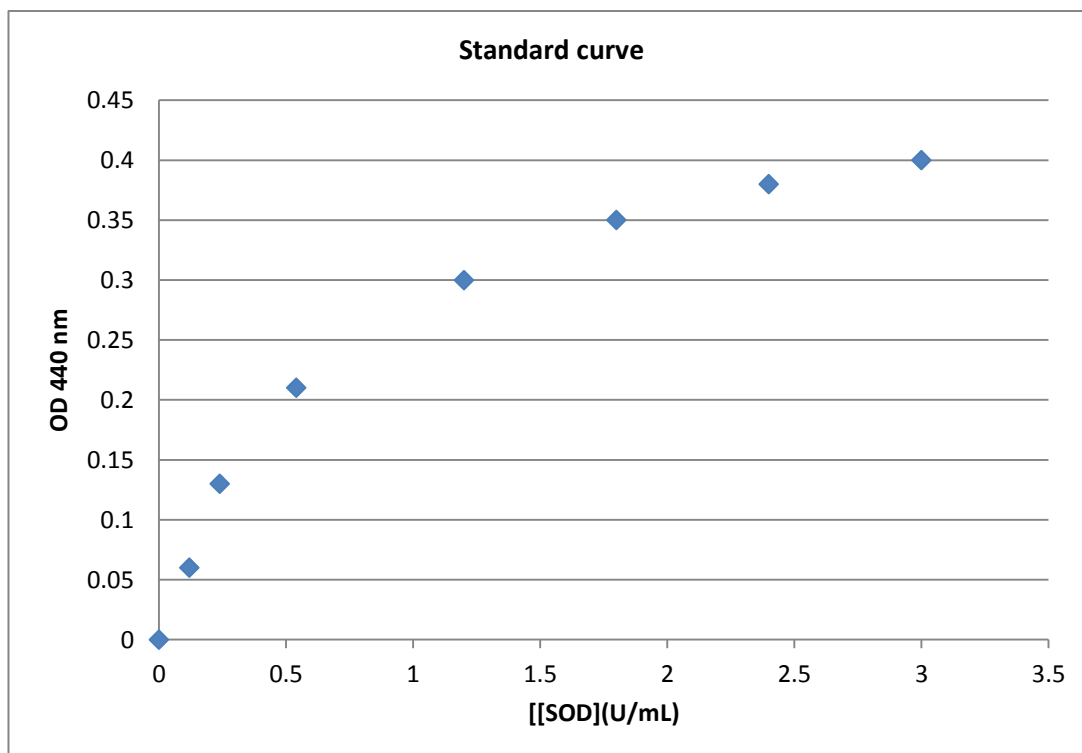
- Working reagent was prepared from mix 160 μ L assay buffer, 5 μ L xanthine and 5 μ L WST-1.

Table 2.17: Work reagent of SOD

	SOD standards	Sample
Working reagent	160 μ L	160 μ L
Standard	20 μ L	-
Sample	-	20 μ L

The working reagent was transferred 160 μ L to separate wells and it was added to 20 μ L SOD standards and 20 μ L samples to working reagent.

Then optical density was read OD 440 nm (OD 420 – 460 nm) (OD_0). the tubes were incubated for 60 min at room temperature (25°C) in the dark. Reading OD 440 nm again (OD_{60}).

**Figure 2.4:** Standard curve of superoxide dismutase.

Calculation

For each standard and sample well, calculations were done as;

Change OD of sample $\Delta OD_s = OD_{60} - OD_0$.

Change OD of standard $\Delta OD_{std} = OD_{60} - OD_0$.

Calculate $\triangle\triangle OD_s = \triangle OD_{std8} - \triangle OD_s$ for each standard and sample where

$\triangle OD_{std8}$ is the $\triangle OD$ for Standard # 8 (the standard with no SOD activity and highest possible absorbance). Plot the standard curve $\triangle\triangle OD$ vs $[SOD](U/mL)$. Use the $\triangle\triangle OD$ for sample to determine SOD activity of sample from the standard curve.

2.8.3 Measurement of Total Antioxidant Capacity

Measures total antioxidant capacity in which Cu^{2+} is reduced by antioxidant to Cu^+ . The resulting Cu^+ specifically forms a colored complex with a dye reagent. The color intensity at 570 nm is proportional to TAC in the sample [103].

Kit components

Reagent A: 12 mL

Reagent B: 1 mL

Trolox Standard: 100 μL 50 mM

Assay procedure

The standard was mixed 5 μL of with 245 μL dH₂O (final 1 mM Trolox).

Standards dilution as shown in the Table 2.18.

Table 2.18: Standard prepare of total antioxidant capacity

NO.	1000 μM Trolox Standard	dH ₂ O	Vol (μL)	Trolox Standard(μM)
Standard 1	100 μL	0 μL	100 μL	1000
Standard 2	60 μL	40 μL	100 μL	600
Standard 3	30 μL	70 μL	100 μL	300
Standard 4	0 μL	100 μL	100 μL	0

To prepare working reagent from 100 μL reagent A and 8 μL reagent B were added 100 μL working reagent to 20 μL of each sample and standards.

Table 2.19: Prepare working reagent of total antioxidant capacity

	Trolox standards	Sample
Working reagent	100 μL	100 μL
Standard	20 μL	-
Sample	-	20 μL

Incubating 10 min at room temperature. Optical density was read at 570 nm.

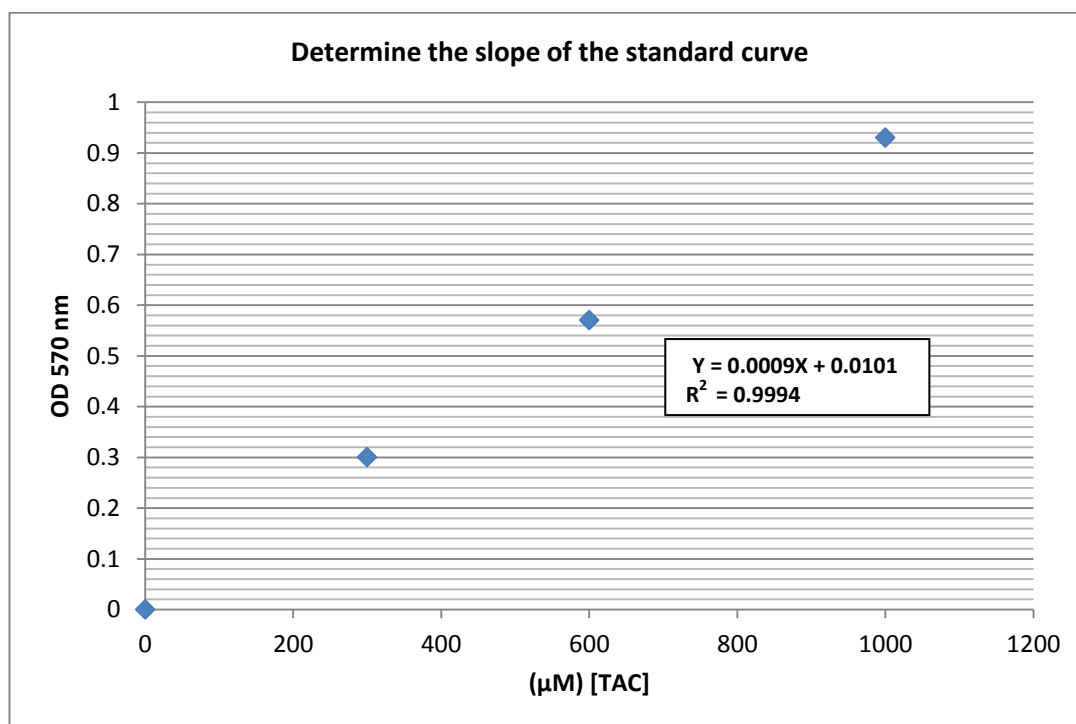
Calculation

TAC μM was calculated that equivalents the trolox concentration of sample.

$$[\text{TAC}] (\mu\text{M}) = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{\text{Slope} (\mu\text{M}^{-1})}$$

A sample and A blank are the OD 570 nm.

Blank is dH_2O (#4).

**Figure 2.5:** The slope of the standard curve of TAC

2.9 Statistical Analyses

Completely randomized design (CRD) was used for this study in the analysis of variance for the means of studied parameters (sugar, lipid, oxidative stress, and antioxidants parameters) by using one-way ANOVA test, t-test, and Dennett's test at a 5% level of significance. Moreover, data were processed and analysis by using statistical program social science (SPSS 22) and the results were expressed as Mean $\pm$ SD [63].

Chapter Three

Results and Discussion

3. Results and Discussion

This study carried out a study of 232 subjects divided according to the following:

- 1- (96) subjects (52 male and 44 female) as healthy control groups.
- 2- (78) subjects (26 male and 52 female) patients have type 2 diabetes mellitus without diabetic retinopathy.
- 3- (58) subjects (24 male and 34 female) of the patients have diabetic retinopathy.

3.1 Anthropometric Parameters

3.1.1 Some Anthropometric Parameters

In this study, anthropometric parameters (age, BMI, SBP, DBP, and disease duration) were determined among three study groups (control, DM and DR) to identify the significant differences. The statistical analysis of these results showed a significant difference between the three studied groups, where p value was < 0.0001 , ($p \leq 0.05$), for all anthropometric parameters, except diastolic blood pressure (DBP), where p value was > 0.05 . While the mean of the systolic blood pressure (SBP) was significantly higher in diabetic retinopathy group compared to the diabetes mellitus group (p was 0.009) and higher than control subjects ($p < 0.0001$). The mean of the age was a significant difference between the three studied groups ($p < 0.0001$) and it was significantly higher in diabetic retinopathy group compared to the diabetes mellitus group and control subjects ($p < 0.0001$). T2DM subjects and DR have higher BMI than the healthy controls group ($p < 0.05$). In addition, the disease duration parameter showed higher significant in DR compared with DM group, where p value was < 0.0001 , as shown in Table 3.1.

Table 3.1: Comparison of anthropometric parameters means among all study groups

Parameters	Control Mean±SD	DM Mean±SD	Retinopathy Mean±SD	P value
Age (years)	49.7±13.7	45±9.2	55.8±9.7	a- <0.0001* b- <0.016* c- <0.003* d- <0.0001*
BMI (Kg/m ²)	28.2±4	31.4±7.3	30±5.8	a- <0.0001* b- <0.0001* c- <0.052 d- 0.311
SBP (mmHg)	101.7±14	118.2±14.3	125.1±17.6	a- <0.0001* b- <0.0001* c- <0.0001* d- 0.009*
DBP (mmHg)	82±9.5	79.1±8.8	81.8±11.4	a- 0.105 b- 0.089 c- 0.991 d- 0.102
Disease duration (years)	-	3.6±2.2	9.8±3.3	d- <0.0001*

* Represents a significant difference at $P \leq 0.05$. letters (a, b, c, d) represent type of the statistical analysis: a- among the study groups (control, DM and retinopathy). b- between control group and DM group, c- between control group and retinopathy group, and d- between patient groups (DM and retinopathy).

Regarding BMI, the results of the current study indicates that obesity is more frequent in T2DM patients. Obesity increases the risk of T2DM and its complications in the great vessels: cardiovascular disease, brain and peripheral vascular disease [62, 49] and this contradicts a study conducted by Sarrafan - Chaharsoughi et al., (2018) [121], who found that there was no significant difference in BMI and diabetic retinopathy. In concerning, duration of disease, many epidemiological studies have already shown that the frequency of microvascular complications in diabetes is clearly correlated to duration of diabetes. Finding of current study go along with study of Garg et al. (2019) who reported that higher statistical significant between duration of disease and develop of diabetic retinopathy at (p value < 0.001) [122]. Additionally, the study that conducted by Biadgo et al. (2017) found that the result

showed that the mean levels of systolic blood pressure was significantly higher ($P < 0.05$) in DR and T2DM compared to controls [43, 123].

3.2 Biochemical parameters for all study groups

3.2.1 FPG and HbA1c

In this study, glucose parameters (FPG and HbA1c) were determined among three study groups (control, DM and DR) to identify the significant differences. The statistical analysis of these results showed a significant difference between the three studied groups, where p value was <0.0001 , ($p \leq 0.05$), for FPS and HbA1c parameters. In addition, there were significant difference in FPS and HbA1c means among DM and DR groups when they were compared control group, where p value was <0.0001 for both comparisons in both sugar parameters. This study compared FPG and HbA1c means between DM and DR groups and the results showed significant difference only in HbA1c parameters, where p value was 0.001, as shown in Table 3.2, Figure 3.1.

Table 3.2: Comparison of FPG and HbA1 means

Sugar Parameters	Control Mean $\pm$ SD	DM Mean $\pm$ SD	Retinopathy Mean $\pm$ SD	P value
FPG (mg/dl)	91.7 $\pm$ 16	300 $\pm$ 108	328 $\pm$ 92	a- $<0.0001^*$ b- $<0.0001^*$ c- $<0.0001^*$ d- 0.110
HbA1c %	4.5 $\pm$ 0.9	9.8 $\pm$ 1.9	11 $\pm$ 2	a- $<0.0001^*$ b- $<0.0001^*$ c- $<0.0001^*$ d- 0.001*

* Represents a significant difference at $P \leq 0.05$. letters (a, b, c, d) represent type of the statistical analysis: a- among the study groups (control, DM and retinopathy). b- between control group and DM group, c- between control group and retinopathy group, and d- between patient groups (DM and retinopathy).

The study showed that T2DM and DR subjects had significantly higher FPG and HbA1c levels compared with control subjects according to the reports of the ADA and the WHO.

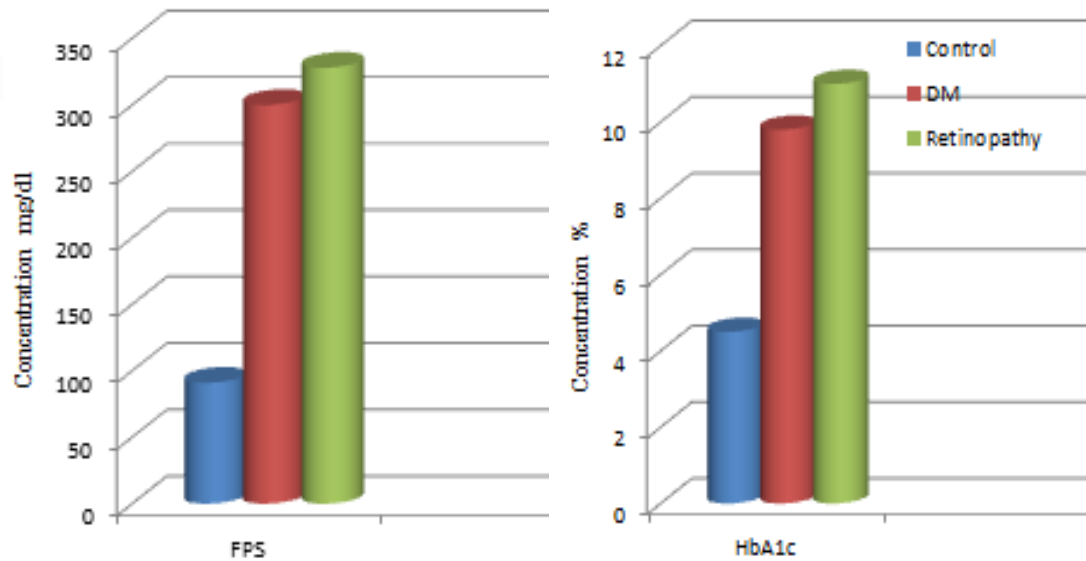


Figure 3.1: Comparison of glucose and HbA1c means among study groups.

Furthermore, increased HbA1c level was observed in DR more than T2DM. The results of the study under hand go along with study entitled (Total Antioxidant Status in Type 2 Diabetic Patients in Palestine) that carried out by Kharroubi et al., (2015) [124], who found that there was a significant relationship between HbA1c and different groups at ($p = 0.002$).

Also, another study that carried out by Pieme et al. (2017) [125], who stated that the FPG and HbA1c levels were respectively 2.05 and 2.32 times higher in the group of patients with diabetes and complications compared to those of healthy persons.

HbA1c is a product of non-enzymatic addition of glucose to hemoglobin and the rate of this conjugation is directly proportional to the level of blood glucose. So, the high % of HbA1c level observed in this study denotes that the type 2 diabetic subjects are under poor glycemic control.

3.2.2 Lipid Profile

As explained in Table 3.3, this study compared the lipid parameters (cholesterol, TG, HDLc, VLDLc, LDLc) means among the study groups (control, DM and DR). The results showed a significant difference among the three studied

groups for cholesterol, TG, VLDLc, and LDLc variables, where p values were < 0.0001 , < 0.0001 , < 0.0001 , 0.007 , respectively. While there was no significant difference, p value is 0.633 among the three studied groups for HDLc means. Moreover, other comparisons were determined in this study with control group or between DM and DR groups. The significant differences were varied in these comparisons as described in Table 3.3 and illustrated in Figure 3.2.

Table 3.3: Comparison of lipid profile parameters means

Lipid parameters	Control Mean $\pm$ SD	DM Mean $\pm$ SD	Retinopathy Mean $\pm$ SD	p value
Cholesterol (mg/dl)	176 $\pm$ 55	203 $\pm$ 41.9	215 $\pm$ 52	a- $<0.0001^*$ b- $<0.0001^*$ c- $<0.0001^*$ d- 0.141
TG (mg/dl)	161 $\pm$ 74	257 $\pm$ 126.6	240 $\pm$ 89	a- $<0.0001^*$ b- $<0.0001^*$ c- $<0.0001^*$ d- 0.347
HDLc (mg/dl)	43 $\pm$ 11	41 $\pm$ 10	42 $\pm$ 11.5	a- 0.633 b- 0.560 c- 0.788 d- 0.801
VLDLc (mg/dl)	32.6 $\pm$ 14.6	51.5 $\pm$ 25	48 $\pm$ 17	a- $<0.0001^*$ b- $<0.0001^*$ c- $<0.0001^*$ d- 0.347
LDLc (mg/dl)	100 $\pm$ 53	109.9 $\pm$ 38	125 $\pm$ 48.5	a- 0.007^* b- 0.316 c- 0.003^* d- 0.049^*

* Represents a significant difference at $P \leq 0.05$. letters (a, b, c, d) represent type of the statistical analysis: a- among the study groups (control, DM and retinopathy). b- between control group and DM group, c- between control group and retinopathy group, and d- between patient groups (DM and retinopathy).

In this study, Table 3.3 shows significantly high levels of total cholesterol, triglyceride, LDLc and VLDLc in patients with T2DM and DR than control. However, no significant difference in the level of HDLc were identified among the three groups. These findings not harmonizing with the study that conducted by Cetin et al. (2013) who stated that mean total cholesterol, triglyceride, LDLc, HDLc and

VLDLc levels were not significantly different among the groups [126]. However, serum TG was significantly higher in the DR (240 mg/dl) group than in the healthy control group (161 mg/dl, $p < 0.0001$). The diabetic group without retinopathy did not show any significant differences when compared with DR group, it is going along with Ganjifrockwala et al. (2016) [127], who found that serum triglyceride (TG) ($p = 0.004$) showed significant differences among groups and no significant differences between patient groups. Moreover, study the proportions of lipid profile disorders were higher in DM patients compared to apparently healthy controls [49, 123].

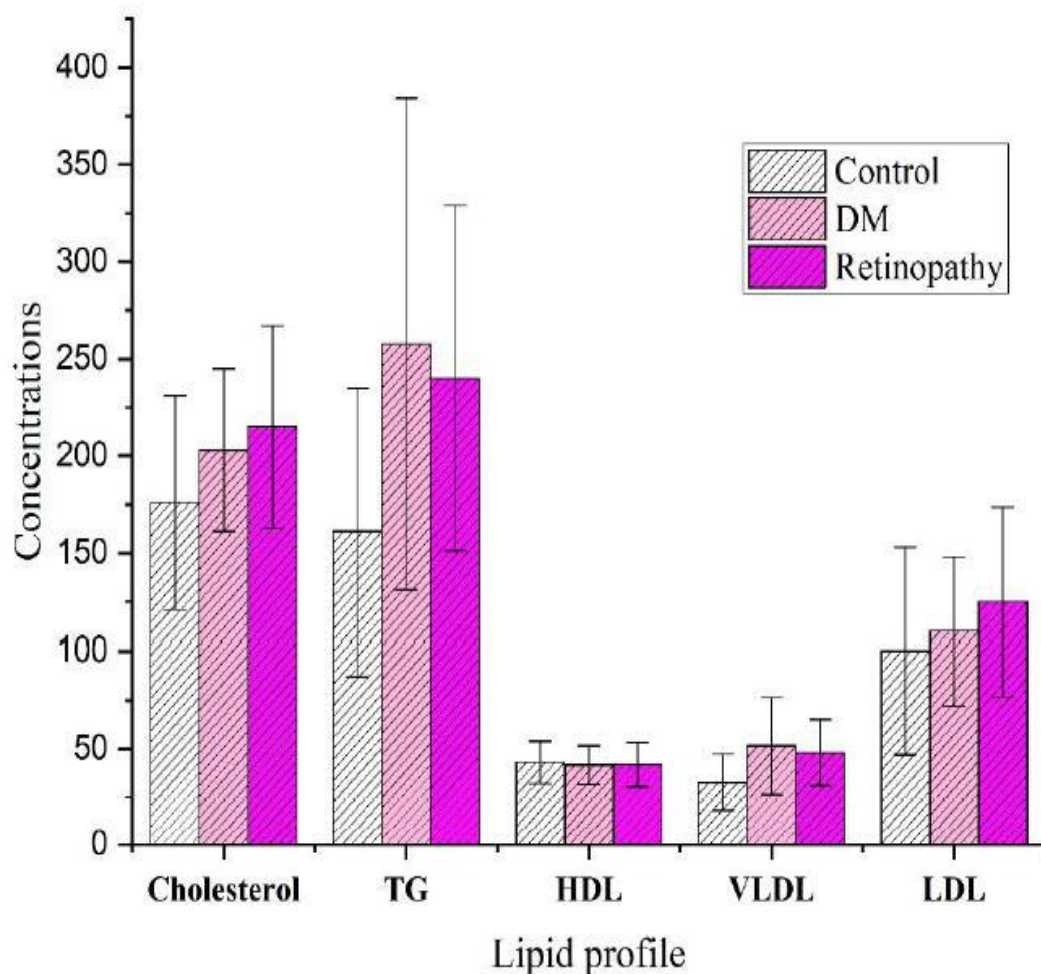


Figure (3.2): Comparison of lipid parameters means among study groups, concentration unite (mg/dl).

3.2.3 Oxidative Stress Parameters

With regard to oxidative stress parameters (uric acid, MDA, SOD, TAC), these parameters were analyzed statistically among study groups (control, diabetes mellitus and diabetic retinopathy). The results showed clear significant differences, $p < 0.05$, among all study groups or between control an unhealthy group (DM and DR) or between unhealthy groups (DM and DR), except of uric acid that showed no significant difference between DM and control groups, where p value 0.064, $p > 0.05$. As shown in Table 3.4 and Figure 3.3.

Table 3.4: Comparison of oxidative stress parameters means

Oxidative parameters	Control Mean $\pm$ SD	DM Mean $\pm$ SD	Retinopathy Mean $\pm$ SD	P value
Uric acid (mg/dl)	4.2 $\pm$ 1.5	4.9 $\pm$ 1.3	5.7 $\pm$ 1.2	a- <0.0001* b- 0.064 c- <0.0001* d- 0.0001*
MDA (μ M)	2.3 $\pm$ 0.7	4.9 $\pm$ 0.5	5.2 $\pm$ 0.6	a- <0.0001* b- <0.0001* c- <0.0001* d- <0.0001*
SOD (U/ml)	7.8 $\pm$ 1.3	4.3 $\pm$ 0.9	3.5 $\pm$ 1.1	a- <0.0001* b- <0.0001* c- <0.0001* d- <0.0001*
TAC (mM)	1.3 $\pm$ 0.6	1 $\pm$ 0.3	0.7 $\pm$ 0.3	a- <0.0001* b- <0.0001* c- <0.0001* d- <0.0001*

* Represents a significant difference at $P \leq 0.05$. letters (a, b, c, d) represent type of the statistical analysis: a- among the study groups (control, DM and retinopathy). b- between control group and DM group, c- between control group and retinopathy group, and d- between patient groups (DM and retinopathy).

The result of current study goes along with the study that conducted by Kassim (2011) [128] to investigate some oxidative stress related parameters in T2DM, who found that significant differences of oxidative stress between patients and control groups. While in regarding to uric acid, the current study shows no significant difference in the level of uric acid between T2DM and healthy controls.

Similar report was observed by Sudhindra R (2012) [129], and that consistent with study that carried out by Munilakshmi et al. (2015) [130], who found that there was a significant differences between uric acid and three different group of study at $p < 0.001$. Glucose intake may affect both the production and excretion of uric acid by several mechanisms. An increased flux of glucose is thought to increase purine generation, while increased anaerobic glycolysis generates increased circulating lactate which leads to a reduction in renal excretion of uric acid [112].

Moreover, in other study that carried out by Pieme et al. (2017) [125], who reported that a statistically higher level of MDA was observed in a group of patients with diabetes and complications compared to those without complications this finding compatible with the study under hand. The high concentration of serum lipids in the T2DM subjects is mainly as a results of increased mobilization of free fatty acids from peripheral depots, due to increased activity action of hormone sensitive lipase. Peroxidation of polyunsaturated fatty acids in blood produces MDA that leads to oxidative damage. T2DM has reported an increased levels of lipid peroxidation (MDA) and reduced antioxidant status may play a major role in diabetic complications [125, 131]. Pavithra et al. reported higher levels of MDA in patients with DM compared with controls and in patients with DR versus those without DR, also suggesting that oxidative stress may play a significant role in the development of DR. As a result, levels of MDA are used as an index of LPO and in consequence of oxidative stress [2]. Hyperglycemia leads to overproduction of ROS through several pathways that may lead to destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress [44].

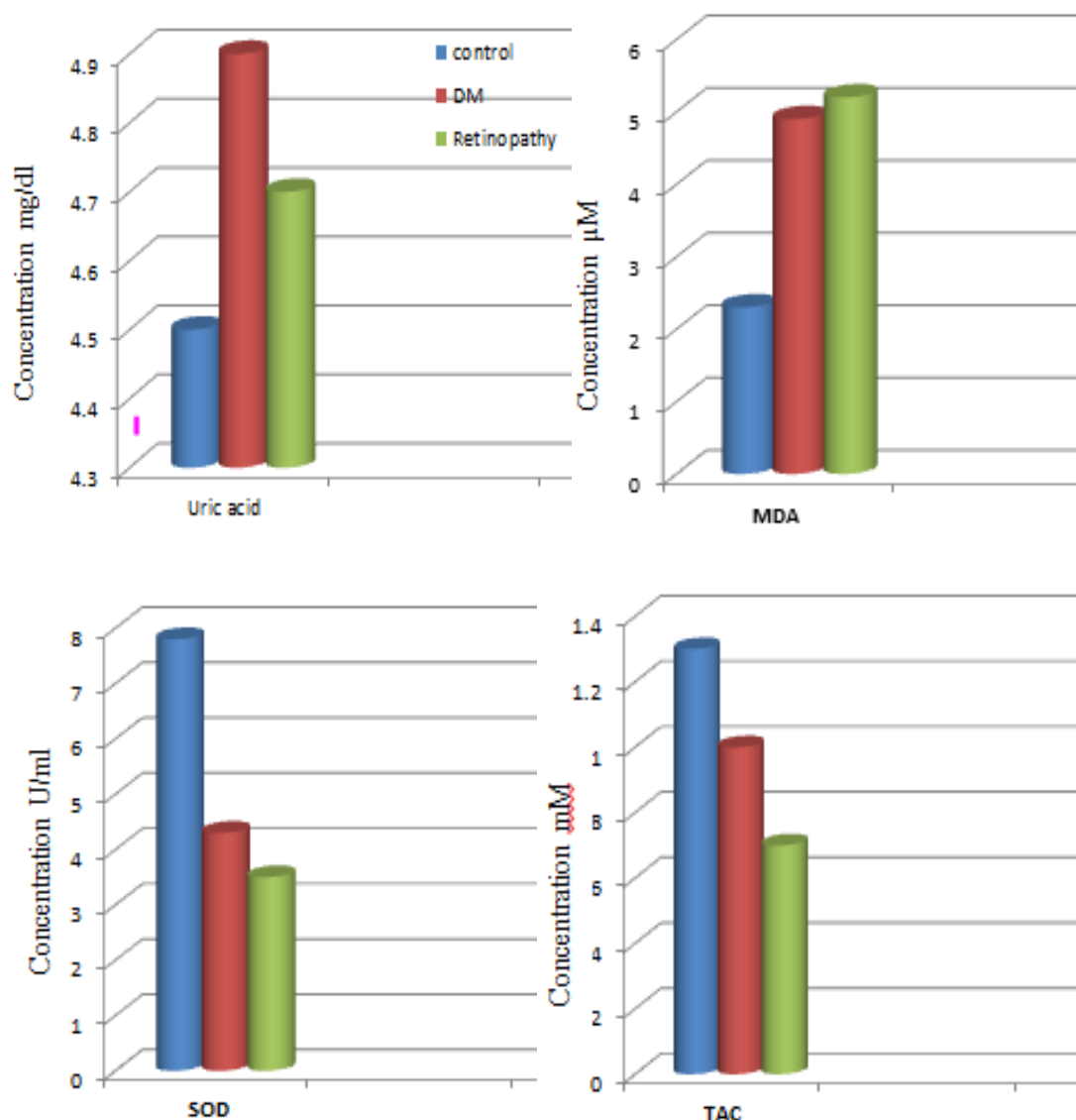


Figure 3.3: Comparison of oxidative stress parameters means among study groups.

Hyperlipidemia has one of the causative factors for increased lipid peroxidation in diabetes mellitus. There was relationship between lipid peroxidation and complication of diabetes mellitus and to estimate the mutual relationship between serum lipoproteins levels and diabetes severity [98]. A statistically significant decrease SOD and TAC levels in the DR group compared to the control group and the DM. The diabetic group without retinopathy also showed a significant decrease in SOD and TAC levels as compared to the healthy control group ($p < 0.0001$). This decrease could be attributed to increased oxidative stress

and lipid peroxidation as evidenced by an increase in MDA. Lipid peroxidation might have resulted in the excess utilization of antioxidants against oxidative stress. Similar observations were reported by Ganjifrockwala et al. (2017) [127].

3.3 Distribution Studied Groups According to Age.

The age groups divided into three groups:

- a. (60) persons of 22-40 years the number of DR patients is (8), in T2DM is (28) and in control group is (24).
- b. (120) persons of 41-59 years the number of DR patients is (22), in T2DM is (48) and in control group is (50).
- c. (52) persons of ≥ 60 years the number of DR patients is (28), in T2DM is (2) and in control group is (22).

3.3.1 Comparison of Study Groups Among Age Groups for Biochemical Parameters

3.3.1.1 FPG and HbA1c Means Among Age groups

To study the impact of age on sugar parameters (FPG and HbA1c) for all studied groups, this study selected three age groups (22-40, 41-59, ≥ 60) years. The results of this study showed no significant difference among age groups for FPG and HbA1c means of control group, where ($p = 0.576$ and 0.836) respectively. While there was significant difference among age groups in DM group only for FPG mean at p value 0.013 .

In contrast, FPG and HbA1c means in diabetic retinopathy group showed a significant difference among age groups, where p value is 0.002 , 0.003 respectively, for FPG and HbA1c. As shown in Table 3.5.

Table 3.5: Comparison of FPG and HbA1c means among age

Sugar parameters	Study groups	Age groups			P value
		22-40	41-59	≥60	
FPG mg/dl	Control	88.8±12.4	93±17.6	91.9±16.6	0.576
	DM	347.7±119	275.6±96.7	256.5±16.7	0.013*
	Retinopathy	293±78.5	384.6±94	408.7±82.8	0.002*
HbA1c %	Control	4.4±0.79	4.5±0.9	4.4±1.1	0.836
	DM	8.8±2	9.9±2	9.8±1.7	0.538
	Retinopathy	11.5±1.6	10.2±1.9	12.1±1.9	0.003*

* Represents a significant difference at $P \leq 0.05$.

These results in the same line with the study that conducted by Ma et al. (2016) [132], who reported that HbA1c levels gradually rose with increasing age. Therefore, HbA1c levels are associated with age in Chinese populations, and this should be considered when selecting HbA1c as a criterion for future diabetes screening.

3.3.1.2 Lipid Parameters Means Among Age Groups.

To study the impact of age on lipid parameters (cholesterol, TG, HDLc, VLDLc, LDLc) for all studied groups, this study selected three age groups. The results of this study showed no significant difference among age groups for cholesterol parameters of all studied groups (control, diabetes mellitus and diabetic retinopathy), where p values were 0.248, 0.048, 0.832, respectively. HDLc parameter of all studied groups, where p values were 0.097, 0.126, 0.695, respectively. In contrast, the other lipid parameters (TG, VLDLc, LDLc) showed a variety in the significant differences among the studied groups. Where, there was significant difference among age groups in control group for TG and VLDLc parameters means at p value 0.034 and 0.013 respectively, and significant difference among age groups in DM group only for LDLc mean at p value 0.01, as mentioned in Table 3.6.

Table 3.6: Comparison of lipid parameters means among age

Lipid parameters	Study groups	Age groups			P value
		22-40	41-59	≥60	
Cholesterol (mg/dl)	Control	164±46	185±60	168.9±50.9	0.248
	DM	190±51.8	208±34	237.5±7.5	0.048
	Retinopathy	226±81	213±38	214±52	0.832
TG (mg/dl)	Control	184±94.9	142.5±56.9	178.7±75	0.034*
	DM	247.6±161	269±105	192±46	0.446
	Retinopathy	270.7±67	248.6±121	226±65	0.404
HDLc (mg/dl)	Control	42.5±8	45±12.6	39±10.4	0.097
	DM	44±9	39.8±10.6	46.5±0.5	0.126
	Retinopathy	45±14	42.6±12	41±10.5	0.695
VLDLc (mg/dl)	Control	38±18	28.5±11	35.7±15	0.013*
	DM	49.5±32	53.8±21	38.4±9.2	0.446
	Retinopathy	54±13.4	49.7±24	45±13	0.404
LDLc (mg/dl)	Control	83±35	111±59.9	93.9±49.8	0.087
	DM	96.5±40	114±35	152.6±2.3	0.01*
	Retinopathy	126.8±81	120.9±29	127.7±49.6	0.890

* Represents a significant difference at $P \leq 0.05$.

The finding of current study compatible with cross sectional analytical study to see the effects of age, gender and diabetes on serum lipid levels, that carried out by Adnan et al. (2013) [133], who reported that no significant association was found between age and lipid parameters.

3.3.1.3 Oxidative Stress Parameters Means Among Age Groups.

To study the impact of age on oxidative stress parameters (uric acid, MDA, SOD, TAC) for all studied groups, this study selected three age groups. The results of this study showed the significant differences were varied among study groups for uric acid parameter. And it showed no significant difference among age groups for MDA parameter of studied groups (exception means in DR group), where p values (0.846, 0.213, 0.015), respectively. While the antioxidant parameters (SOD, TAC)

showed significant differences among age groups in all studied groups, as mentioned in Table 3.7.

Table 3.7: Comparison of oxidative stress parameters means among age groups

Oxidative parameters	Study groups	Age groups			P value
		22-40	41-59	≥60	
Uric acid (mg/dl)	Control	4±0.61	4.6±1.7	4.8±1.8	0.180
	DM	4.4±1.2	5.2±1.3	5.8±0.7	0.013*
	Retinopathy	5.4±0.7	5.4±1.5	6.1±0.6	0.143
MDA (μM)	Control	2.3±0.8	2.3±0.6	2.2±0.6	0.846
	DM	4.8±0.5	4.9±0.5	4.5±0.4	0.213
	Retinopathy	5.4±0.3	4.9±0.6	5.4±0.5	0.015*
SOD (U/ml)	Control	8.9±0.9	8.1±0.5	5.8±0.8	<0.0001*
	DM	5.4±0.3	3.8±0.6	2.7±0.3	<0.0001*
	Retinopathy	5.9±0.4	3.5±0.4	3±0.7	<0.0001*
TAC (mM)	Control	2.2±0.4	1.2±0.2	0.6±0.1	<0.0001*
	DM	1.3±0.3	0.9±0.1	0.5±0.05	<0.0001*
	Retinopathy	1.2±0.02	0.8±0.1	0.5±0.2	<0.0001*

* Represents a significant difference at $P \leq 0.05$.

These findings go along with a study that carried out by Basaran et al. (2016) [134] to examined the association between uric acid and glycemic status by the aspects of age and sex, who stated that age effect on uric acid in type 2 diabetic patients at p-value $p < 0.001$). Oxidative stress has been implicated in many diseases associated with ageing and in the ageing process itself, recently demonstrated that advanced age, in the absence or presence of disease, is associated with decreased antioxidant defense and increased oxidative damage [127, 131].

3.4 Distribution Studied Groups According to BMI

The BMI groups divided into three groups:

- a. (44) peoples to BMI < 25 the number of control group is (20), in T2DM is (14) and in DR group is (10).

b. (86) peoples to BMI 25-29.9 the number of control group is (44), in T2DM is (20) and in DR group is (22).

c. (102) peoples to BMI ≥ 30 the number of control group is (32), in T2DM is (44) and in DR group is (26).

3.4.1 Comparison of Biochemical Parameters Among BMI Groups

3.4.1.1 FPG and HbA1c Means Among BMI Groups

To study the impact of BMI on sugar parameters (FPG and HbA1c) for all studied groups, this study selected three BMI groups. The results of this study showed no significant difference among body mass index (BMI) groups for sugar parameters (FPG and HbA1c) means of all studied groups, (exception FPG means in DM group and HbA1c means in DR group), where p values were 0.539, 0.631, for control and DR groups of FPG, and 0.495, 0.212, for control and DM groups of HbA1c. In contrast, FPG means in DM group showed a significant difference among BMI groups, where p value was < 0.0001 , and HbA1c means in DR group showed a significant difference also among BMI groups, where p value was 0.006. As shown in Table 3.8.

Table 3.8: Comparison of FPG and HbA1c means among BMI groups

Sugar parameters	Study groups	BMI groups			P value
		< 25	25-29.9	≥ 30	
FPG (mg/dl)	Control	89.9 $\pm$ 17	92 $\pm$ 17	94.7 $\pm$ 11	0.539
	DM	310 $\pm$ 70	262 $\pm$ 93	406.7 $\pm$ 125	<0.0001*
	Retinopathy	315 $\pm$ 87.6	331 $\pm$ 91.7	349 $\pm$ 111	0.631
HbA1c %	Control	4 $\pm$ 0.9	4.5 $\pm$ 1	4.7 $\pm$ 0.9	0.495
	DM	9.2 $\pm$ 1.8	9.9 $\pm$ 1.8	10 $\pm$ 2	0.212
	Retinopathy	10 $\pm$ 1.5	10.8 $\pm$ 2	12.9 $\pm$ 1.5	0.006*

* Represents a significant difference at $P \leq 0.05$.

According to the study that carried out by Prema et al. (2016) [135] found that body mass index was directly related to fasting blood sugar. In regarding,

HbA1c the finding of present study is consistent with the study that was conducted by Babikr et al. (2016) [136], who stated that Pearson correlation coefficient of HbA1c in diabetic patients with BMI was statistically significant at the 0.0001 level.

3.4.1.2 Lipid Parameters Means Among BMI Groups

To study the impact of BMI on lipid parameters (cholesterol, TG, HDLc, VLDLc, LDLc) for all studied groups, this study selected three BMI groups. The results of this study showed a significant difference among body mass index (BMI) groups only in the control group of HDLc parameter, where p value was 0.035. In contrast, the other study groups of HDLc and all other lipid parameters (cholesterol, TG, VLDLc, LDLc) in all studied groups showed no significant differences among BMI groups, p value > 0.05. As mentioned in Table 3.9.

Table 3.9: Comparison of lipid parameters means among BMI groups .

Lipid parameters	Study groups	BMI groups			P value
		< 25	25-29.9	≥ 30	
Cholesterol (mg/dl)	Control	156±47.7	176±50	185±60.8	0.160
	DM	186.7±55	203.8±37	213±39.9	0.196
	Retinopathy	207±48	213.9±56	220±51	0.790
TG (mg/dl)	Control	164.9±81	152.5±79.5	170±61.5	0.554
	DM	249.7±216	248±64	284±149	0.556
	Retinopathy	220.7±72	248±89.8	262±118	0.402
HDLc (mg/dl)	Control	45.5±11.8	44.9±11.5	39±9.5	0.035*
	DM	43.5±10	42±10	38.8±9	0.311
	Retinopathy	46.4±16	42±11.5	40.5±9	0.402
VLDLc (mg/dl)	Control	34.7±15	30.5±15.9	34±12	0.432
	DM	49.9±43	49.6±13	56.8±29	0.566
	Retinopathy	44±14	49.6±17.9	52.4±23	0.402
LDLc (mg/dl)	Control	76.7±40	102.7±51.8	109±57	0.076
	DM	93.2±41	111.8±36.5	117±38.5	0.172
	Retinopathy	108±45.6	127.5±54	129.9±44.8	0.475

* Represents a significant difference at $P \leq 0.05$.

These findings are compatible with a study that was carried out by Mohandoss and Jaiganesh (2017) [137], who found that BMI showed direct & significant correlation

with total cholesterol, TG, LDLc & VLDLc & inverse correlation with HDLc in diabetics patients.

3.4.1.3 Oxidative Stress Parameters Means Among BMI Groups

To study the impact of BMI on oxidative stress parameters (uric acid, MDA, SOD, TAC) for all studied groups, this study selected three BMI groups. The results of this study showed no significant difference among BMI groups for uric acid parameter of all studied groups, where p values were 0.995, 0.199, 0.279, respectively. In contrast. The significant differences were varied among study groups for MDA parameter. While the other oxidative stress parameters (SOD, TAC) showed significant differences among BMI groups in all studied groups, as mentioned in Table 3.10.

Table 3.10: Comparison of oxidative stress parameters means among BMI groups

Oxidative parameters	Study groups	BMI groups			p value
		< 25	25-29.9	≥ 30	
Uric acid (mg/dl)	Control	4.5±0.6	4.5±1.6	4.5±1.8	0.995
	DM	5±1.4	4.5±1.3	5±1.3	0.199
	Retinopathy	5.9±1	5.9±0.7	5.4±1.4	0.279
MDA (μM)	Control	2±0.6	2.5±0.6	2±0.7	0.027*
	DM	4.8±0.5	4.8±0.6	5±0.5	0.366
	Retinopathy	5±0.7	5±0.5	5.3±0.3	0.830
SOD (U/ml)	Control	7.7±1.3	8.2±1	7.3±1	0.014*
	DM	5.4±0.4	4.2±1	4±0.9	<0.0001*
	Retinopathy	4.9±1	3±1	3.3±0.7	<0.0001*
TAC (mM)	Control	1.8±0.8	1.3±0.4	1±0.4	<0.0001*
	DM	1.3±0.2	1.2±0.4	0.9±0.2	<0.0001*
	Retinopathy	1±0.1	0.6±0.2	0.7±0.2	<0.0001*

* Represents a significant difference at $P \leq 0.05$.

The findings of the present study compatible with results of a study that conducted by Patrick (2013) [138] to evaluating lipid peroxidation and antioxidant status along with lipid profile T2DM patients and BMI - matched healthy subjects.

He reported that statistical significant differences between MDA, SOD and BMI at p-value $p < 0.0001$.

3.5 Study of Diabetic Groups Based on the Diabetic Duration

The duration of diabetes groups divided into three groups:

- a. (64) patients of 1-5 years the number of DR patients is (6), in T2DM is (58).
- b. (35) patients to 6-10 years the number of DR patients is (23), in T2DM is (12).
- c. (37) patients >10 years the number of DR patients is (29), in T2DM is (8).

3.5.1 Comparison of Biochemical Parameters Among Diabetic Duration

3.5.1.1 FPG and HbA1c Means Among Diabetic Duration

To study the impact of diabetic duration on sugar parameters (FPG and HbA1c) for two study groups (DM and DR), this study selected three diabetic duration groups. The results of this study showed no significant difference among diabetic duration groups for FPG means of both two studied groups, where p values were 0.376, 0.470, respectively. In contrast, the results of this study showed a significant difference among diabetic duration groups for HbA1c means of both two studied groups, where p values were 0.0001, 0.008, respectively. As shown in Table 3.11.

Table 3.11: Comparison of FPG and HbA1c means among diabetic duration groups

Sugar parameter	Study groups	Duration of diabetes			p value
		1-5 Years	6-10 Years	>10 Years	
FPG (mg/dl)	DM	306±113	263±78	348±7	0.376
	Retinopathy	289±68	341±98	318.6±89.7	0.470
HbA1c %	DM	9.5±1.5	10.8±2.6	14.8±0.2	<0.0001*
	Retinopathy	8.1±0.6	11.2±2.2	11.4±1.6	0.008*

* Represents a significant difference at $P \leq 0.05$.

The finding of present study is incongruent with the study that conducted by Sinha and Garg (2019) [139], who found that there was no significant correlation between duration of diabetes with HbA1c levels. While in our study, HbA1c levels increased with increased duration of diabetes.

3.5.1.2 Lipid Parameters Means Among Diabetic Duration

To study the impact of diabetic duration on lipid parameters (cholesterol, TG, HDLc, VLDLc, LDLc) for both two studied patient groups (DM and DR), this study selected three diabetic duration groups. Regarding cholesterol, the results of this study shown a no significant difference among diabetic duration groups, while there was significant difference among diabetic duration groups in DR group, p values were 0.01, 0.005, 0.001 of the TG, HDLc, and VLDLc means respectively. While there were no significant differences in LDLc means among diabetic duration groups for both studied groups, $p > 0.05$. As mentioned in Table 3.12.

Table 3.12: Comparison of lipid parameters means among diabetic duration.

Lipid parameters	Study groups	Duration of diabetes			P value
		1-5 Years	6-10 Years	>10 Years	
Cholesterol (mg/dl)	DM	205.7±40.5	187±50.5	214±1.4	0.356
	Retinopathy	170±12.7	216.8±56.6	221.3±47.5	0.187
TG (mg/dl)	DM	252±111	291.5±200	227±1.4	0.585
	Retinopathy	170.5±20	212±90	286.9±71.9	0.01*
HDLc (mg/dl)	DM	42.5±10.9	36.8±9.7	46±1.4	0.169
	Retinopathy	58.5±2.8	42.7±12.8	38.7±7.8	0.005*
VLDLc (mg/dl)	DM	50.4±22	58±40	45±0.42	0.584
	Retinopathy	34.1±4	42.4±18	57.3±14.3	0.001*
LDLc (mg/dl)	DM	112.8±37	92±41.9	121.7±0.9	0.211
	Retinopathy	77.4±19.6	131.7±55.2	125.2±38.5	0.108

* Represents a significant difference at $P \leq 0.05$.

These finding of study under hand in consistent with the study that carried out by Thagele et al. (2018) [140] to study the impact of disease duration on lipid profile in type 2 diabetes mellitus patients, who found that there was significantly positive correlation occurs between diabetic duration with triglycerides and VLDLc and HDLc at p-value 0.05.

Moreover, in regarding, LDLc the finding of present study not harmony with the result of a study that conducted by Biadgo et al. (2017) [123], who stated that duration of T2DM statistical significantly correlated with LDLc.

3.5.1.3 Oxidative Stress Parameters Means Among Diabetic Duration

To study the impact of diabetic duration on oxidative stress parameters (uric acid, MDA, SOD, TAC) for two studied patient groups (DM and DR), this study selected three diabetic duration groups. The results of this study showed significant differences among diabetic duration groups for uric acid levels, where $p < 0.05$ that were 0.041 and 0.032 respectively for DM and DR and it showed significant differences among diabetic duration groups MDA in DM group, where P value was 0.0001.

Table 3.13: Comparison of oxidative stress parameters means among diabetic duration.

Oxidative parameters	Study groups	Duration of diabetes			P value
		1-5 Years	6-10 Years	>10 Years	
Uric acid (mg/dl)	DM	4.9±1.3	5±1.4	7.4±0.5	0.041*
	Retinopathy	4.2±1.8	5.7±1.1	5.9±1.1	0.032*
MDA (µM)	DM	4.7±0.5	5.5±0.2	5.9±0.0	<0.0001*
	Retinopathy	5.3±0.4	5.2±0.6	5.3±0.5	0.639
SOD (U/ml)	DM	4.3±1	4.2±0.9	4.3±0.2	0.859
	Retinopathy	3.8±1	3.6±0.2	3.2±1.2	0.111
TAC (mM)	DM	1±0.3	1.1±0.3	0.9±0.01	0.568
	Retinopathy	0.8±0.2	0.7±0.3	0.6±0.02	0.326

* Represents a significant difference at $P \leq 0.05$.

In contrast, there was no significant difference in SOD and TAC means among diabetic duration groups in both studied groups, where $p > 0.05$. However, there were a decrease in SOD and TAC parameters with increasing diabetic duration. As shown in as mentioned in Table 3.13.

3.6 Distribution of the Studied Groups According to Smoking

a. (52) subjects (22.4 %) Smoking the number of control patients is (23), in diabetes is (15) and in diabetic retinopathy group is (14).

b. (180) subjects (77.6) Non-smoking the number of control patients is (73), in T2DM is (63) and in diabetic retinopathy group is (58).

3.6.1 Effect of Smoking on Biochemical Parameters

3.6.1.1 Effect of Smoking on FPG and HbA1c means

This study has tried to determine the impact of smoking behavior on sugar parameters (FPG and HbA1c) for all study groups (control, DM and DR). The results revealed that there were significant differences in control group for both FBS and HbA1c parameters, where $p < 0.05$, while there were no significant differences between smokers or non-smokers on both FPG and HbA1c parameters in DM and DR groups, where $p > 0.05$ Table 3.14.

Table 3.14: Effect of smoking on FPG and HbA1c means

Sugar parameters	Study Groups	Smoking		P value
		Yes 52	No 180	
FPG (mg/dl)	Control	85.9±14.6	93.5±16	0.039*
	DM	345±140	289±97.5	0.163
	Retinopathy	353±89	320±93	0.235
HbA1c %	Control	4±0.7	4.6±1	0.023*
	DM	9.5±1.5	9.9±2	0.377
	Retinopathy	11.6±1.9	10.9±2	0.284

* Represents a significant difference at $P \leq 0.05$.

The finding of current study identical with results of study that carried out by Sari et al. (2018) [141], who found that the no significant difference at ($p < 0.05$) among fasting blood glucose and HbA1c in smoking diabetic patients compared to non-smoking diabetic patients at ($p = 0.325$), ($p = 0.412$) respectively.

3.6.1.2 Effect of Smoking on Lipid Parameters

This study has tried to determine the impact of smoking behavior on lipid parameters (cholesterol, TG, HDLc, VLDLc, and LDLc) for all study groups. The results revealed that there were significant differences in DM group for TG, HDLc, VLDLc parameters, where $p < 0.05$. Moreover, there were no significant differences between smokers or non-smokers for other lipid parameters in other study groups, where $p > 0.05$. As described in Table 3.15.

Table 3.15: Effect of smoking on lipid parameters means

Lipid parameters	Study groups	Smoking		p value
		No	Yes	
Cholesterol (mg/dl)	Control	166±67	179±51	0.398
	DM	198±32	204±44	0.559
	Retinopathy	201.8±64.6	219.8±47	0.350
TG (mg/dl)	Control	146.7±54.8	165.7±79	0.201
	DM	188.6±27	274±135	<0.001*
	Retinopathy	238.7±79	240.5±92	0.942
HDLc (mg/dl)	Control	44±12	40±7.8	0.08
	DM	47.7±8.9	40±9.8	0.009*
	Retinopathy	42.9±11.6	39.8±11.3	0.394
VLDLc (mg/dl)	Control	29.9±10.9	33.6±15.6	0.148
	DM	37.7±5.4	54.8±27	<0.0001*
	Retinopathy	47.7±15.8	48±18.5	0.942
LDLc (mg/dl)	Control	96±64.6	101±49.7	0.736
	DM	112.8±35	109±39	0.727
	Retinopathy	114±64	128.7±42.7	0.432

* Represents a significant difference at $P \leq 0.05$.

These findings of the study under hand in the same line with the study that conducted by Athyros et al. (2013) [142] to demonstrated the adverse effects of smoking on the risk of microvascular complications of diabetic patients, who found that the smokers had higher levels of TG and VLDLc ($p < 0.01$), as well as lower levels of HDLc ($p < 0.01$) than never smokers.

3.6.1.3 Effect of Smoking on Oxidative Stress Parameters

This study has tried to determine the impact of smoking behavior on oxidative stress parameters. Regarding TAC parameter, the results revealed that there were no significant differences in all study groups, where $p > 0.05$. In comparison, the other oxidative stress parameters (uric acid, MDA, SOD) shown significantly different results between smoking behavior groups in the studied groups. Uric acid parameter showed significant differences in control group, where p value was 0.026. So in same group has significant differences in MDA parameter. While SOD parameter recorded significant differences in DM group, as described in Table 3.16.

Table 3.16: Effect of smoking on oxidative stress parameters

Oxidative parameters	Study groups	Smoking		P value
		Yes	No	
Uric acid (mg/dl)	Control	4±0.8	4.6±1.7	0.026*
	DM	5±1.3	4.9±1.3	0.533
	Retinopathy	5.8±1.5	5.6±1	0.636
MDA (µM)	Control	3.2±0.28	2±0.53	<0.0001*
	DM	5±0.5	4.8±0.5	0.287
	Retinopathy	5.7±0.06	5.1±0.6	<0.0001*
SOD (U/ml)	Control	8±1	7.7±1.4	0.225
	DM	4.9±0.8	4.2±0.9	0.012*
	Retinopathy	4±1.3	3.4±1	0.094
TAC (mM)	Control	1.5±0.7	1.3±0.5	0.276
	DM	1.1±0.15	1±0.35	0.062
	Retinopathy	0.73±0.35	0.75±0.3	0.843

* Represents a significant difference at $P \leq 0.05$.

The finding of current study goes along with the study that carried out by (Mouhamed et al., 2011) [143], who stated that smoking was closely associated with serum uric acid. In regarding, MDA the findings of study under hand compatible with the study that carried out by Nsonwu-Anyanwu et al. (2018) [144], who reported that MDA significantly higher in smokers compared to nonsmokers. In concerning TAC , the finding of current study consistent with study that carried out by Nsonwu-Anyanwu et al. (2018) [144] who found non statistical different between smoking and nonsmoking with TAC at p value (0.458).

Many studies have shown that the adverse effects of smoking on diabetes mellitus were not only diabetic macrovascular complications but the causal nature of its association with diabetes and the progression of diabetic microvascular complications [145].

Smoking also increases inflammation and oxidative stress, to directly damage β -cell function and to impair endothelial function.

3.7 Distribution Studied Groups According to Residence

- a- (133) urban people (57.3%) of total that divided as control (47), diabetes mellitus (50), and diabetic retinopathy (36).
- b- (99) rural people (42.7%) of total that divided as control (49), diabetes mellitus (28), and diabetic retinopathy (22).

3.7.1 Effect of Residence of Patient on Biochemical Parameters

3.7.1.1 Effect of Residence of Patient on FPS and HbA1c means

This study has evaluated the impact of location of studied people on sugar parameters (FPG and HbA1c) for all study groups (control, DM and DR).

With regard to FPS, there were significant differences between urban and rural groups of samples in control and retinopathy groups, where $p < 0.05$, while DM

group showed no significant difference between urban and rural groups of samples, where p value was 0.091. Interestingly, HbA1c parameter showed no significant difference between urban and rural groups of samples in all studied groups, where p values were 0.063, 0.363, 0.732, respectively. Table 3.17.

Table 3.17: Effect of residence of patient on FPG and HbA1c

Sugar parameters	Study groups	Residence		p value
		Rural	Urban	
FPG mg/dl	Control	95.5±13.8	87.8±17.6	0.02*
	DM	274.7±87.8	314.8±116	0.091
	Retinopathy	294.6±79	348.7±95	0.024*
HbA1c %	Control	4.3±0.96	4.7±0.92	0.063
	DM	10±2	9.6±1.6	0.363
	Retinopathy	11±1.9	11.2±2.3	0.732

* Represents a significant difference at $P \leq 0.05$.

The finding of current study almost in the same line with the study that conducted by Veghari et al. (2010) [146] in Iran to the prevalence of DM and some related factors among adults in the Golestan province (north of Iran) in 2006., who found that significant difference between hyperglycemia and urban , and rural area at p-value < 0.003.

3.7.1.2 Effect of Residence of Patient on Lipid Parameters

This study has evaluated the impact of location of studied people on lipid parameter (cholesterol, TG, HDLc, VLDLc, LDLc) for all study groups (control, DM and DR). The results revealed that there were significant differences in control group for cholesterol, HDLc, LDLc parameters, where $p < 0.05$ that were 0.002, 0.0001 and 0.036, respectively.

The mean plasma for HDLc which was found to be higher in rural than in urban in control group. Moreover, there were no significant differences between urban and

rural groups of samples for other lipid parameters in other study groups, where $p > 0.05$. As described in Table 3.18.

Table 3.18: Effect of residence n of patient on lipid

Lipid parameters	Study groups	Residence		p value
		Urban	Rural	
Cholesterol mg/dl	Control	158.4±40	193±62	0.002*
	DM	199±44.6	209.7±33	0.275
	Retinopathy	214.6±51.7	216±53.9	0.882
TG mg/dl	Control	151±60	170±84	0.188
	DM	244±97	281.8±166	0.278
	Retinopathy	244.9±89.5	232±89.6	0.606
HDLc mg/dl	Control	38.7±8.9	47.6±11.7	<0.0001*
	DM	42±9.7	40.6±10.8	0.494
	Retinopathy	41±11	43.6±12	0.456
VLDLc mg/dl	Control	31±11.9	34±16.9	0.306
	DM	48.8±19	56±33	0.278
	Retinopathy	48.9±17.9	46.4±17.9	0.606
LDLc mg/dl	Control	88.6±41.5	111±61	0.036*
	DM	108±41	112.7±32	0.605
	Retinopathy	124±47	126.6±52	0.870

* Represents a significant difference at $P \leq 0.05$.

The finding of current study is incongruent with the study that carried out by Quartey (2012) [147] who found that significant difference between total cholesterol, HDL and LDLc in diabetic type 2 patients.

3.7.1.3 Effect of Residence of Patients on Oxidative Stress Parameters

This study has evaluated the impact of location of studied people on oxidative stress parameters (uric acid, MDA, SOD, TAC) for all study groups (control, DM and diabetic retinopathy).

The results revealed that there were no significant differences between urban and rural groups of samples for all tested oxidative stress parameters in all studied groups, where $p > 0.05$. As described in Table 3.19.

Table 3.19: Effect of residence of patients on oxidative stress parameters

Oxidative parameters	Study groups	Residence		p value
		Urban	Rural	
Uric acid mg/dl	Control	4.7±1.7	4.3±1.3	0.186
	DM	4.8±1.4	5.2±1.2	0.197
	Retinopathy	5.6±1.13	5.8±1.3	0.545
MDA μM	Control	2.4±0.7	2.2±0.7	0.167
	DM	4.9±0.6	4.7±0.54	0.239
	Retinopathy	5.1±0.5	5.4±0.6	0.062
SOD U/ml	Control	8±1.3	7.6±1.3	0.146
	DM	4.4±0.9	4.2±1	0.316
	Retinopathy	3.7±1.3	3.4±0.7	0.293
TAC mM	Control	1.4±0.68	1.2±0.5	0.068
	DM	1±0.2	1.15±0.4	0.337
	Retinopathy	0.7±0.3	0.7±0.2	0.498

* Represents a significant difference at $P \leq 0.05$.

The results of study under hand goes along with the study that conducted by Quartey (2012) [147], who reported that there was no significant difference in the mean serum uric acid levels between the urban and rural diabetic patients at p-value > 0.05, while in contrast with same study according to MDA, whereas founding significant with location.

3.8 Distribution According to Blood Pressure

a. (56) subjects with high blood pressure (24.1%) of total that divided to control (12), diabetes mellitus (24), and diabetic retinopathy (20).

b. (176) subjects with normal blood pressure (75.9 %) of total that divided to control (84), diabetes mellitus (54), and diabetic retinopathy (38).

3.8.1 Effect of Blood Pressure of Patient on Biochemical Parameters

3.8.1.1 Effect of Blood Pressure on FPG and HbA1c

This study has evaluated the impact of the levels of blood pressure on sugar parameters (FPG and HbA1c) for all study groups (control, DM and DR). The results

showed that there were no significant differences for both sugar parameters between high and normal blood pressure groups in all studied groups (except of retinopathy group of HbA1c), where $p < 0.05$. The only significant difference was detected in diabetic retinopathy group of HbA1c, where p value was 0.006, As described in Table 3.20.

Table 3.20: Effect of blood pressure of patient on FPG and HbA1c

Sugar parameters	Study groups	Blood pressure (mmHg)		P value
		High	Normal	
FPG mg/dl	Control	90±10.5	91±16.8	0.655
	DM	293.7±78	303±119	0.674
	Retinopathy	302.5±84.7	341.7±95	0.115
HbA1c %	Control	4.9±1	4.4±0.9	0.158
	DM	10±2	9.6±1.8	0.155
	Retinopathy	11.5±2.1	10.16±1.5	0.006*

* Represents a significant difference at $P \leq 0.05$.

The finding of current study consistent with results of a study that carried out by Kaštelan et al. (2013) [148] to investigate whether body mass index (BMI) independently or in correlation with other risk factors is associated with DR progression, who found that there was statistical significant between systolic and diastolic blood pressure with HbA1c at p -value < 0.01 , where association with the progression of DR in T2DM.

3.8.1.2 Effect of Blood Pressure on Lipid Profile

This study has evaluated the impact of the levels of blood pressure on lipid parameter (cholesterol, TG, HDLc, VLDLc, LDLc) for all study groups (control, DM and DR). Regarding HDLc, the results showed that there were no significant differences between high and normal blood pressure groups for all three studied The groups, where $p > 0.05$.

The results of other lipid parameters (cholesterol, TG, VLDLc, LDLc) were different regarding each study group, as documented in Table 3.21.

Table 3.21: Effect of blood pressure of patient on lipid parameters means

Lipid parameters	Study groups	Blood pressure (mmHg)		p value
		High	Normal	
Cholesterol mg/dl	Control	192.6±54.6	173.7±55	0.280
	DM	219.5±31	195.8±44	0.009*
	Retinopathy	233.7±58.7	205.8±46	0.075
TG mg/dl	Control	209.8±60	154±73	0.011*
	DM	248.5±46	261.6±149	0.559
	Retinopathy	262±89	228±87	0.181
HDLc mg/dl	Control	41±11.7	43.5±11	0.554
	DM	39.5±9	42.6±10	0.182
	Retinopathy	40.5±10.5	43±12	0.411
VLDLc mg/dl	Control	41.9±12	31±14.5	0.013*
	DM	49.7±9.2	52±29.8	0.559
	Retinopathy	52.4±17.8	45.7±17.5	0.181
LDLc mg/dl	Control	109±52	98.9±53.7	0.528
	DM	130±30	100.8±38	0.001*
	Retinopathy	140.8±52.5	117±44.8	0.096

* Represents a significant difference at $P \leq 0.05$.

The results of present study is congruent with the study that carried out by Biadgo et al. (2018) [123] to aim to assess the correlation between serum lipid profile with anthropometric and clinical variables among T2DM patients, who found that there was correlation between systolic BP among various lipid profiles showed statistically significant correlation in T2DM and control groups.

3.8.1.3 Effect of Blood Pressure on Oxidative Stress Parameters

This study has evaluated the impact of the levels of blood pressure on oxidative stress parameters (uric acid, MDA, SOD, TAC) for all study groups (control, DM and DR). Regarding MDA, the results showed that there were no significant differences between high and normal blood pressure groups for all three

studied groups, where $p > 0.05$. The results of other oxidative stress parameters (uric acid, SOD, TAC) were different regarding each study group, as documented in Table 3.22.

Table 3.22: Effect of blood pressure of patients on oxidative stress parameters

Oxidative parameters	Study groups	Blood pressure (mmHg)		p value
		High	Normal	
Uric acid mg/dl	Control	4.7±1.8	4.4±1.5	0.599
	DM	6±0.9	4.4±1	<0.0001*
	Retinopathy	5.9±0.8	5.5±1.3	0.155
MDA μM	Control	2.2±0.9	2.3±0.6	0.743
	DM	5±0.6	4.8±0.5	0.093
	Retinopathy	5.3±0.6	5.2±0.5	0.642
SOD U/ml	Control	6.6±0.5	7.9±1.3	<0.0001*
	DM	3.6±0.8	4.6±0.9	<0.0001*
	Retinopathy	3.7±1.3	3.5±0.98	0.572
TAC mM	Control	0.8±0.19	1.4±0.6	<0.0001*
	DM	0.9±0.2	1.18±0.3	<0.0001*
	Retinopathy	0.72±0.3	0.76±0.2	0.696

* Represents a significant difference at $p \leq 0.05$.

These finding of current study goes along with the study that carried out by Rao and Vanukuri (2016) [149] who found that statistically significant a strong positive association between hypertension and serum uric acid levels in T2DM.

3.9 Distribution Based on the Gender

The gender groups divided into two groups:

- a. (102) male (44%) of total that divided to control (52), diabetes mellitus (26), and diabetic retinopathy (24).
- b. (130) female (56%) of total that divided into control (44), diabetes mellitus (52), and diabetic retinopathy (34).

3.9.1 Comparison of Biochemical Parameters Between Male and Female

3.9.1.1 FPG and HbA1c Means Between Male and Female

This study has evaluated the impact of gender on sugar parameters (FPG and HbA1c) for all study groups (control, DM and DR). Regarding FPG, the results showed that there were a significant difference between male and female groups in control group, where p value was 0.004. While there were no significant differences in DM and diabetic retinopathy groups. On the other hand, HbA1c results showed a significant difference between male and female groups in DM group, p value was 0.002, while there were no significant differences in control and DR groups, as documented in Table 3.23.

Table 3.23: Comparison of FPG and HbA1c means between male and female

Sugar parameters	Study groups	Gender		p value
		Male	Female	
FPG mg/dl	Control	87.5±17	96.7±13	0.004*
	DM	314±140.9	293.5±88	0.496
	Retinopathy	337±85	321.8±98	0.525
HbA1c %	Control	4.4±0.9	4.6±0.9	0.254
	DM	10±1.9	8.9±1.6	0.002*
	Retinopathy	11±1.7	10.9±2.2	0.394

* Represents a significant difference at $P \leq 0.05$.

Concerning to HbA1c, the finding of present study goes along with the study Ma et al. (2016) [132], who found that HbA1c levels of the male group were significantly higher than those of the female group ($p < 0.0001$). Therefore, HbA1c levels were associated with gender in Chinese populations, and this should be considered when selecting HbA1c as a criterion for future diabetes screening. The sugar parameters levels of diabetes is higher in men than women, but there are more

women with diabetes than men, This fully corresponds to a study by George et al. (2019) [8].

3.9.1.2 Lipid Parameters Means Between Male and Female

This study has evaluated the impact of gender on lipid parameter means (cholesterol, TG, HDLc, VLDLc, LDLc) for all study groups (control, DM and DR). This study determined the significant differences between male and female only in DM group of two lipid parameters TG and VLDLc, where p value was 0.008 for both. In exception, there were no significant differences detected in other lipid parameters and other study groups, as illustrated in Table 3.24.

Table 3.24: Comparison of lipid parameters means between male and female

Lipid parameters	Study groups	Gender		p value
		Male	Female	
Cholesterol mg/dl	Control	174.6±60	177.8±49	0.774
	DM	195.9±28	206±47	0.212
	Retinopathy	216±56.5	214.8±49.6	0.920
TG mg/dl	Control	158.6±82	164±64	0.706
	DM	215.6±57.9	287.6±145.7	0.008*
	Retinopathy	255.8±88.8	229±88.7	0.264
HDLc mg/dl	Control	42.9±10.6	43.5±12	0.790
	DM	40±9.5	44.9±10.5	0.055
	Retinopathy	42.9±11	41.6±12	0.679
VLDLc mg/dl	Control	32±16	32.7±12	0.921
	DM	43±11.5	55.7±29	0.008*
	Retinopathy	51±17.7	45.8±17.7	0.264
LDLc mg/dl	Control	99±58	101±48	0.832
	DM	107.8±31	110.9±41.6	0.718
	Retinopathy	122±52	127±46	0.699

* Represents a significant difference at $P \leq 0.05$.

The findings of study under hand is congruent with the study that carried out by Adnan et al. (2013) [133] to aim to see the effects of age, gender and diabetes on serum lipid levels, who reported that female gender had higher high density

lipoprotein cholesterol and illustrated that men were significantly less prone to dyslipidemia than women.

3.9.1.3 Oxidative Stress Parameters Between Male and Female

This study has evaluated the impact of gender on oxidative stress parameters (uric acid, MDA, SOD, TAC) for all study groups (control, DM and DR). This study determined the significant differences between male and female only in control and DM groups of MDA parameter, where p values were <0.0001 and 0.008 , respectively. In exception of that, there were no significant differences detected in oxidative stress parameters and in other study groups, as illustrated in Table 3.25.

Table 3.25: Comparison of an oxidative stress parameters according to gender

Oxidative parameters	Study groups	Gender		p value
		Male	Female	
Uric acid mg/dl	Control	4.5±1.6	4.4±1.4	0.781
	DM	4.9±1.14	4.9±1.4	0.992
	Retinopathy	5.6±1.2	5.7±1.1	0.796
MDA μM	Control	2.8±0.5	1.7±0.4	$<0.0001^*$
	DM	5.1±0.47	4.7±0.6	0.008^*
	Retinopathy	5.4±0.5	5.1±0.6	0.103
SOD U/ml	Control	7.8±1.34	7.84±1.35	0.905
	DM	4.6±0.9	4.2±1	0.071
	Retinopathy	3.8±1.2	3.3±0.9	0.138
TAC mM	Control	1.4±0.6	1.34±0.5	0.639
	DM	1.1±0.18	1±0.3	0.654
	Retinopathy	0.74±0.3	0.76±0.2	0.812

* Represents a significant difference at $P \leq 0.05$.

The finding of present study is inconsistent with results of a study that carried out by (Quartey, 2012) [147] who found that there was non-significant statistical different between men and women with MDA. While significant with uric acid in the same study. In other study that carried out by Morteza et al. (2012) [150] the aim of study the correlation of serum urea as an indicator of protein metabolism with MDA

and SOD, as indicators of oxidative stress in T2DM patients, who found that negative correlation of uric acid with MDA and SOD, only in men with T2DM.

Chapter Four

Conclusion

and

Recommendation

4. Conclusion and Recommendation

4.1 Conclusion

Based on the findings of the current study, it is concluded that:

1. Smoking, blood pressure, and duration of DM have been a great effect on lipid profile levels of T2DM and DR.
2. Overweight type 2 diabetics patients are at high risk for diabetic complications as retinopathy.
3. Hyperlipidemia and systolic blood pressure have been a great effect on DR, and T2DM.
4. Sugar parameters values of DM and retinopathy patients more influenced by age, residence and DM duration.
5. Smoking, BMI, blood pressure, and age play important role of impact on oxidative stress levels of DM patients and its complications.
6. Duration of diabetes has very important effect on both MDA and uric acid levels for diabetic patients with/without retinopathy.
7. Oxidative parameters value not influenced by residence as well as; body mass index not has any effect on uric acid level.

4.2 Recommendation

The current study suggests the following recommendation

1. Periodic medical checkup and suitable life style change is recommended for seemingly healthy controls to improve the quality of interventions not only to accomplish weight loss aims but also to control dyslipidemia and reduce type2 diabetic complications and regular retinal exams are essential.
2. Dietary counseling and lifestyle modification should be emphasized and subjects with diabetes should be encouraged to see a dietitian.

3. Subjects at high risk of developing hyperlipidemia may benefit from treatment with antioxidants such as vitamin which might assist endogenous antioxidant capacity and reduce peroxidation rates. Supplementation might possibly delay or even prevent complications provide health education for T2DM patients about regular follow-up and appropriate glycemic control to prevent and delay the progression of the disease.
4. Further studies about other types of antioxidant to determine its correlation with T2DM due to very effective on DR.
5. Study of the effect of antioxidants on T1DM with diabetic retinopathy; especially for those under the age of 18 to limit the incidence.
6. Study of the effect of antioxidants on T2DM and its effect on other diabetes mellitus complications such as; DFU or cardiovascular.

Reference

Reference :

- [1] Cahill GF. Pathophysiology of Diabetes. *The Medical clinics of North America*, vol. 49, No. 6, pp. 881–892, 2020.
- [2] Pavithra D, Praveen D, Chowdary PR, Aanandhi MV. A review on role of Vitamin E supplementation in type 2 diabetes mellitus. *Drug Inven Today*, vol. 10, No. 2, pp. 236–240, 2018.
- [3] Chattopadhyay S, Samanta S, Ganguly S, Banerjee J, Officer M, Welfare F, et al. Types, Causes, Effect and Common Treatment of Diabetic Mellitus (DM), A Long Term Endocrine Metabolic Disorder: A Review, *Sustainable Humanosphere*; vol. 16, no. 9, pp. 416-424, (February)2020.
- [4] Wołoszyn-Durkiewicz A, Myśliwiec M. The prognostic value of inflammatory and vascular endothelial dysfunction biomarkers in microvascular and macrovascular complications in type 1 diabetes. *Pediatr Endocrinol Diabetes Metab*, vol. 25, no. 1, pp. 28–35, 2019.
- [5] Biester T, Nir J, Remus K, Farfel A, Muller I, Biester S, et al. An open-label, randomized, cross-over study to evaluate the safety and efficacy of day and night closed-loop control by comparing the MD-Logic automated insulin delivery system to sensor augmented pump therapy in patients with type 1 diabetes at h. *Diabetes, Obesity and Metabolism*, vol. 21, no. 4, pp. 822-828 , 2019.
- [6] Boland BB, Brown C, Boland ML, Cann J, Sulikowski M, Hansen G, et al. Pancreatic β -cell REST replenishes insulin secretory capacity and attenuates diabetes in an extreme model of obese type 2 diabetes. *Diabetes*, vol. 68, no. 1, pp. 131–140, 2019.

- [7] Benhalima K, Van Crombrugge P, Moyson C, Verhaeghe J, Vandeginste S, Verlaenen H, et al. Estimating the risk of gestational diabetes mellitus based on the 2013 WHO criteria: a prediction model based on clinical and biochemical variables in early pregnancy. *Acta Diabetologica*, vol. 57, no. 5, pp. 661-671, January 2020.
- [8] George, Shiji, et al. Assess the knowledge regarding home remedies for diabetes mellitus among diabetic patients. *The Pharma Innovation Journal*, vol. 8, no. 5, pp. 531-534, 2019.
- [9] Sanz-Nogués, Mustafa, Burke, O'Brien, Coleman. Knowledge, Perceptions and Concerns of Diabetes - Associated Complications Among Individuals Living with Type 1 and Type 2 Diabetes Mellitus. *Healthcare*, vol. 8, no. 1, pp. 25, 2020.
- [10] Huang D, Refaat M, Mohammedi K, Jayyousi A, Al Suwaidi J, Abi Khalil C. Macrovascular Complications in Patients with Diabetes and Prediabetes. *BioMed Research International* , vol. 8, no. 3, pp. 234-239, 2017.
- [11] Rajalakshmi R, Shanthirani CS, Anandakumar A, Anjana RM, Murthy GVS, Gilbert C, et al. Assessment of diabetic retinopathy in type 1 diabetes in a diabetes care center in South India—Feasibility and awareness improvement study. *Indian Journal of Ophthalmology*, vol. 68, no.13, pp. 92, 2020.
- [12] Likidlilid A, Patchanans N, Peerapatdit T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. *J. Med. Assoc. Thail.*, vol. 93, no. 6, pp. 682–693, 2010.
- [13] Ashour A. Dicarbonyl stress and dysfunction of the glyoxalase system in

periodontal diseases. *PhD Thesis. University of Warwick*. 2016.

[14] Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutrition journal*, vol. 15, no. 1, 2016.

[15] Chandramathi S, Suresh K, Anita ZB, Kuppusamy UR. Urinary advanced oxidation protein product: A potential oxidative damage marker for cancer. *West Indian Medical Journal*, vol. 65, no. 3, pp. 505–510, 2016.

[16] Dhama K, Latheef SK, Dadar M, Samad HA, Munjal A, Khandia R, et al. Biomarkers in stress related diseases/disorders: Diagnostic, prognostic, and therapeutic values. *Frontiers in molecular biosciences*, vol. 6, no. 4, pp. 91-112, October 2019.

[17] Simó R, Ciudin A, Simó-Servat O, Hernández C. Cognitive impairment and dementia: a new emerging complication of type 2 diabetes—The diabetologist's perspective. *Acta Diabetologica*, vol. 54, no. 5, pp. 417–424, 2017.

[18] Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*, vol. 8, no. 3, pp. 72, 2019.

[19] Rajeshwari CU, Andallu B. Oxidative stress in NIDDM patients: Influence of coriander (*Coriandrum sativum*) seeds. *Biological and Chemical Sciences*, vol. 2, no. 1, pp. 31-41, 2011.

[20] Yan Z, Huang C, Guo H, Zheng S, He J, Lin J, et al. Isobenzofuranone monomer and dimer derivatives from the mangrove endophytic fungus

Epicoccum nigrum SCNU-F0002 possess α -glucosidase inhibitory and antioxidant activity. *Bioorganic Chemistry*, vol. 94, no. 4, pp. 103-107, 2017.

[21] Banerjee M, Vats P, Kushwah AS, Srivastava N. Interaction of antioxidant gene variants and susceptibility to type 2 diabetes mellitus. *British journal of biomedical science*, vol. 76, no. 4, pp. 166-171, 2019.

[22] Peluso I, Raguzzini A. Salivary and Urinary Total Antioxidant Capacity as Biomarkers of Oxidative Stress in Humans. *Pathology research international*, vol. 54, no. 3, pp. 14-23, 2016.

[23] Saha S, Murgod R. Evaluation of Iron Profile in Type II Diabetes Mellitus Cases. *International Journal of Biotechnology and Biochemistry*, vol. 15, no. 1, pp. 27-37, 2019.

[24] Duran-Salgado MB, Rubio-Guerra AF. Diabetic nephropathy and inflammation. *World Journal Diabetes*, vol. 5, no. 3, pp. 393-398, June 2014.

[25] Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. *Nature reviews Disease primers*, vol. 3, no. 1, pp. 1-17, 2017.

[26] King A, Bowe J. Animal models for diabetes: understanding the pathogenesis and finding new treatments. *Biochemical pharmacology*, vol. 99, no. 5, pp. 1-10, 2016.

[27] Balaji R, Duraisamy R, Kumar MP. Complications of diabetes mellitus: A review. *Drug Invention today*, vol. 12, no. 1, pp. 98-103, 2019.

[28] Fayfman M, Pasquel FJ, Umpierrez GE. Management of hyperglycemic

crises: diabetic ketoacidosis and hyperglycemic hyperosmolar state. *Medical Clinics*, vol. 101, no. 3, pp. 587–606, 2017.

[29] Hassan M. Measurement of Prolactin Level among Type2 Diabetic Patients, Aldaraga Health Center, Wad Medani, Gezira State, Sudan (2017). University of Gezira, 2018.

[30] Mahajan P V, Morey A, Subramanian S, Bandre A, Ware H. Management of Type 1 diabetes mellitus with autologous mesenchymal stem cells. *Journal of Health and Research*, vol. 6, no. 1, pp. 64-75, 2019.

[31] Al-Tu'ma AF, Ali ZM, Elyassin HD. Molecular basis of human leukocyte antigens DQB1* 0201 allele and its association with type 1 diabetes mellitus of Iraqi children. 2019.

[32] Neal B, Perkovic V, Mahaffey KW, De Zeeuw D, Fulcher G, Erondou N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *New England Journal of Medicine*, vol. 377, no. 7, pp. 644–57, 2017.

[33] Del Prato S, Bianchi C, Daniele G. 12 Abnormalities of Insulin Secretion and β -Cell Defects in Type 2 Diabetes. *Text book Diabetes*. 2016.

[34] Pichu S, Patel BM, Apparsundaram S, Goyal RK. Role of biomarkers in predicting diabetes complications with special reference to diabetic foot ulcers. *Biomarker Medical*, vol. 11, no. 4, pp. 377-388, 2017.

[35] Gilor C, Niessen SJM, Furrow E, DiBartola SP. Classification of diabetes mellitus in veterinary medicine and why it matters. *Journal of Veterinary Internal Medicine*, vol. 30, no. 4, pp. 927–940, 2016.

- [36] Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. *Diabetes, metabolic syndrome and obesity: targets and therapy*, vol. 7, no. 3, pp. 587–591, 2014.
- [37] Burkart AM, Tan K, Warren L, Iovino S, Hughes KJ, Kahn CR, et al. Insulin resistance in human iPS cells reduces mitochondrial size and function. *Scientific reports*, vol. 6, no. 1, pp. 1-12, 2016.
- [38] Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. *Diabetes Care*, vol. 39, no. 11, pp. 2065–2079, 2016.
- [39] Troisia J, Cavallod P, Coluccia A, Pierrii L, Scalab G, Symesf S, et al. Metabolomics in genetic testing. *Advances in Clinical Chemistry*, Vol. 94, no. 4, pp. 85-153, 2020.
- [40] Teh WT, Teede HJ, Paul E, Harrison CL, Wallace EM, Allan C. Risk factors for gestational diabetes mellitus: implications for the application of screening guidelines. *Australian and New Zealand Journal of Obstetrics and Gynaecol*, vol. 51, no. 1, pp. 26-30, 2011.
- [41] Fowler MJ. Diagnosis, classification, and lifestyle treatment of diabetes. *Clinical Diabetes*, vol. 28, no. 2, pp. 79-86, 2010.
- [42] Association AD. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. *Diabetes Care*, vol. 42, no. 3, pp. 13-28, 2019.
- [43] Adampah, Timothy, et al. "Mixed-Effects Model for Longitudinal Study of Type-2-Diabetes. *International Journal of Statistics and Applications*, vol. 5, no.

2, pp. 47–55, 2015.

[44] Alqahtani AA, Alzidany AM, Alenazi SM, Dhifallah T, Alotaibi B. Long Term Complication of Poor Glycemic Control in Diabetic Patients. *Ec Microbiology*, vol. 2, no. 2, pp. 1-7, 2020.

[45] Nentwich MM, Ulbig MW. Diabetic retinopathy-ocular complications of diabetes mellitus. *World Journal Diabetes*, vol. 6, no. 3, pp. 489-499, 15 April 2015.

[46] Cerf ME. Beta cell dysfunction and insulin resistance. *Front Endocrinol*, vol. 4, no. 3, pp. 37-49, 2013.

[47] Chen, S. Genetic Research and Type 2 Diabetes Mellitus (Master's thesis). 2012.

[48] Cuschieri S. The genetic side of type 2 diabetes – A review. Diabetes and Metabolic Syndrome: *Clinical Research and Reviews*, vol. 13, no. 4, pp. 2503–2506, 2019.

[49] Martina, Adisasmita AC. Association between Physical Activity and Obesity with Diabetes Mellitus in Indonesia. *International Journal of Caring Sciences*, vol. 12, no. 3, pp. 1703-1709, 2019.

[50] Sajjadi S, Hejazi S, Firoozi A, Akbari Rad F, Azami G, Afrazeh M, et al. The Relationship Between Diabetes Mellitus and Pulmonary Diseases: A Systematic Review. *Journal of Cardio-Thoracic Medicine*, vol. 6, no. 2, pp. 274-281, 2018.

[51] Sonwani S, Arya A, Saxena RS. A Prospective Study of Risk Factors,

Clinical Profile and Outcome in Patients of Diabetic Ketoacidosis (DKA) in Type II Diabetes Patients. *Indian Journal of Contemporary Medical Research*, vol. 5, no. 4, pp. 21-24, 2018.

[52] Pang B, Li M, Song J, Li Q, Wang J, Di S, et al. Luo Tong formula attenuates retinal inflammation in diabetic rats via inhibition of the p38MAPK / NF - κ B pathway. *Chinese Medicine*, vol. 15, no. 1, pp. 1-12, 2020.

[53] Porwal P, Pachade S, Kokare M, Deshmukh G, Son J, Bae W, et al. IDRiD: Diabetic Retinopathy – Segmentation and Grading Challenge. *International Symposium on Biomedical Imaging*, vol. 19, no. 4, pp. 59-68, 2020.

[54] Van de Kreeke, J. A., Darma, S., Chan Pin Yin, J. M., Tan, H. S., Abramoff, M. D., Twisk, J. W., & Verbraak, F. D. The spatial relation of diabetic retinal neurodegeneration with diabetic retinopathy. *Journal pone*, vol. 15, no. 4, 2020.

[55] Cabrera AP, Monickaraj F, Rangasamy S, Hobbs S, McGuire P, Das A. Do Genomic Factors Play a Role in Diabetic Retinopathy? *Journal of Clinical Medicine*, vol. 9, no.1, 2020.

[56] Pearce I, Simó R, Lövestam-Adrian M, Wong DT, Evans M. Association between diabetic eye disease and other complications of diabetes: Implications for care. A systematic review. *Diabetes, Obesity and Metabolism*, vol. 21, no. 3, pp. 467-478, 2019.

[57] Kowluru RA, Mohammad G, Sahajpal N. Faulty homocysteine recycling in diabetic retinopathy. *Eye and Vision*, vol. 7, no. 1, pp. 1-11, 2020.

[58] Calderon GD, Juarez OH, Hernandez GE, Punzo SM, De La Cruz ZD. Oxidative stress and diabetic retinopathy: Development and treatment. *Eye and*

Vision, vol. 31, no. 8, pp. 1122-1130, 2017.

[59] Eftekhari A, Dizaj SM, Chodari L, Sunar S, Hasanzadeh A, Ahmadian E, et al. The promising future of nano-antioxidant therapy against environmental pollutants induced-toxicities. *Biomedicine & Pharmacotherapy*, vol. 103, no. 3, pp. 1018-1027, 2018.

[60] Hatai B, Banerjee SK. The role of reactive oxygen species (ROS) in osteoarthritis patients. *Pharmacognosy and Phytochemistry*, vol. 8, no. 3, pp. 2287-2289, 2019.

[61] Ahmadinejad F, Møller SG, Hashemzadeh-Chaleshtori M, Bidkhor G, Jami MS. Molecular mechanisms behind free radical scavengers function against oxidative stress. *Antioxidants*, vol. 6, no. 3, pp. 1-15, 2017.

[62] Rafighi Z, Arab S, Mohd yusof R, Shiva A. The Effect of Vitamin C and E on Lipid Profile in Type 2 Diabetes Mellitus Patients. *Global Journal of Health Science*, vol. 3, no. 2, pp. 35-48, 2019.

[63] Das P, Biswas S, Mukherjee S, Bandyopadhyay SK. Association of oxidative stress and obesity with insulin resistance in type 2 diabetes mellitus. *Mymensingh Medical Journal*, vol. 25, no. 1, pp. 148-152, 2016.

[64] Jagdale AD, Bavkar LN, More TA, Joglekar MM, Arvindekar AU. Strong inhibition of the polyol pathway diverts glucose flux to protein glycation leading to rapid establishment of secondary complications in diabetes mellitus. *Juornal Diabetes Complications*, vol.30, no.3, pp. 398-450, 2016.

[65] Behl T, Kaur I, Kotwani A. Implication of oxidative stress in progression of diabetic retinopathy. *Survey of ophthalmology*, vol. 61, no. 2, pp. 187-196, 2016.

- [66] Lien Ai Pham-Huy, Hua He, Chuong Pham-Huy. Free Radicals, *International journal of Biomedical science*, vol. 4, no. 2, pp. 89-96, 2008.
- [67] D'Autréaux B, Toledano MB. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nature reviews Molecular cell biology*, vol. 8, no.10, pp. 813-824, 2007.
- [68] Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. *Trends in pharmacological sciences*, vol.38, no.7, pp. 592-607, 2017.
- [69] ΚΟΝΤΟΖΗΣΗ, Ευτυχία. Μελέτη της αντιοξειδωτικής δράσης του εκχυλίσματος του φυτού *Conium divaricatum* σε ενδοθηλιακά κύτταρα. Study of antioxidant activity of *Conium divaricatum* extract on endothelial cells. *Master's Thesis*, 2019.
- [70] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, USA, 2015.
- [71] Winterbourn CC. Reactive oxygen species in biological systems.test book, chapter 8. 98–117, 2019
- [72] Tan, Zou, et al. Quantification of reactive oxygen species for photodynamic therapy. In: Optics in Health Care and Biomedical Optics VII. *International Society for Optics and Photonics*, vol. 25, no. 4, pp. 100-141, 2016.
- [73] Kembro JM, Aon MA, Winslow RL, O'Rourke B, Cortassa S. Integrating mitochondrial energetics, redox and ROS metabolic networks: A two-compartment model. *Biophysical Journal*, vol. 104, no. 2, pp. 332–343, 2013.

- [74] Umeno A, Biju V, Yoshida Y. In vivo ROS production and use of oxidative stress-derived biomarkers to detect the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. *Free Radical Research*, vol. 51, no. 4, pp. 413–427, 2017.
- [75] Lupette J, Jaussaud A, Vigor C, Oger C, Galano JM, Réversat G, et al. Non-enzymatic synthesis of bioactive isoprostanoids in the diatom *Phaeodactylum* following oxidative stress. *Plant physiology*, vol. 178, no. 3, pp. 1344–1357, 2018.
- [76] Ridwan EN, Utomo MT, Notopuro H. The Effect of Cigarette Smoke Exposure in the Fetal Growth and Fetal Development of Mice (*Mus musculus*). *Journal Ilmiah Mahasiswa Kedokteran Universitas Airlangga*, vol. 11, no. 1, pp 13-17, 2020.
- [77] Lushchak, V. I. Environmentally induced oxidative stress in aquatic animals. *Aquatic toxicology*, vol. 101, no. 1, pp. 13-30, 2011.
- [78] Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: Futuristic strategies based on these targets. *International Journal of Endocrinology*, vol. 14, no. 5, pp. 1-10, 2014.
- [79] Akıllıoğlu HG, Gökmen V. Advanced Glycation End Products (AGEs). *Chem Hazards Therm Foods*, vol. 3, no. 4, pp. 121-151, 2019.
- [80] Hotta N, Kawamura T, Umemura T. Are the polyol pathway and hyperuricemia partners in the development of non-alcoholic fatty liver disease in diabetes. *Journal Diabetes Investigation*, vol. 7, no. 4, pp.1-3, 2019.

- [81] Fantus IG, Goldberg HJ, Whiteside CI, Topic D. The Hexosamine Biosynthesis Pathway: Contribution to the Pathogenesis of Diabetic Nephropathy. : *Contemporary Diabetes The Diabetic Kidney*, vol. 5, no. 4, pp. 117-133, 2010.
- [82] Yin L, Fang Y, Song T, Lv D, Wang Z, Zhu L, et al. FBXL10 regulates cardiac dysfunction in diabetic cardiomyopathy via the PKC β 2 pathway. *Journal of cellular and molecular medicine*, vol. 23, no. 4, pp. 2558-2567, 2019.
- [83] Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications review-Article. *Cell death & disease*, vol. 9, no. 2, pp. 162-178, 2018.
- [84] Ankamah E, Sebag J, Ng E, Nolan JM. Vitreous antioxidants, degeneration, and vitreo-retinopathy Exploring the links. *Antioxidants*, vol. 9, no. 1, pp. 1-20, 2020.
- [85] Qaid EYA, Zakaria R, Sulaiman SF, Yusof NAM, Shafin N, Othman Z, et al. Role of antioxidants in hypoxia-induced learning and memory deficit. *International Medical Journal*, vol. 27, no. 1, pp. 62-66, 2020.
- [86] Tafani M, Sansone L, Limana F, Arcangeli T, De Santis E, Polese M, et al. The Interplay of Reactive Oxygen Species, Hypoxia, Inflammation, and Sirtuins in Cancer Initiation and Progression. *Oxidative medicine and cellular longevity*, pp. 1-18, 2016.
- [87] Alkadi H. A review on free radicals and antioxidants. *Infectious Disorders - Drug Targets (Formerly Current Drug Targets - Infectious Disorders)*, vol. 20, no. 1, pp. 16-26, 2020.

- [88] Salimi A, Motallebi A, Ayatollahi M, Seydi E, Mohseni AR, Nazemi M, et al. Selective toxicity of persian gulf sea cucumber holothuria parva on human chronic lymphocytic leukemia b lymphocytes by direct mitochondrial targeting. *Environmental toxicology*, vol. 32, no. 4, pp. 1158-1169, 2017.
- [89] Perumal S, Langeswaran K. Diosmin anti-tumor efficacy against hepatocellular carcinoma . *Biomedical Research*, vol. 3, no. 5, pp. 1-10, 2019.
- [90] Palmieri B, Sblendorio V. Oxidative stress tests: Overview on reliability and use. Part II. *European review for medical and pharmacological sciences*, vol. 11, no. 6, pp. 383-399, 2007.
- [91] Sedaghat Doost A, Van Camp J, Dewettinck K, Van der Meeren P. Production of thymol nanoemulsions stabilized using Quillaja Saponin as a biosurfactant: Antioxidant activity enhancement. *Food Chemistry*, vol. 293, no 21, pp. 134-143, 2019.
- [92] Hulbert AJ, Martin N, Else PL. Lipid peroxidation and animal longevity. *Lipid Peroxidation: Inhibition, Effects and Mechanisms*, vol. 8, no. 12, pp. 1-18, 2017.
- [93] Sony NM, Ishikawa M, Hossain MS, Koshio S, Yokoyama S. The effect of dietary fucoidan on growth, immune functions, blood characteristics and oxidative stress resistance of juvenile red sea bream, *Pagrus major*. *Fish Physiol Biochemistry*, vol. 45, no. 1, pp. 439-454, 2019.
- [94] Sousa BC, Pitt AR, Spickett CM. Chemistry and analysis of HNE and other prominent carbonyl-containing lipid oxidation compounds. *Free Radical Biology and Medicine*, vol. 111, no. 4, pp. 294-308, 2017.

- [95] Félix R, Valentão P, Andrade PB, Félix C, Novais SC, Lemos MFL. Evaluating the In Vitro Potential of Natural Extracts to Protect Lipids from Oxidative Damage. *Antioxidants*, vol. 9, no. 3, pp. 231-245, 2020.
- [96] Pasupathi SP. Chronic tobacco smoking and gastric cancer: a review. *International Journal of Current Pharmaceutical Research*, vol. 1, no. 2, pp. 48-66, 2011.
- [97] Gandhi SD. Comparison of Analytical Tehcniques To Evaluate the Effect. 2012.
- [98] Shamsa AJM. Evaluation of lipid peroxidation, lipid profile and antioxidant status in patients with non-insulin dependent diabetes mellitus in Najaf/Iraq. *kufa Journal for Nursing sciences*, vol. 2, no. 3, pp. 143-151, 2012.
- [99] Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, vol. 54, no. 4, pp. 287-293, 2018.
- [100] Wang W, Zhang X, Deng F, Yuan R, Shen F. Genome-wide characterization and expression analyses of superoxide dismutase (SOD) genes in *Gossypium hirsutum*. *BMC Genomics*, vol. 18, no. 1, pp. 1-25, 2017.
- [101] Bartosz G. Total antioxidant capacity. *Advances in clinical chemistry*, vol. 37, no.3, pp. 219-292, 2003.
- [102] Young IS. Measurement of total antioxidant capacity. *Journal Clinical Pathology*, vol. 54, no. 5, pp. 339, 2001.

- [103] Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum. *BMC veterinary research*, vol. 12, no. 1, pp. 1-7, 2016.
- [104] Smith BA, Mottishaw CR, Hendricks AJ, Mitchell J, Becker S, Ropski PS, et al. Potential roles of inorganic phosphate on the progression of initially bound glucopyranose toward the nonenzymatic glycation of human hemoglobin: Mechanistic diversity and impacts on site selectivity. *Cogent biology*, vol. 4, no. 1, pp. 2025-2331, 2018.
- [105] Ezegbogu MO, Abdulsalam K. Glycated Haemoglobin (HbA1c): An Update on Available Methods. *Bayero Journal of Pure and Applied Sciences*, vol. 11, no. 1, pp. 8-14, 2018.
- [106] Gupta S, Jain U, Chauhan N. Laboratory diagnosis of HbA1c: a review. *Journal of Nanomedicine Research*, vol. 5, no.4, pp. 120-130, 2017.
- [107] Feingold KR, Grunfeld C. Lipids: A key player in the battle between the host and microorganisms. *Journal Lipid Research*, vol. 53, no. 12, pp. 2487-2490, 2012.
- [108] Langlois, M. R., Nordestgaard, B. G., Langsted, A., Chapman, M. J., Aakre, K. M., Baum, H., ... & Collinson, P. Quantifying atherogenic lipoproteins for lipid-lowering strategies: consensus-based recommendations from EAS and EFLM. *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 58, no. 4, pp. 496-517, 2020.
- [109] Lim LS, Wong TY. Lipids and diabetic retinopathy. *Expert opinion on biological therapy*, vol. 12, no. 1, pp. 93-105, 2012.

- [110] Sacks FM, Jensen MK. From high-density lipoprotein cholesterol to measurements of function: prospects for the development of tests for high-density lipoprotein functionality in cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology*, vol. 38, no. 3, pp. 487-499, 2018.
- [111] Khalil S, Khayyat S, Al-Khadra Y, Alraies MC. Should all diabetic patients take statin therapy regardless of serum cholesterol level. *Expert Review of Cardiovascular Therapy*, vol. 17, no. 4, pp. 237-239, 2019.
- [112] K. McMullen M. Fructose Increases Uric Acid Contributing to Metabolic Syndrome - Herbal, Nutritional and Dietary Strategies to Reduce Uric Acid. *OBM Integrative and Complementary Medicine*, vol. 3, no. 3, pp. 1-40, 2018.
- [113] Xia J, Wang Z, Zhang F. Association between related purine metabolites and diabetic retinopathy in type 2 diabetic patients. *International journal of endocrinology*, vol. 5, no. 2, pp. 198-210, 2014.
- [114] Misra A, Dhurandhar N V. Current formula for calculating body mass index is applicable to Asian populations. *Nutrition and Diabetes*, vol. 9, no. 1, pp. 1-12, 2019.
- [115] Muntner P, Shimbo D, Carey RM, Charleston JB, Gaillard T, Misra S, et al. Measurement of blood pressure in humans: A scientific statement from the american heart association. *Hypertension*, vol. 73, no. 5, pp. 35-66, 2019.
- [116] Boroushaki, Mohammad Taher, et al. "Reno-protective effect of Rheum turkestanicum against gentamicin-induced nephrotoxicity. *Journal of basic medical sciences*, vol. 22, no. 3, pp. 328-247, 2019.
- [117] DAY, Caroline; BAILEY, Clifford J. HbA1c-changing units. *The British*

Journal of Diabetes & Vascular Disease, vol. 9, no. 3, pp. 134-136, 2009.

[118] Nagaraju R, Joshi AKR, Rajini PS. Estimation of plasma triglycerides with correction for free glycerol by orlistat inhibition of lipoprotein lipase activity.

Analytical biochemistry, vol. 439, no. 1, pp. 44-46, 2013.

[119] Ibrahim, S., Khayat, M. I., & Awama, M. A. Comparative Study for the measurement of HDL Cholesterol between a direct Assay and a Precipitation Method. *Research Journal of Pharmacy and Technology*, vol. 11, no. 3, pp. 1035-1038, 2018.

[120] Saphier O, Barak T, Hamou B, Silberstein T. Caffeic and Gallic Acids Protect Commercial Infant Milk Formula from Oxidative Damage. *Advantage dairy Research*, vol. 7, no. 2, pp. 4-11, 2019.

[121] Sarrafan-Chaharsoughi Z, Manaviat MR, Namiranian N, Yazdian-Anari P, Rahmanian M. Is there a relationship between body mass index and diabetic retinopathy in type II diabetic patients. A cross sectional study. *Journal Diabetes Metabolsim Disorder*, vol. 17, no. 1, pp. 63-69, 2018.

[122] Garg P, Mishra S, Mullick R. A Correlative Study of Duration of diabetes mellitus, microalbuminuria, hyperlipidemia with the severity of diabetic retinopathy. *International journal Ophthalmic Research*, vol. 5, no. 1, pp. 308-313, 2019.

[123] Biadgo B, Abebe SM, Baynes HW, Yesuf M, Alemu A, Abebe M. Correlation between serum lipid profile with anthropometric and clinical variables in patients with type 2 diabetes mellitus. *Ethiopian journal of health sciences*, vol. 27, no. 3, pp. 215-226, 2017.

- [124] Kharroubi AT, Darwish HM, Akkawi MA, Ashareef AA, Almasri ZA, Bader KA, et al. Total antioxidant status in type 2 diabetic patients in Palestine. *Journal Diabetes Research*, vol. 12, no. 3, pp. 123-141, 2015.
- [125] Pieme CA, Tatangmo JA, Simo G, Nya PCB, Moor VJA, Moukette BM, et al. Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes. *BMC Research Notes*, vol. 10, no. 1, pp. 141, 2017.
- [126] Cetin EN, Bulgu Y, Ozdemir S, Topsakal S, Akin F, Aybek H, et al. Association of serum lipid levels with diabetic retinopathy. *International journal Ophthalmic Research*, vol. 6, no. 3, pp. 346, 2013.
- [127] Ganjifrockwala F, Joseph J, George G. Serum oxidized LDL levels in type 2 diabetic patients with retinopathy in Mthatha Region of the Eastern Cape Province of South Africa. *Oxidative medicine and cellular longevity*, vol. 13, no. 7, pp. 24-37, 2016.
- [128] Kassim MN. Oxidative stress and metabolic control in type 2 diabetic patients. *AL-TAQANI*, vol. 24, no. 4, pp. 70-78, 2011.
- [129] Rao S, Sahayo BJ. A study of serum uric acid in diabetes mellitus and prediabetes in a south Indian tertiary care hospital. *Journal of Health and Allied Sciences NU*, vol. 2, no. 2, pp. 18-23, 2012.
- [130] Munilakshmi U, Prabhavathi K, Shashidhar KN, Reddy M, Lakshmaiah V. Association of serum uric Acid with Anthropometric, hbA1c And Lipid profile in diabetic retinopathy. *International Journal of Current Research and Review*, vol. 7, no. 4, pp. 20, 2015.

- [131] Kedari GSR. Evaluation of thyroid status, oxidant stress and antioxidant status in patients with type 2 diabetes mellitus. *Journal of Clinical and Diagnostic Research*, vol. 5, no. 2, pp. 254-261, 2011.
- [132] Ma Q, Liu H, Xiang G, Shan W, Xing W. Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus. *Biomed reports*, vol. 4, no. 6, pp. 737-740, 2016.
- [133] Adnan M, Shabbir I, Ali Z, Ali SF, Rahat T. Impact of age, gender and diabetes on serum lipid levels. *Journal of Medical Research*, vol. 52, no. 1, pp. 22, 2013.
- [134] Basaran N, Evliyaoglu O, Sucu V, Dikker O, Bulut L, Tezcan F, et al. Changing of uric acid levels by age and sex in patients with diabetes mellitus. *Journal of Clinical and Experimental Investigations*, vol.7, no.1, pp. 1-6, 2016.
- [135] Prema GS, Anitha R, Padmini O. Relation between Body Mass Index (BMI) and Fasting Blood Sugar (FBS). *International Journal of Contemporary Medical Research*, vol.3, no.1, pp. 2454-7379, 2016.
- [136] Babikr WG, Alshahrani ASA, Hamid HGM, Abdelraheem AHMK, Shalayel MHF. The correlation of HbA1c with body mass index and HDL-cholesterol in type 2 diabetic patients. *An International Journal of Medical Sciences*, vol.27, no. 4, pp. 976-1683, 2016.
- [137] KS P, Mohandoss R, Jaiganesh D. Correlation between lipid profile with duration, HbA1c values, BMI in type 2 DM patients. *Indian Journal of Basic and Applied Medical Research*, vol. 6, no. 3, pp. 524-529, June 2017.
- [138] Patrick GL. An introduction to medicinal chemistry. Oxford university

press, 2013.

[139] Sinha A, Garg R. Relationship between duration of disease, BMI and blood glucose with HbA1c levels in type II diabetes mellitus patients in India. *Indian Journal of Applied Research*, vol. 9, no. 9, pp.65-67, 2019.

[140] Thagele SN, Sharma AK, Singh N, Kumar M, Rawat D, Sharma S. Impact of disease duration on lipid profile in type 2 diabetes mellitus patients. *Journal of Medical Science Clinical Research*, vol. 9, no. 3, pp. 697-701, 2018.

[141] Sari MI, Sari N, Darlan DM, Prasetya RJ. Cigarette smoking and hyperglycaemia in diabetic patients. *Open access Macedonian journal of medical sciences*, vol. 6, no. 4, pp. 634, 2018.

[142] Athyros VG, Katsiki N, Doumas M, Karagiannis A, Mikhailidis DP. Effect of tobacco smoking and smoking cessation on plasma lipoproteins and associated major cardiovascular risk factors: a narrative review. *Current medical research and opinion*, vol. 29, no. 10, pp. 1263-1274, 2013.

[143] Mouhamed DH, Ezzaher A, Neffati F, Douki W, Gaha L, Najjar MF. Effect of cigarette smoking on plasma uric acid concentrations. *Environmental health and preventive medicine*, vol. 16, no. 5, pp. 307-312, 2011.

[144] Nsonwu-Anyanwu AC, Offor SJ, John II. Cigarette Smoke and Oxidative Stress Indices in Male Active Smokers. *Reactive Oxygen Species*, vol. 5, no. 15, pp. 199-208, 2018.

[145] Canoy D, Wareham N, Luben R, Welch A, Bingham S, Day N, et al. Risk Factors and Chronic Disease Cigarette Smoking and Fat Distribution in 21 , 828 British Men and Women : A Population- based Study. *Obesity research*, vol. 13,

no. 8, pp. 1466-1475, 2005.

[146] Veghari G, Sedaghat M, Joshaghani H, Hoseini A, Niknezhad F, Angizeh A, et al. The prevalence of obesity and its related risk factor in the north of Iran in 2006. *Journal of Research in Health Sciences*, vol. 10, no. 2, pp. 116-121, 2010;

[147] Quartey P. A comparative study of oxidative stress and type 2 diabetes mellitus in rural and urban communities in the Ashanti Region, Ghana. 2012.

[148] Kaštelan S, Tomić M, Gverović Antunica A, Ljubić S, Salopek Rabatić J, Karabatić M. Body mass index: a risk factor for retinopathy in type 2 diabetic patients. *Mediators Inflammation*, vol. 13, no. 3, pp. 1-8, 2013.

[149] Rao TMV, Vanukuri NK. A study on serum uric acid levels in type 2 diabetes mellitus and its association with cardiovascular risk factors. *Certified Instructors of Infant Massage*, vol. 3, no. 12, pp. 148-155, 2016.

[150] Morteza A, Jenab Y, Nargesi AA, Ghazizadeh Z, Salabati M. Urea and oxidative stress in type 2 diabetes. *Journal Metabolic Syndrome*, vol. 1, no. 2, pp. 1-5, 2012.

Appendix

Appendix 1. QUESTIONNAIRE OF THE STUDY

Name

Signature

PERSONAL DETAILS

SEX: Male
 Female Are you currently pregnant?

Address:

Date of birth : or age : years

Height (m) Weight (Kg):

BMI: BP:

LIFE STYLE

Smoking:

Smoker: has smoked cigarettes regularly for at least 6 months

Ex-smoker: stopped for at least 1 year after regular smoking

Never

MEDICAL HISTORY

Has a doctor ever told you that you have any of the following conditions?
 (If yes please specify date of diagnosis)

Diabetes:	Yes	No
Diabetic retinopathy:	Yes	No
High blood pressure (doctor gave pills):	Yes	No
Do have any disease apart from the diabetes?	Yes/	No

If yes what kind of disease is it?

Family history

Diabetes:	Father	mother	both
Hypertension:	Father	mother	both

MEDICATIONS:

List any medications that you are currently taking for diabetes

Have you ever taken any antioxidant supplements for at least the past month?

Yes

No

(If yes please specify):

Appendix 2.

Normal Values for Lab Tests

Test	SI units	Conversionvalue
Fasting (plasma or serum) Glucose	3.9-6.1 mmol/l	70-110 mg/dL
High density lipoproteins (HDL)	> 0.91 mmol/L	> 35 mg/dL
Cholesterol (serum)	< 5.2 mmol/L	< 200 mg/dL
Low density lipoproteins (LDL)	< 3.4 mmol/L	< 130 mg/dL
Triglycerides	0.45-1.71 mmol/L	40-150 mg/dL
Uric acid (serum)	120-420 μ mol/L	2.0-7.0 mg/dL
HbA1C	Less than 6.5 %	

الخلاصة

مجال البحث الحالي هو العلاقة بين الإجهاد التأكسدي وداء السكري من النوع 2 مع / بدون اعتلال الشبكية السكري. الإجهاد التأكسدي هو اختلال التوازن بين إنتاج أنواع الأكسجين التفاعلية ومضادات الأكسدة التي قد تلعب دورًا في مسببات داء السكري من النوع 2 وحدوث اعتلال الشبكية السكري الناتج عن زيادة نشاط الجذور الحرة وتراكم منتجات بيروكسيد الدهون. تم تقسيم الموضوعات 232 إلى 96 مجموعة تحكم ، و 78 شخصًا يعانون من داء السكري من النوع 2 بدون اعتلال الشبكية السكري ، و 58 مصابًا باعتلال الشبكية السكري. النطاق العمري لجميع المواد الدراسية أكثر من 21 عامًا. تم تجنيدهم من المناطق الحضرية والريفية في محافظة المثنى. تم جمع البيانات الاجتماعية الديموغرافية من الموضوعات. القياسات الأنثروبومترية بما في ذلك ضغط الدم الانقباضي والانقباضي والوزن والطول. تم جمع عينات الدم بعد 12 ساعة من الصيام طوال الليل لتحليل مستويات الجلوكوز ، نسبة الهيموجلوبين السكري ، نسبة الدهون ، مؤشرات الإجهاد التأكسدي وغيرها من العوامل الكيميائية الحيوية في هذه الدراسة ، وجد أن مستويات مضادات الأكسدة في البلازما كانت أعلى في مرضى السكري من المرضى الذين يعانون من اعتلال الشبكية بينما تم العثور على مستويات مالونديالدهيد أعلى في موضوعات اعتلال الشبكية مقارنة بمرضى السكري. يلعب التدخين ومؤشر كتلة الجسم وضغط الدم والعمر تأثيرًا مهمًا على مستويات الإجهاد التأكسدي لدى المرضى ومضاعفاته. لا تتأثر قيم المعلمات المؤكسدة بمكان السكن. هناك علاقة ارتباط قوية بين مدة المرض وتطور المرض إلى حدوث اعتلال الشبكية السكري ، حيث كان في اعتلال الشبكية السكري أعلى من مرضى السكري غير المصابين باعتلال الشبكية. توصي الدراسة الحالية بإجراء فحوصات طبية دورية وتغيير نمط الحياة المناسب ، وفقدان الوزن أيضًا للسيطرة على اضطراب شحميات الدم وتقليل مضاعفات مرض السكري من النوع 2 العلاج بمضادات الأكسدة مثل الفيتامينات التي قد تدعم قدرة مضادات الأكسدة الذاتية وتقليل معدلات البيروكسيد لمنع حدوث مضاعفات.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة المثنى / كلية العلوم
قسم الكيمياء

تقدير مضادات الأكسدة والشدة التأكسدية عند مرضى اعتلال الشبكية السكري

رسالة مقدمة الى كلية العلوم جامعة المثنى كجزء من متطلبات نيل درجة الماجستير
في علوم الكيمياء مقدمة من قبل

يسرى علي حميد
بكلوريوس علوم كيمياء 2006

بأشراف
ا. د. جواد كاظم مريح

2020