Anti-oxidant status in the sera and leukocytes of NIDDM patients

A Thesis

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By
Ahmed Younis Abd Al Janaby
B.Sc. University of Baghdad
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ABSTRACT

The present study involved the examination of (50) patients (24 M and 26 F) suffering from Diabetes Mellitus type II (NIDDM), and (42) of healthy control (22 M and 20 F), from Al-Mahmodia general Hospital in Al-Mahmodia city bteween April 2004 and October 2004 .

The concentration of vitamin C was measured in serum and Leukocytes. In addition the concentration of antioxidant such as glutathione, uric acid and creatinine were measured.The concentration of total cholesterol, malondialdehyde and the activity of creatine kinase were measured also.

Compared with healthy control, vitamin C concentration was found significantly decreased in serum and leukocytes except in diabetic female patient, which decreased but not significantly.

The concentration of glutathione was found significantly decreased compared with healthy control, while the concentraion of uric acid and creatinine were found significantly increased as compared with healthy control.

The activity of creatine kinase was found significantly increase , as well as, the concentration of malondialdihyde and total cholesterol compared with that found in healthy control.

ACKNOWLEGMENTS

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A word of thanks also goes to every one my pen has overlooked but my heart cherishes.

CONTENTS

LIST OF ABBREVIATIONS

A	absorbence
ACD	acid-citrate-dextrose
BMI	body mass index
CK	creatine kinase
COX	Cyclooxygenase
CRF	chronic renal failure
Crn	creatinine
DHA	dehydroascorbic acid
DM	Diabetes mellitus
DNPH	2,4-dinitrophenylhydrazine
DTNB	5,5-Dithio bis(2-nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic acid
ESR	Electron spin resonance
FFA	free fatty acid
FG	impaired fasting glycaemia
FPG	fasting plasma glucose
GAA	guanidinoacetic acid
GAD	Auto antibodies to glutamic acid decarboxylase
GDM	gestational diabetes mellitus
GFR	glomerular filtration rate
GSH	glutathione
GST	Glutatione s-transferases
IAAs	Auto antibodies to insulin
ICAs	islet cell antibodies
IDDM	insulin dependent diabetes mellitus
IFG	impaired fasting glycaemia
IGT	impaired glucose tolerance
MDA	malondialdehyde
m-hpo_3	Meta phosphoric acid
NIDDM	Non insulin dependent diabetes
OGTT	oral glucose tolerance test
RNS	Reactive nitrogen species

PAGE	Polyacrylamide gel electrophoresis
P	probability
PUFAs	Polyunsaturated fatty acids
RCF	relative centrifugal force
ROS	Reactive oxygen species
SCC	serum creatinine concentration
SD	standared deviation
STC	serum total cholesterol
TCA	Trichloroacetic acid

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CHAPTER ONE

1. INTRODUCTION

1.1 General inroduction

Diabetes Mellitus (DM) is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin.[1] or it is a metabolic disorder in which the ability to oxidize carbohydrates is more or less completely lost; usually due to faulty pancreatic activity, especially of the islets of langerhans, and consequent disturbance of normal insulin mechanism .[2]

Clinical diabetes may be divided into four general subclasses as follows :-[1,3,4]

1-Type 1 Diabetes ; called insulin dependent diabetes mellitus (IDDM) , “Juveenile-Onset diabetes”(5% to 10% of the population with diabetes) , this is divided to

I-Immune destruction

II-Idiopathic

2-Type 2 Diabetes ; called Non-insulin-dependent diabetes mellitus (NIDDM) , “Adult – onset diabetes”(90% to 95% of the population with diabetes).

3-Gestational diabetes mellitus :-

I-Impaired fasting glucose.

II-Impaired glucose to lerance

4-Other specific types of diabetes:-

These groups together account 1% to 2% of patients with diabetes:-

I-Genetic defects of B-cell function.

II- Genetic defects in insulin action.

III- Diseases of the exocrine pancreas.

IV-Endocrinphies.

V-Drug-or chemical-induced.

VI-Anti-insulin receptor antibodies.

VII-Infection.

VIII-Other genetic syndromes sometimes associated with diabetes.

1.2 General characteristics of DM

1.2.1 Type 1 diabetes

Type 1 diabetes is charactrized by cellular-mediated autoimmune destruction of islet *β*-cells.

Markeres:-

-islet cell antibodies(ICAs).

-Auto-antibodies to insulin(IAAs)

-Auto-antibodies to glutamic acid decarboxylase(GAD)

-Auto-antibodies to tyrosine phosphatases.

Laboratory finding

-Hyperglycemia.

-Ketonuria.

-Low or undetectable serum insulin and c-peptide levels

-Auto –antibodies aginst components of the islet *β*-cells.[4]

1.2.2 Type 2 diabetes

Type 2 diabetes is due to insulin insensitivity combined with a failure of insulin secretion to overcome this by hypersecretion,resulting

in relative insulin deficiency. There is a strong genetic predisposition. Type 2 diabetes is more common in individuals with family history of the disease, in individuals with hypertention or dyslipidaemia and in certain ethnic groups.[4]

The risk of developing type 2 diabetes increases with:-

-Family history of diabetes.

-Obesity(more than 20% over ideal body weight or body mass index(BMI) more than 25 Kg / m^2)

-Membership of some ethnic groups

-Age more than 45 years.

-Previously identified impaired fasting glycaemia (IFG) or impaired glucose tolerance (IGT).

-Hypertention (more than 140/90 mmHg in adults).

-HDL cholesterol level less than 1.0 mmol/L and/or a triglyceride level more than 2.3 mmol/L.

-Reduced physical activity.

-History of gestational diabetes mellitus (GDM) or delivery of babies more than 4.5 Kg.

Laboratory finding:-

-Hyperglycemia.

-Hyperlipidaemia.

-High serum insulin/c-peptide level.

-Defective insulin secretion.

-Insulin resistance.[4]

1.2.3 Gestational diabetes mellitus (GDM)

Definition:- Any degree of clinical glucose intolerance with onset or first recognition during pregnancy.[4]

The following problems may develop with (GDM):-

-Altered duration of pregnancy.

-Placental failure.

-Hypertention/pre-eclampsia.

-High birth weight of the newborn.

Diagnosis of GDM:- Fasting plasma glucose level more than 7.0 mmol/L or casual plasma glucose more than 11.1 mmol/L , confirmed on a subsequent day.[4]

Laboratory strategy to diagnose GDM :-

One step approach : oral glucose tolerance test (OGTT)
(75 g glucose) 2 mg/kg

Two step approach : 1- First OGTT with 50 g glucose
Load ; cut-off value after 1 hour
Plasms glucose more than 7.8 mmol/L
2- second OGTT with 75 g glucose
load and evaluation as the
standard OGTT.[4]

Six weeks after pregnancy or later the women should be re-examined for the presence of diabets mellitus or IGT.[4]

Figure 1 shows the diagnostic strategy for diabetes mellitus.

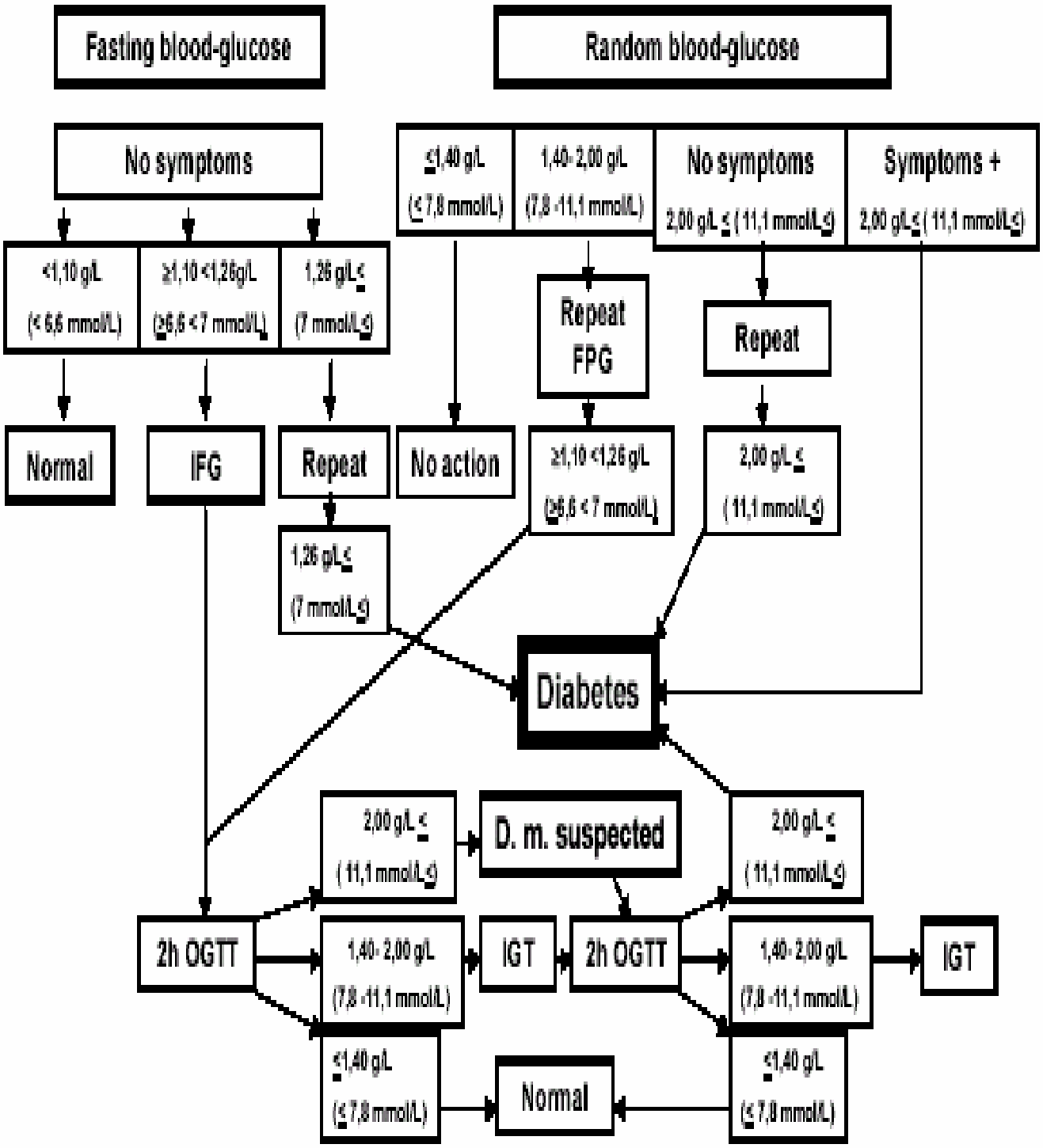


Figure1-1 Diagnostic Strategy for diabetes. (FG) impaired fasting glycaemia,(FPG) fasting plasma glucose , (OGTT) oral glucose tolerance test , (D.m.) diabetes mellitus , (IGT) impaired glucose tolerance.[4]

1.3 Reactive oxygen species and reactive nitrogen species

1.3.1 Reactive oxygen species (ROS)

Reactive Oxygen Species can be classified into Oxygen-centered radicals and Oxygen-centered nonradicals. Oxygen – centered radicals are superoxide anion ($O^{-\cdot}_2$) , hydroxyl radical ($^{\cdot}OH$) , alkoxyl radical ($RO^{\cdot}$) , and peroxyl radical ($ROO^{\cdot}$) .While Oxygen-centered nonradicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$).[5,6]

1.3.2 Reactive nitrogen species (RNS)

Reactive Nitrogen Species such as nitric oxide free radical ($NO^{\cdot}$ **),** nitric dioxide ($NO_2^{\cdot}$ **)** as example for free radical , and peroxynitrate ($OONO^-$) as non radical .[6,7]

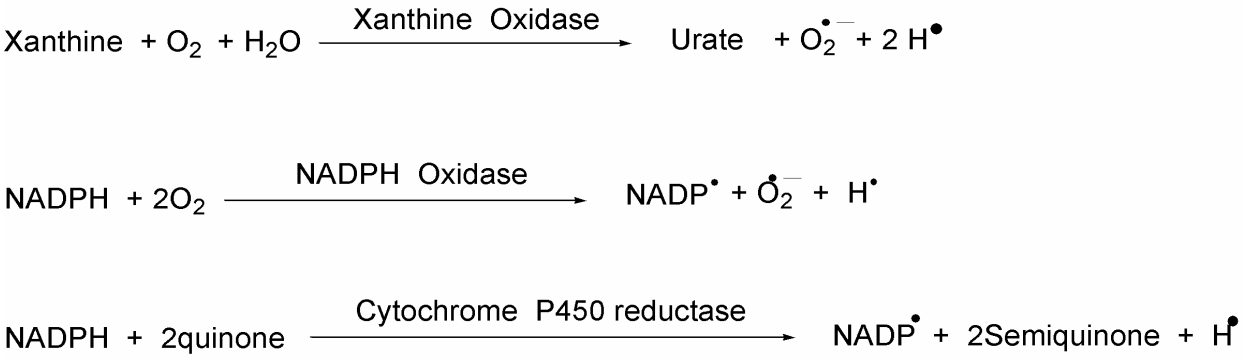
Free radicals are defined as any species that have one or more unpaired electrons and are , therefore , highly reactive molecules, chemically , they are designated by a signal “ς”.[7]

1.3.3 Production of ROS and RNS in human body :-

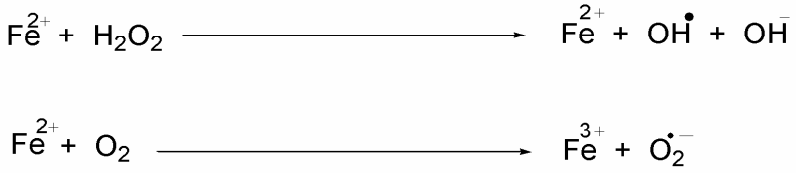
ROS and RNS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays , ozone , cigarette smoking , air pollutants and industrial chemicals.[8,9]

Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. [8] (Figure 1-2).

Enzymatic free radical formation



Nonenzymatic free radical formation



Lipid oxidation by radical attack (L= lipid)

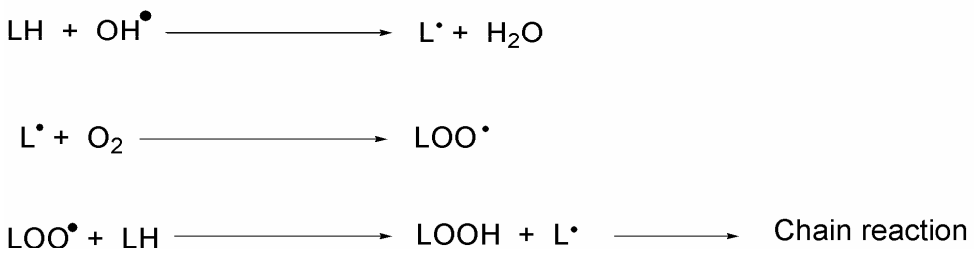


Figure1-2 Examples of free radical formation. [8]

Oxygen is required for the generation of all ROS , RNS and reactive chlorine species. The major reactions for the production of oxygen and nitrogen free radicals in the body are illustrated in Figure (1-3). [7]

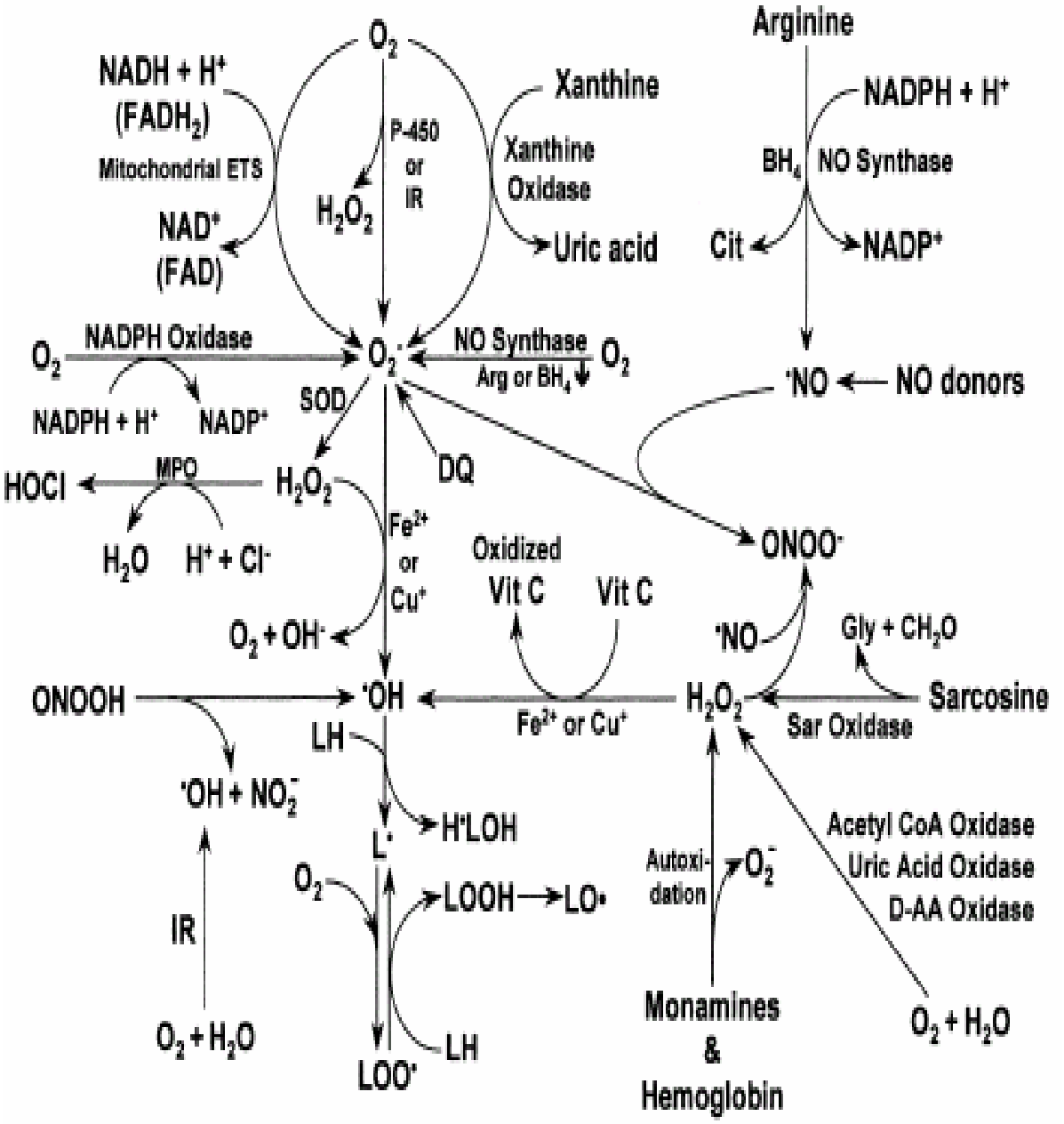


Figure1-3 Production of oxygen and nitrogen free radicals and other reactive species in mammalian cells. AA, amino acid; Arg, L-arginine; BH_4, (6R)-5,6,7,8,-tetrahydro-L-biopterin; CH_2O, formaldehyde; Cit, L-citrulline; DQ, diquat; ETS, electron transport system; FAD, flavin adenine dinucleotide (oxidized); $FADH_2$, flavin adenine dinucleotide (reduced); Gly, glycine; H_2O2, hydrogen peroxide; HOCl, hypochlorous acid; H•LOH, hydroxy lipid radical; IR, ionizing radiation; L•, lipid radical; LH, lipid (unsaturated fatty acid); LO•, lipid alkoxyl radical; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; MPO, myeloperoxidase; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); •NO, nitric oxide; O_2^- , superoxide anion radical; •OH, hydroxyl radical; $ONOO^-$, peroxynitrite; P-450, cytochrome P-450; PDG, phosphate-dependent glutaminase; Sar, Sarcosine; SOD, superoxide dismutase; Vit C, vitamin C; Vit E, vitamin E (α -tocopherol).[7]

1.4 Antioxidant: -

An antioxidant is any substance that, when present at low concentration compared to those of an oxidizable substance, significantly delays or prevents the oxidation of that substrate. The term " oxidizable substrate " includes almost everything found in living cell, including proteins, lipids, carbohydrates and DNA. [10]

Bagchi and Puri [8] defined an antioxidant as a molecule stable enough to donate an electron to rampaging free radical and to neutralize it, thus reducing its capacity to damage. [8]

Antioxidants are a defense mechanism to counter attack by free radicals and other ROS and RNS. There are different oxidants as well as in different cellular compartments. These mechanisms include enzymes such as glutathione peroxidase, superoxid dismutase (SOD) and catalase. The other mechanism of defense against free radical damage is the presence of antioxidants, such as Vitamin C, Vitamin E and reduced glutathione (GSH). [8,12,13,14]

The mechanism of antioxidant action can include removal of oxygen, scavenging ROS and RNS, inhibition of ROS and RNS formation, binding with metal ion needed for catalysis of ROS, generation and upregulation of endogenous antioxidant defenses. [11]

Oxidative stress originating from improper control of oxygen reduction is believed to play a role in the tissue and cellular damage caused by a variety of conditions, including diabetes, neurodegenerative disease infection, aging, and ionizing radiation. [15]

1.4.1 Measurement of oxidative stress: -

Because antioxidant defenses are not completely efficient, increased free radical formation in the body is likely, increase damage. The term " oxidative stress" is often used to refer to this effect. [8]

Free radicals, in biological sample have a short half-life. So it is difficult to be measured directly. Electron spin resonance (ESR) is the technique used, and it is often requires previous "trapping" of the radicals in order to produce more stable molecules that can accumulate in larger amounts. However, estimation of free radical damage is more easily performed. Radicals easily react with macromolecules of different classes, more stable molecules are formed, which can subsequently be measured. [16]

The relation between free radicals (and other reactive species) and antioxidants are shown in Figure (1- 4). This relation at the levels of oxidants and antioxidants may be considered as a biochemical marker of free radical generation, which is in turn, is useful to measure the oxidative stress associated with disease. [17]

One of the most frequently used measures of lipid peroxidation is the thiobarbituric acid method. [16] Protein fragmentation can be measured by Polyacrylamide Gel Electrophoresis (PAGE). Also, free radical damage to plasma proteins can be measured by quantitative determination of plasma protein profile. [18]

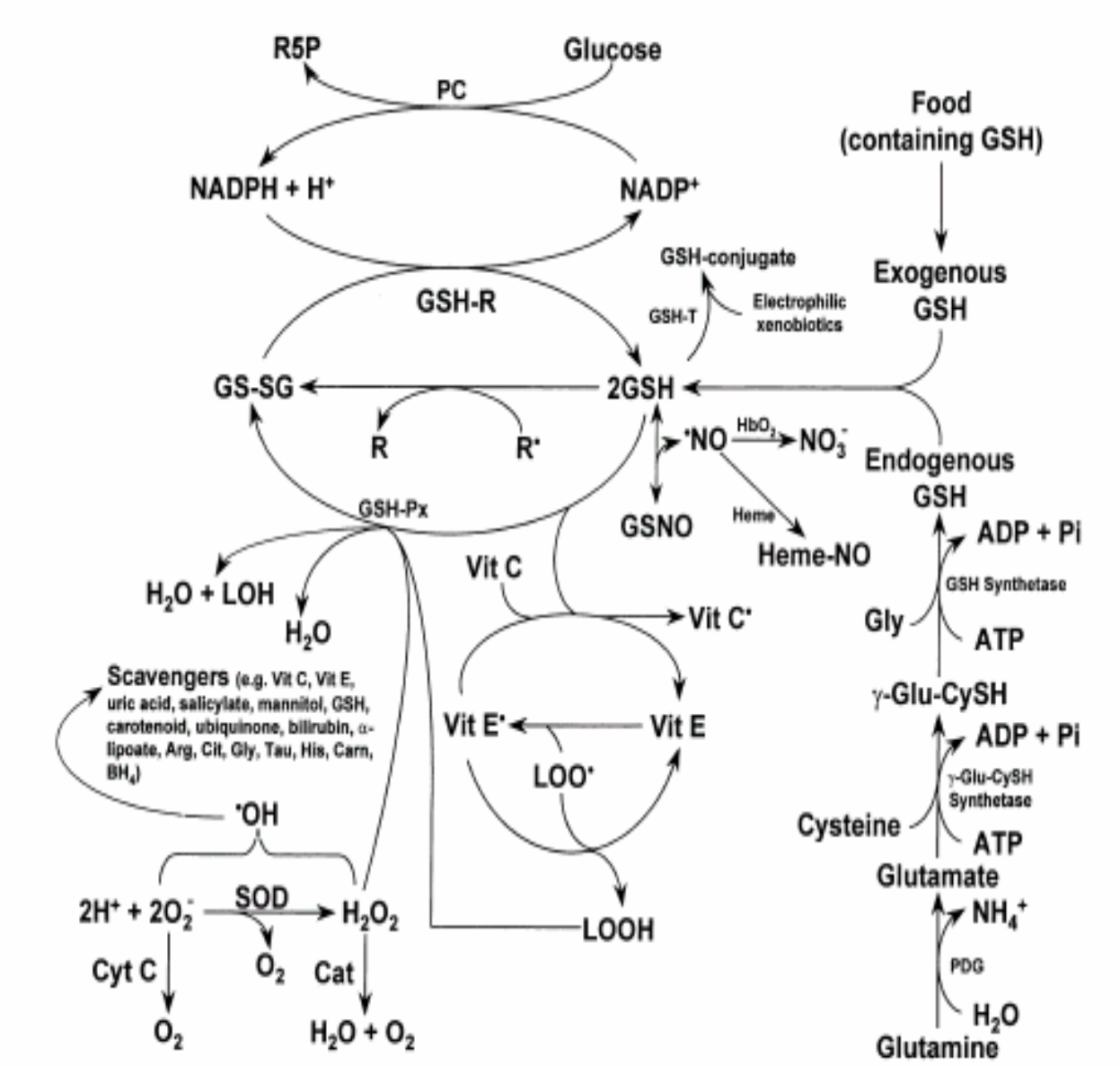


Figure1-4 Removal of oxygen and nitrogen free radicals and other reactive species in mammalian cells. ADP, adenosine diphosphate; Arg, arginine; BH_4, (6R)-5,6,7,8,-tetrahydro-L-biopterin; Carn, carnosine; Cat, catalase; Cit, citrulline; Cyt C, cytochrome C; ETS, electron transport system; Glu, L-glutamate; Gly, glycine; γ-Glu-CySH, γ-glutamyl-cysteine; GS-SG, oxidized glutathione (glutathione disulfide); GSH, glutathione (reduced form); GSH-Px, glutathione peroxidases; GSH-R, glutathione reductase; GSH-T, glutathione S-transferase; GSNO, nitrosylated glutathione; HbO_2, oxyhemoglobin; Heme-NO, heme-nitric oxide; His, histidine; LOH, lipid alcohol; $LOO^{\bullet}$, lipid peroxyl radical; LOOH, lipid hydroperoxide; $^{\bullet}NO$, nitric oxide; NO_3^-, nitrate; O_2 superoxide anion radical; $ONOO^-$, peroxynitrite; PC, pentose cycle; $R^{\bullet}$, radicals; R, non-radicals; R5P, ribulose 5-phosphate; SOD, superoxide dismutase; Tau, taurine; Vit C, vitamin C (ascorbic acid); Vit $C^{\bullet}$, vitamin C radical; Vit E, vitamin E (α-tocopherol); Vit $E^{\bullet}$, vitamin E radical. [7]

1.4.2 Glutathione: -

The term glutathione is typically used as a collective term to refer a tripeptide L-gamma-glutamyl-L-cysteinylglycine in both its monomeric and oxidized forms. Monomeric glutathione is also known as reduced glutathione which most commonly called glutathione (GSH).[20] and it is known chemically as N-(N-L-gamma-glutamyl-L-cysteinylglycine) and its dimer is as oxidized glutathione,orglutathione disulfide and diglutathione.[19] Form which is refer as L-gamma-glutamyl-L-cysteinyle-glycine disulfide and its abbreviation is GSSG[19,20]

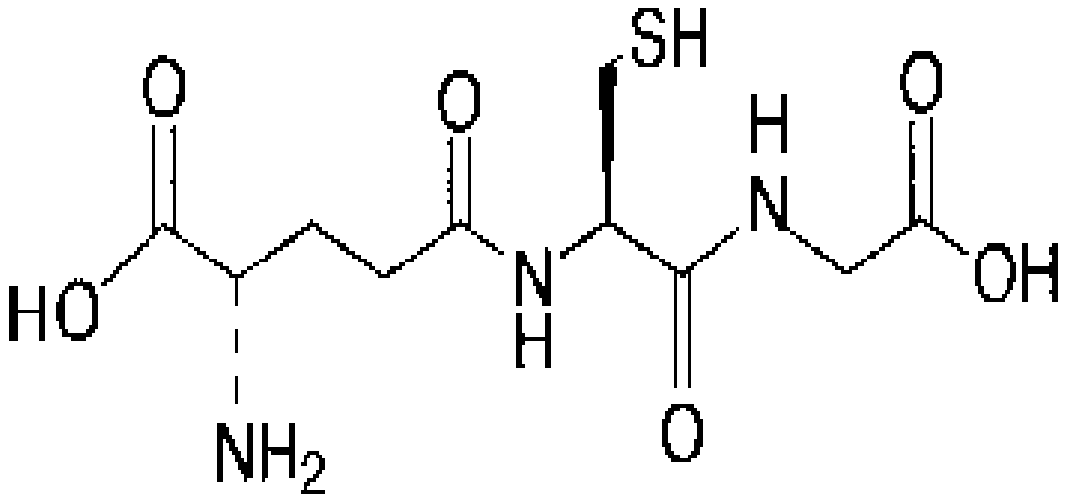


Figure1-5 Glutathione (GSH) .[20]

GSH is a relatively small molecule ubiquitous in living system.[21,22,23] Occurring naturally in all human cells, GSH is a water-

phase orthomolecule. It's intracellular depletion ultimately results in cell death and it's clinical relevance has been researched for decades. [19,24,25]

GSH is synthesized in the gamma-glutamyl cycle by the consecutive action of gamma-glutamylcysteine synthetase and glutathione synthetase .[26](Figure 1-6).

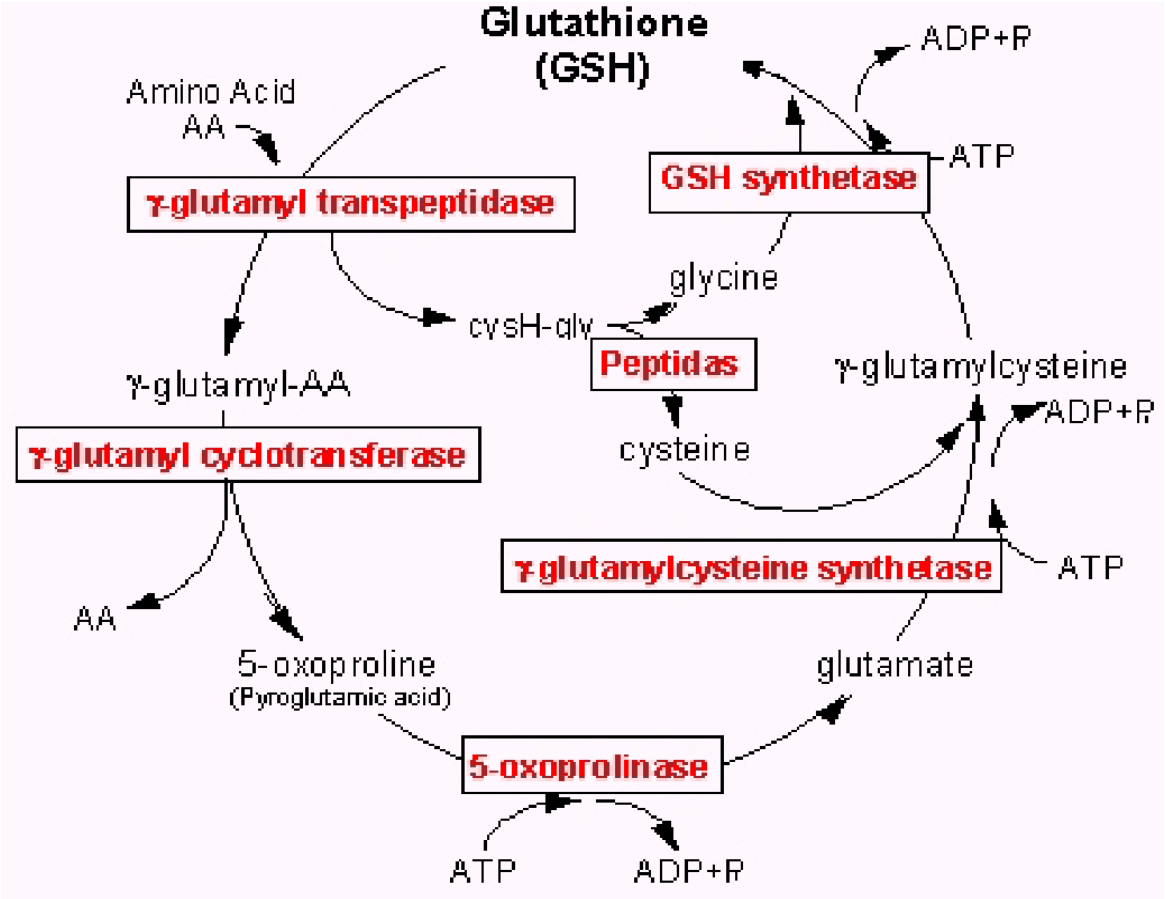


Figure1-6 The gamma-glutamyl cycle .[26]

GSH is used as a cofactor by : -

1-Multiple peroxidase enzymes, to detoxify peroxides generated from oxygen radical attack on biological molecules.

2-Transhydrogenases , to reduce oxidized centers on DNA, proteins, and other biomolecules.

3-Glutatione S-transferases (GST) is to conjugate GSH with endogenous substances (e.g., estrogens) and to exogenous electrophiles (e.g., arene oxides, unsaturated carbonyls, organic halides), and diverse xenobiotics.[20]

GSH is the major antioxidant produced by the cell, protecting it from free radicals, which, if left unchecked, will damage or destroy key cell components (e.g., membranes, DNA). [24,27,28] In addition GSH recycles other well known antioxidants such as vitamin C and vitamin E, keeping them in their active state (Figure 1-7) .[29,30]

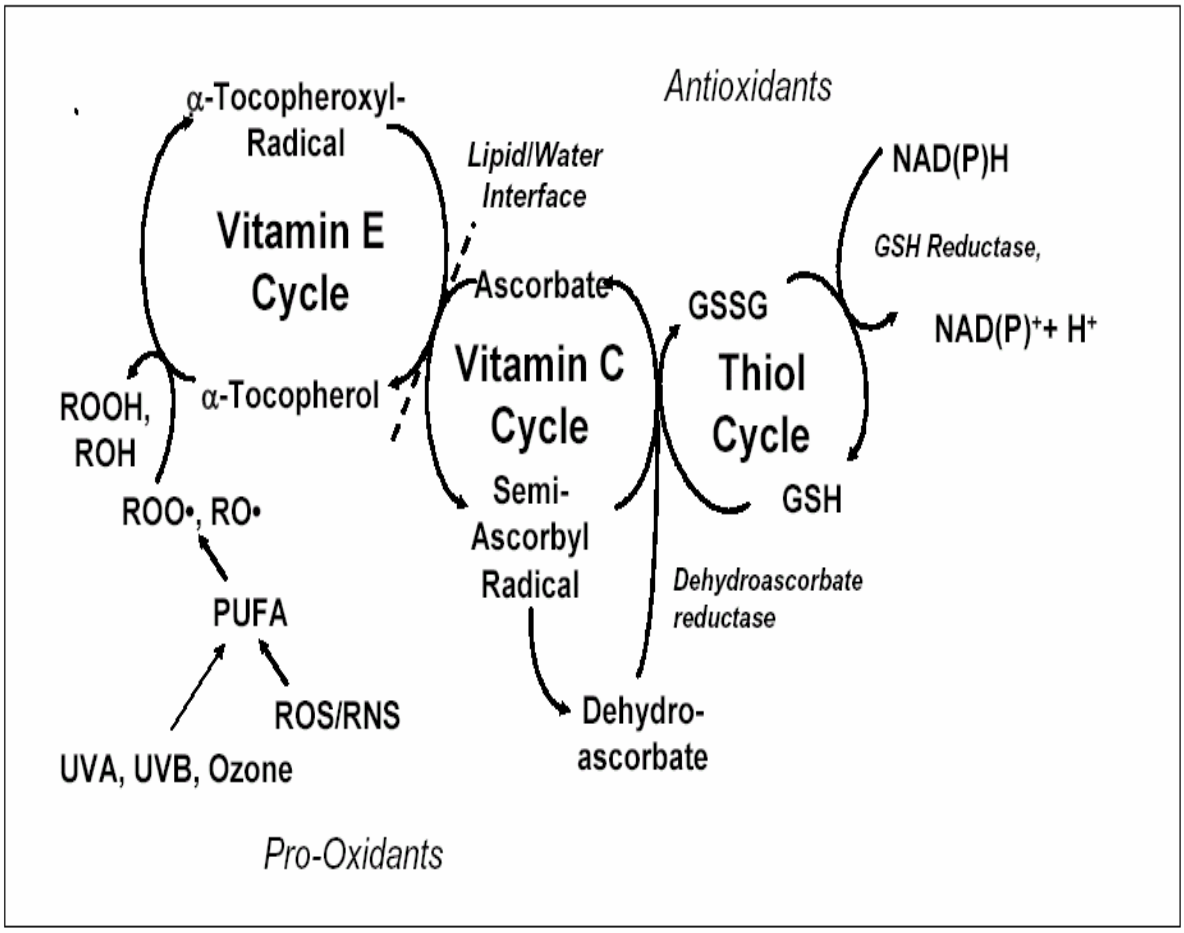


Figure1-7 The antioxidant network .[31]

GSH is an extremely important cell protectant. It directly quenches reactive hydroxyl free radicals, other oxygen-centerd free radicals, and radical centers on DNA and other biomolecules.[21] GSH is a primary

protectant of skin, lens, cornea, and retina against radiation damage, and the biochemical foundation of P450 detoxication in the liver, kidneys, lungs, intestinal epithelia, and other organs.[20]

GSH and it's metabolites also interface with energetics and neurotransmitter synthesis, through several prominent metabolic pathways.[20,32]

GSH availability down-regulates the pro-inflammatory potential of leukotrienes and other cicosanoids. Recently discovered S-nitroso metabolites, generated in vivo from GSH and nitric oxide further diversify GSH's impact on metabolism. [20]

1.4.3 Vitamin C

Ascorbic acid and dehydroascorbic acid are the biologically active formes of vitamin C in human.[33] (Figure1-8).

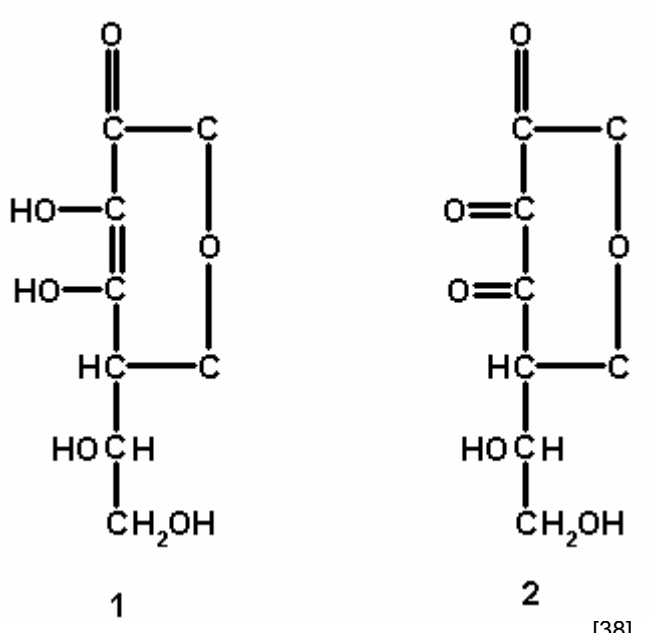


Figure1-8 (1) Ascorbic acid , (2) Dehydroascorbic acid. [38]

Vitamin C (Vit. C) is an electron donor, and this property accounts for all it's known functions.[35]

Vit. C is a primary antioxidant in plasma and within cells, but it can also interact with the plasma membrane by donating electrons to α-tocopheroxyl radical and a trans-plasma membrane oxidoreductase activity.[35] Recycling of α-tocopherol (vitamin E) by ascorbate helps to protect membrane lipids from peroxidation. [36]

Vit. C generally functions as an antioxidant by directly reacting with ROS and has a vital role in defenses against oxidative stress. Also, dehydroascorbic acid (DHA) stimulates the antioxidant defenses of cells.[37]

Ascorbic acid is one of the important water soluble vitamins.It is essential for collagen, carnitine and neurotransmitters biosynthesis. All commercial forms of ascorbic acid except ascorbyl palmitate are soluble in water.[38]

Vitamin C is an effective antioxidant for sevsral reasons. Firstly, both ascorbate and the ascorbyl radical (formed by one electron oxidative of ascorbate) have low reduction potentials and can react with most other biologically relevant radicals and oxidants. Secondly, the ascorbyl radical has low reactivity due to resonance stabilization of the unpaired electron and readily dismutates to ascorbate and DHA (Fig. 1-9). Ascorbate can be regenerated from both the ascorbyl radical and DHA by enzyme-dependent and independent pathways.[39]

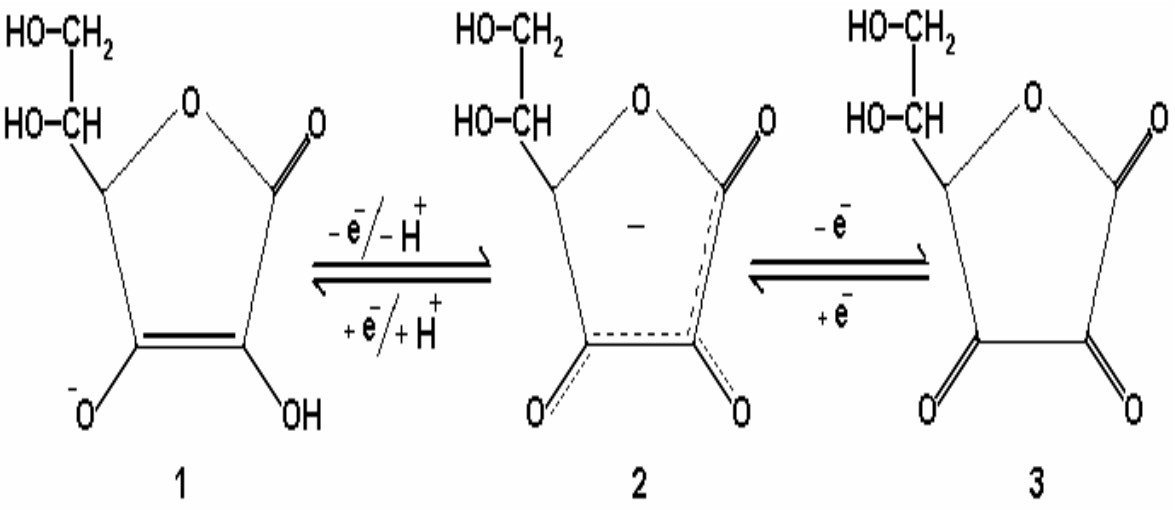


Figure1-9 Oxidation of ascorbate (AH^-) by two successive one electron oxidation steps to give the ascorbyl radical ($A^{\cdot -}$) and DHA , respectively . [39]

1.4.4 Lipid peroxidation

Lipids is a heterogeneous group of compounds having several important functions in the body as being an efficient source of energy, constituents in cell membranes and nerve tissues, thermal and electrical insulators and acting as local hormones etc. Energy is produced from lipids in the body when lipids are *β*-oxidised and oxygen is reduced to water in the respiratory chain by mitochondria in different tissues.[40]

When lipids are oxidized without release of energy, unsaturated lipids go rancid due to oxidative deterioration when they react directly with molecular oxygen. This process is called lipid peroxidation, and the insertion of an oxygen molecule is catalyzed by free radicals (non-enzymatic lipid peroxidation) or enzymes (enzymatic lipid peroxidation)[40,41] . The mechanism of free radical induced lipid peroxidation was established in the 1940 by Farmer and his collaborators working at the research laboratories of the British Rubber Producers Association. Later in the 1950, the relevance of lipid peroxidation to biological systems and medicine began to be extensively explored. [40]

Lipid peroxidation is implicated in the etiology of wide variety of pathophysiological conditions, such as aging, Alzheimer's disease, diabetes, ischemia-reperfusion injury, muscle fatigus or soreness, parkinson's disease, retinopathies, and reheumatoid arthritis. [42]

1.4.4.1 Non enzymatic lipid peroxidation

Polyunsaturated fatty acids(PUFAs) are particularly susceptible to peroxidation and once the process is initiated, it proceeds as a free radical-mediated chain reaction involving initiation, propagation and termination.[40]

Initiation of lipid peroxidation is caused by attack of any species that has sufificient reactivity to abstract a hydrogen atom from methylene group upon a PUFA, [40] (Figure 1-10). Since a hydrogen atom in principle is a free radical with a single unpaired electron, it's removal leaves behind an unpaired electron on the carbon atom to which it is originally attached. The carbon-centred radical is stabilized by a molecular rearrangement to form a conjugated diene, followed by reaction with oxygen to give a peroxyl radical. Peroxyl radicals are capable of abstracting a hydrogen atom from another adjacent fatty acid side-chain to form a lipid hydroperoxide, but can also combine with each other or attack membrane proteins. When the peroxyl radical abstracts a hydrogen atom form a fatty acid, the new carbon-centerd radical can react with oxygen to form another peroxyl radical, and so the propagation of the chain reaction of lipid peroxidation can continue. Hence, a single substrate radical may result in conversion of multiple

fatty acid side chains into lipid hydroperoxides. The length of the propagation chain befor termination depends on several factores e.g. the oxygen concentration and the amount of chain-breaking antioxidants present. Hydroperoxides are fairly stable molecules, but their decomposition can be stimulated by high temperatures or by exposure to transition metal ions (iron and copper ions). Decomposition of hydroperoxides generates a complex mixture of secondary lipid peroxidation products such as hydrocarbon gases (e.g. ethane and pentane) and aldehydes (e.g. malondialdehyde “MDA” and 4-hydroxynonenal). [40]

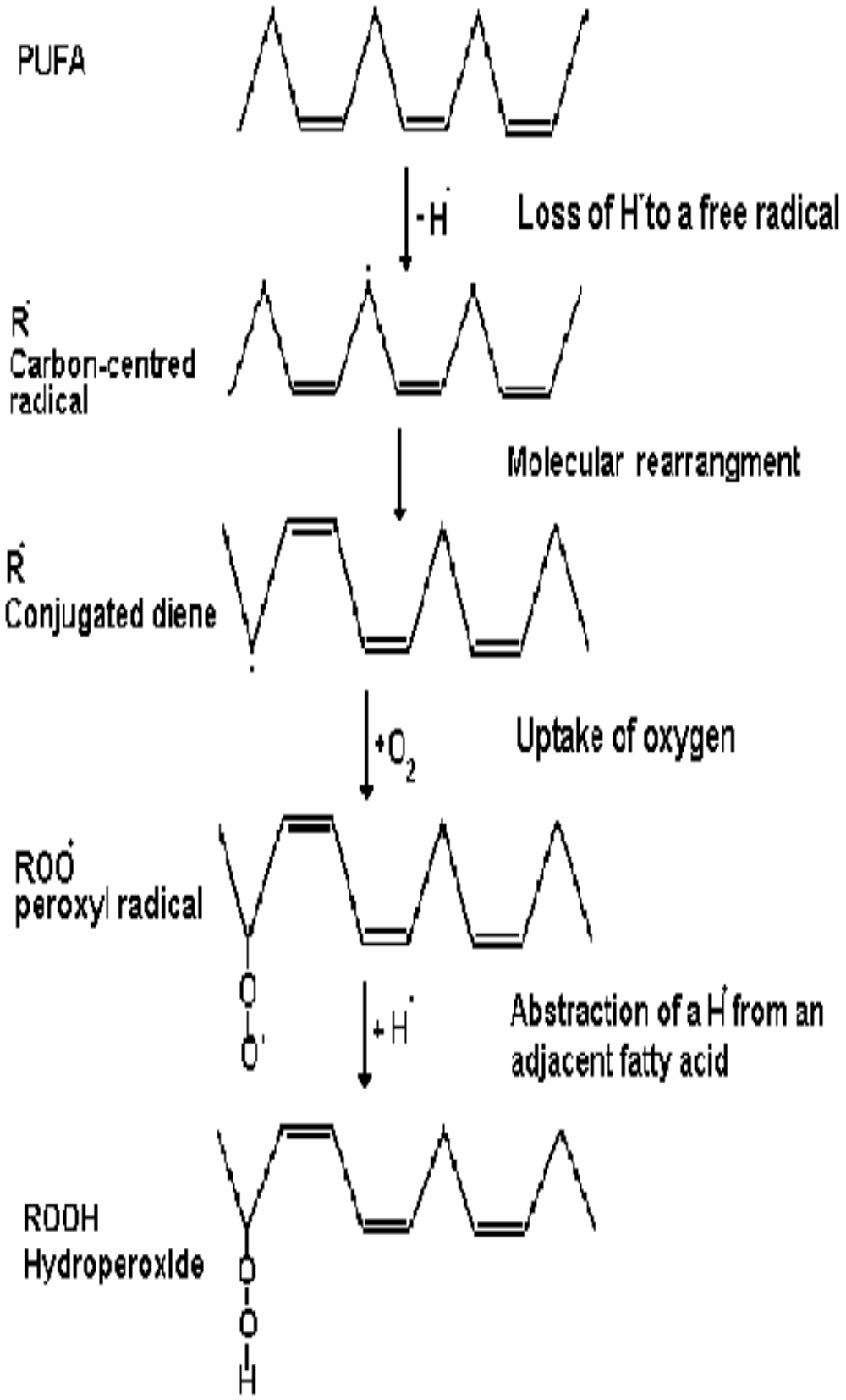


Figure1-10 Mechanism of non-enzymatic lipid peroxidation. [40]

1.4.4.2 Enzymatic lipid peroxidation

Enzymatic lipid peroxidation may refer only to generation of lipid hydroperoxides achieved by insertion of an oxygen molecule at the active centre of an enzyme. Free radicals are probably important intermediates in the enzymatically-catalysed reaction, but are localised to the active site of the enzyme. Cyclooxygenase (COX) and lipoxygenase fulfil the definition for enzymatic lipid peroxidation when they catalyse the controlled peroxidation of various fatty acid substrates. The hydroperoxides and endoperoxides produced from enzymatic lipid peroxidation become stereospecific and have important biological functions upon convertion to stable active compound. Both enzymes are involved in the formation of eicosanoids, which comprise a large and complex family of biologically active lipids derived from PUFAs with 20 carbon atoms. Prostaglandins are formed by COX-catalysed peroxidation of arachidonic acid. COX exists in at least two isoforms. COX-1 is present in cells under physiological conditions, wherease COX-2 is induced in macrophages, epithelial cells and fibroblasts by several inflammatory stimuli leading to release of prostaglandins. [40]

1.4.5 Cholesterol

Cholesterol is a softy, waxy compound belonging to the steroid family of molecules and is found among the lipids in the bloodstream as well as in all cell of the body. [40]

Cholesterol is an essential component of several biochemical functions such as :-

-Formation and maintenance of cell membranes “ helps the cell to resist changes in temperature and protects and isolates nerve fibres “

-Formation of sex hormones “ progesterone, testosterone, estradiol, cortisol “

-Production of bile salts, which help to digest food.

-Convertion into vitamin D in the skin when exposed to sunlight. [43,44]

1.4.5.1 Cholesterol metabolism in diabetes mellitus

A disorder in metabolism in diabetes mellitus occurs chiefly due to insulin insufficiency. The metabolism of carbohydrates, fat and proteins is distrubed. Not only insulin but also the contrainsular hormones glucagon, smoatastatine and glucorticoids are included in the chain reaction of the damaging hormonal units. [45] In biochemical investigation of metabolism processes (under conditions of insulin insufficiency), there is a lower penetration of glucose into the muscle and fat cells, disturbance of oxidative phosphorylation and lowering of glucogen synthesis. [46,47] The disturbed participation of glucose in the supply of energy to an organism includes fats and proteins into energy metabolism as a compensation. As a result, an increased amount of free fatty acid (FFA), ketone bodies, and amino acids appears in the blood. The increased supply of FFA into the liver causes a greater synthesis of triglycerides. [48] A deficiency of insulin underlies the inhibition of lipogenesis and activation of lipolysis. [45]

Deficiency of insulin increase the level of FFA and at the same time lowers the utilization of glucose, so that muscle cells, are switched over to FFA as an energy donor . Glucose accumulates in the fat cells and transforms into triglycerides if the amount of insulin is sufficient. [45]

The manifestation of the disordered lipid metabolism is so prominent that diabetes has been called “ more a disease of lipid than of carbohydrate metabolism “.[49]

Cholesterol is the most abundant sterol in mammals . It is a vital component of cell membranes; a precursor of bile acid, which is required for digestion and the precursor of the steroid hormones and vitamin D_3 . [50,51]

Cholesterol is readily absorbed from the intestine if bile, fatty acids, and pancreatic juice are present. The plasma cholesterol level is usually elevated, and this may play a role in the accelerated development of the arteriosclerotic vascular disease, that is major long-term complication of diabetes in human. [49]

In the liver, cholesterol is converted to bile acid that are secreted via the bile duct, partly reabsorbed in the intestine and returned to the liver. The only way cholesterol is normally removed from the body is in the form of bile salts. [50]

The concentration of cholesterol in the blood apparently depends on the dietary intake of sterols, neutral fats and the synthesis of cholesterol by the liver. Hepatic synthesis of cholesterol appears to be inhibited by newly absorbed dietary cholesterol. [52]

1.4.6 Uric acid: -

Serum uric acid (or more correctly, its monoanion uric acid at physiological pH value) has been thought to be in humans as a metabolically inert end product of purine metabolism without physiological significance (except gouty diathesis). However, serum uric acid has been recently associated with insulin resistance. [53]

Purines arise from metabolism of dietary and endogenous nucleic acids, and are degraded ultimately to uric acid in man, through the action of the enzyme xanthine oxidase (figure 1-12). [54]

Uric acid is a weak acid (pk_a 5.8) [55.56]; distributed throughout the extracellular fluid compartment as sodium urate, and cleared from the plasma by glomrular filtration. [54]

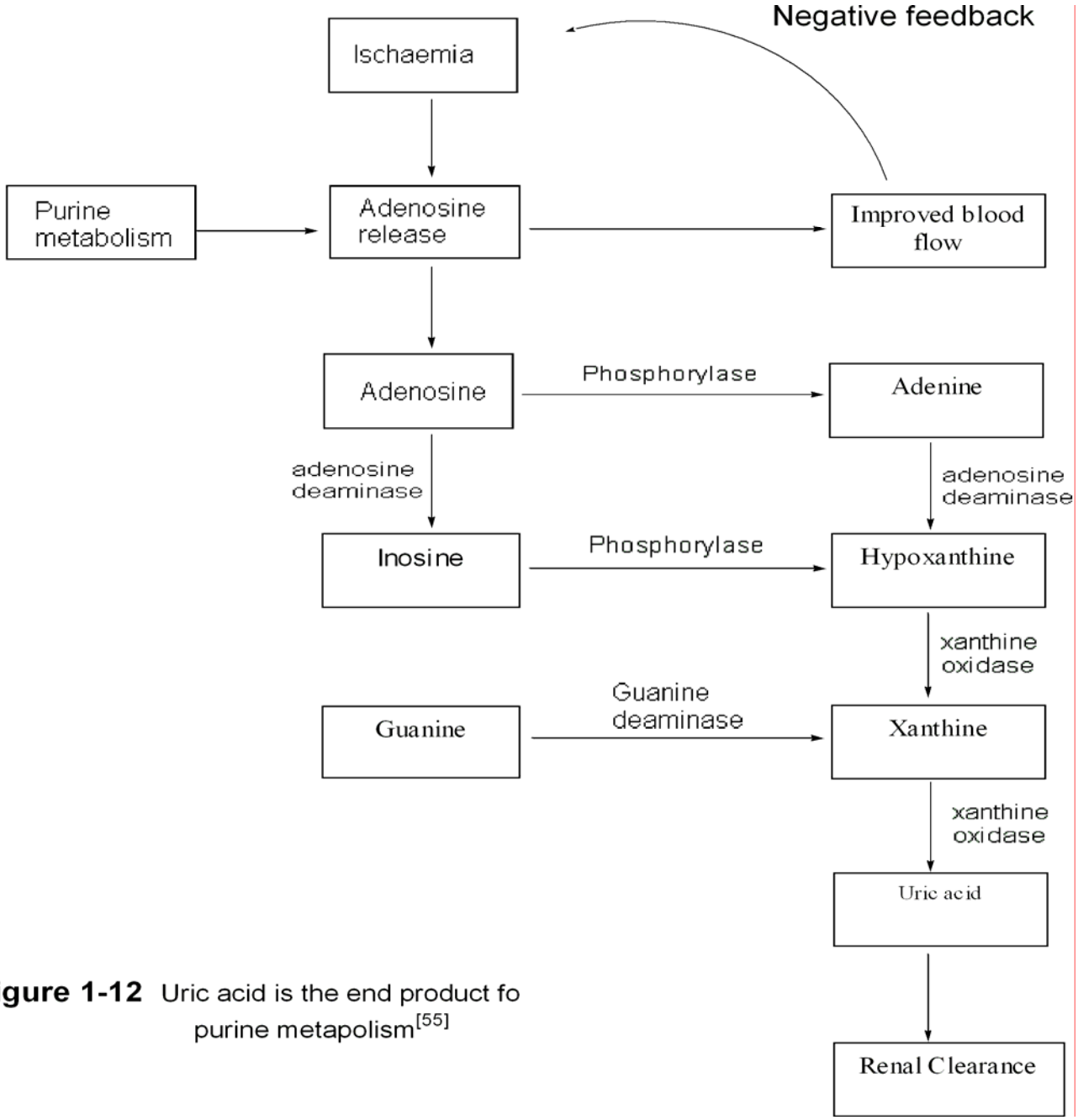


Figure 1-12 Uric acid is the end product fo purine metapolism[55]

The association of serum uric acid with cardiovascular disease has been appreciated for nearly half a century.[56] The clinical evidence suggests that uric acid plays a role in platelet adhesiveness, formation of free radicals, and oxidative stress. [55,56]

It has been proposed that because uric acid is a powerful scavenger of free radicals, it plays an important role in protecting hominoids from oxidative damage. [57]

1.4.7 Creatinine

Serum creatinine (Crn) is associated with all cause mortality or stroke in middle aged and elderly people and in patients with insulin-independent diabetes or previous cardiovascular disorder [58]

Serum creatinine level is a function of creatinine production and renal excretion. [59]

1.4.7.1 Creatinine metabolism

The pathways of creatinine metabolism in vertebrates seem simple (Figure1-13). [60]

The transfer of the amino group of Arg to Gly yield L-ornithine and guanidinoacetic acid (GAA) represents the first of two steps in biosynthesis of Creatinine. GAA, by the action of S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase, is then methylated at the amino group to give creatine. Creatinine will produce from conversion of creatine by non-enzymatic reaction.[60]

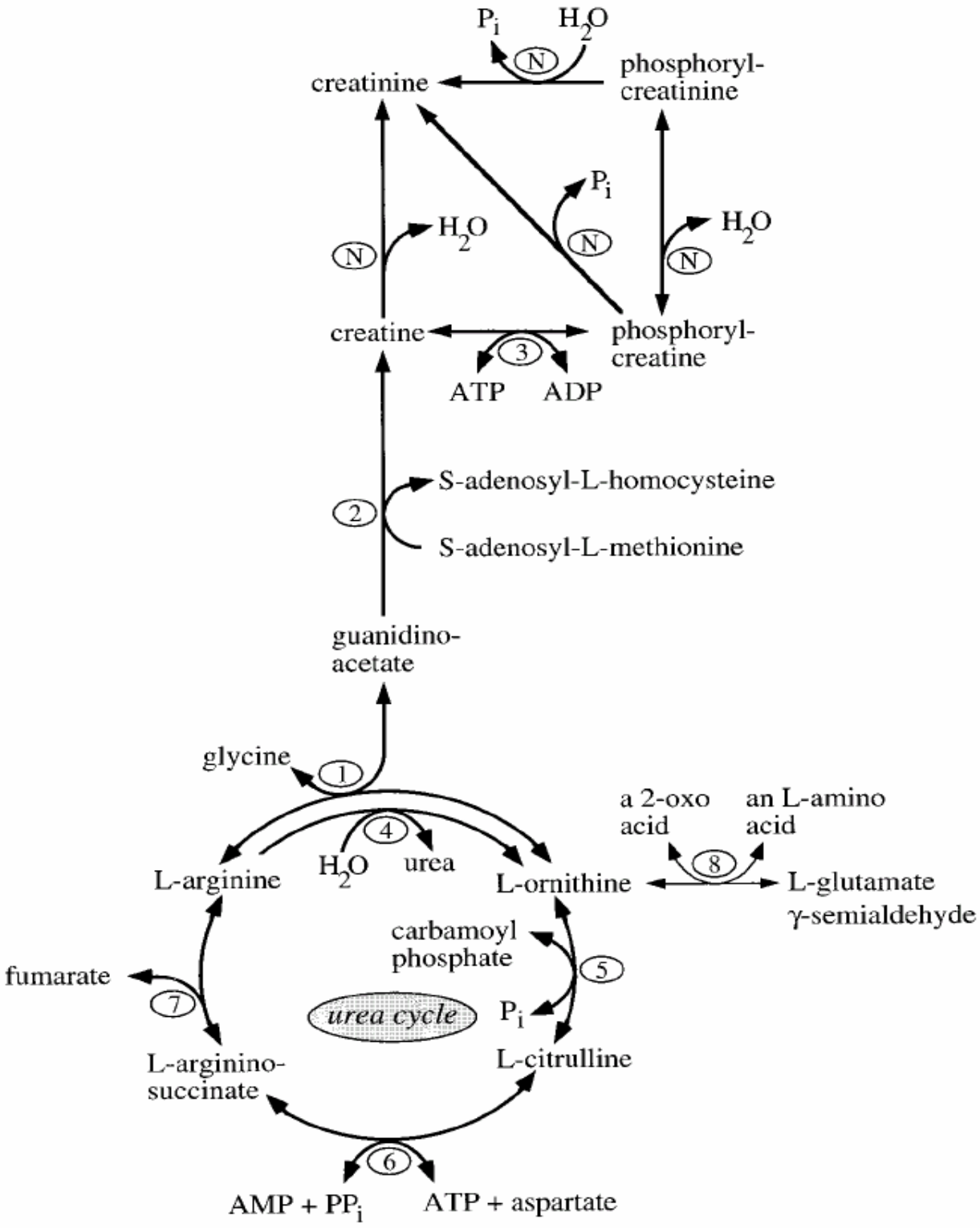


Figure1-12 Schematic representation of the reactions and enzymes involved in vertebrate creatine and creatinine metabolism. The respective enzymes are denoted by numbers: ***1***) L-arginine:glycine amidinotransferase (AGAT; EC 2.1.4.1); ***2***) *S*-adenosyl- L-methionine:*N*-guanidinoacetate methyltransferase (GAMT; EC 2.1.1.2); ***3***) creatine kinase (CK; EC 2.7.3.2); ***4***) arginase (L-arginine amidinohydrolase; EC 3.5.3.1); ***5***) ornithine carbamoyltransferase (EC 2.1.3.3); ***6***) argininosuccinate synthase (EC 6.3.4.5); ***7***) argininosuccinate lyase (EC 4.3.2.1); ***8***) L-ornithine:2-oxo-acid aminotransferase (OAT; EC 2.6.1.13); ***N***) nonenzymatic reaction.[60]

The equilibrium of the reversible and nonenzymatic cyclization of creatine to creatinine is both pH dependent and temperature dependent. Creatine is favored at high pH and low temperature, whereas creatinine is favored at elevated temperature and in acidic solution. [60] (Figure 1-14).

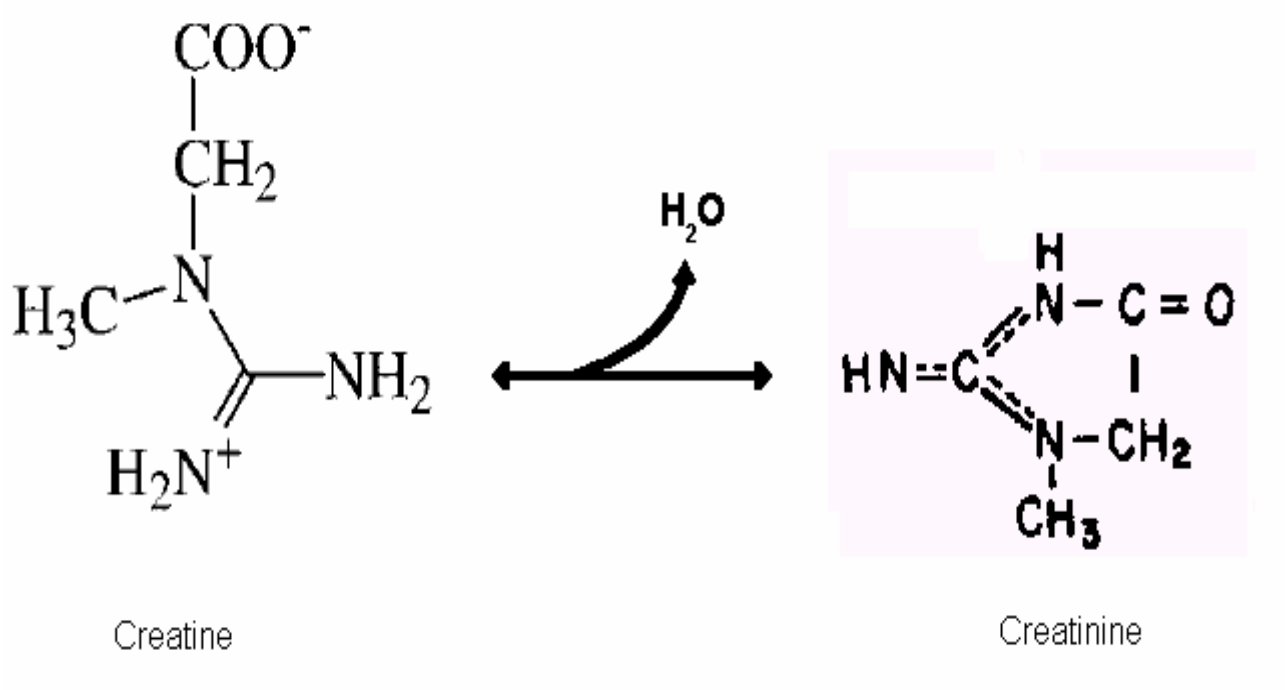


Figure1-13 Nonenzymayic cyclization of creatine to creatinine.[61]

1.4.8 Creatine Kinase

Creatine kinase (CK, EC 2.7.2.3) is a key enzyme of cellular energy metabolism, catalyzing the reversible phosphoryl transfer from phosphocreatine to ADP.[62,63] The CK reaction is important for rapid resynthesis of ATP when the heart increases its work. [64] Substrates are bound specifically in a rapid equilibrium, random mechanism with product release being the rate-limiting step under physiyological conditions. In addition to the productive reaction, CK also forms a

dead-end inhibition or transition state analog complex with Mg-ADP, nitrate and creatine. [62]

Several isoforms of the enzyme have been reported:cytosolic (M,muscle type; B, brain type) and mitochondrial isoenzymes. Three cytosolic isoenzymes exist in homo-or heterodimeric form (BB-, MB-, and MM-CK) and two mitochondril CKs occur mainly as octamers (Mi_a- and Mi_b – CK) . CK is a member of the family of guanidino kinases (ATP:guanidinophosphotransferases), enzymes with closely related primary sequences and large structural similarities. Lobster arginine kinase is another member of this family, being a monomeric protein of 40 kDa, whereas the cytosolic form of CK can be regarded either as dimeric member of this protein family, having relative molecular masses of 86 kDa, or as octomers of 340 kDa, that can be dissociated into dimers in vitro, as found for Mi_b –CK.[62]

Mi-CKs have been found in the intermembrane space of the mitochondria, being attached to the inner membrane. The enzyme is thereby enabled to utiliz intramitochondrially synthesized ATP for the synthesis of phosphocreatine, which is shuttled out of the mitochondria for the regeneration of ATP by the cytosolic CK isoenzymes. This interplay of mitochondrial and cytosolic CKs has been referred to as the phosphocreatine circuit. [62]

Mi_b –CK from chicken heart has been crystllized,and its X-ray structure in the absence and presence of ATP (not been in the absence of Mg^{2+}) has been solved. The CK monomer displays a two-domine organization, with a small amino-terminal(residues 1-100) and a large carboxyl-terminal domina (residues 120-380). The latter is dominated by an eight-standed antiparallel β-pleated sheet,

surrounded by seven α-helices, whereas the amino-terminal domain exhibits helical structure elements only. Furthermore, a single β-strand formed by amino acids separates the large β-sheet of the carboxyl-terminal domain in two halves, which, together with their surrounding structures, can be regarded as subdomains of the carboxy domain.[65,66,67]

AIM OF THE STUDY

1- To investigate the relationship between serum and leukocytes vitamin C in NIDDM.

2- Investigation the activity of CK in control and NIDDM patients.

3- Determination the oxidative stress associated with NIDDM, by measuring the concentration of substance that act as antioxidant. such as vit. C, glutathione, and MDA as end product of lipid peroxidation.

4- Investigation of the relationship between uric acid and NIDDM.

5- Investigation the relationship between Crn and NIDDM.

CHAPTER TWO

2. MATERIALS AND METHODES

2.1 Materials

2.1.1 Chemicals

All chemicals were used as supplied without further purification.

Chemicals	Purity %	Supplied Company
Meta-phosphoric acid $(m\text{-}hpo_3)_n$	90	Fluka
Thiourea	99	Fluka
Copper sulfate anhydrous	99	Fluka
Sulfuric acid	95	Fluka
Ethylenediaminetetraacetic acid dihydrate (EDTA)· 2 H_2O	99.5	Fluka
Tris(hydroxymethylene) Aminomethane	99.5	Fluka
Methanol	99.8	Fluka
Triton X‑100	99.5	Fluka
Glutathione	99.5	Biochemical
Ascorbic acid	99	Biochemical
Trichloroacetic acid	99	Hopkin and Willams
Chemicals	**Purity %**	**Supplied Company**
Thiobarbituric acid	99.5	Sigma chemical

5,5-Dithio bis(2-nitrobenzoic acid) (DTNB)	99	
Sodium chloride	99	Sigma chemica
Dextrin	99	BDH
2,4-Dinitrophenyl hydrazne	96	Linar Chemicals
Creatine kinase kit		Biocon
Creatinine kit		Biolabo
Total cholesterol kit		Biomaghreb
Uric acid kit		Biomaghreb

2.1.2 Instrumental Analysis and Equipment

Instrumental	Supplied Company
pH meter	Jenway(Germany)
Sensitive Blanca	Stanton 461 AN(Germany)
Vortex mixer	Karlkole(Germany)
Water Bath	Karlkole(Germany)
Shaker water bath	Tecam(England)
Oven	Hearson (England)
Magnatic stirrer	Gallin Kamp(England)
Instrumental	**Supplied Company**
Spectrophotometer type 21	Molton Roy(Switzerland)

Centrifuge	Heraeus(Germany)
Filter paper	Whatman No.1(England)
Centrifuge tube	Mes(1 gm) Fisonsm(England)
Plane tube	Afma-Dispo-(Jordan)
Micropiptte 20	Gilson(France)
Micropiptte 100 - 1000	Gilson(France)
Micropiptte 100	Oxford Sampler Micropipetting system(USA)
Micropiptte 40 - 200	Oxford Sampler Micropipetting system(USA)
Micropiptte 50	Nichiryo(Japan)
Micropiptte 500	Nichiryo(Japan)
Micropiptte 1000	Socorex(Swiss)

2.2 Methodologies

2.2.1 Patients and controls.

Fifty patients of diabetes mellitus type 2 were examined and tested (24 male, 26 female), with age mean of (47±15) years. The patients were the out patients of the hospital of Mahmudia during the period between April 2004 to October 2004. The control group consisted of forty two healthy volunteers (20 female, 22 male), with age mean of (43±17) years .

2.2.2 collection of blood and serum preparation: -

Blood samples were collected by veipuncture using 20 ml disposable syringes. Blood was divided into two parts: -

First part :- 10 ml was put in the centrifuge tube, then it was centrifuged for approximately 10 minutes at a relative centrifugal force (RCF) of 1000xg - 2000xg [68,69,70] . While the second part (10 ml) was put in EDTA tube, the blood was mixed gently, used for leukocyte essay.

2.2.3 Determination of serum reduced glutathione (GSH): -

All analytical methods such as flourometric, photometry enzymatic,[52,70,71] and HPLC methods[73], that used to determine tissues homogenate,erthrocyte,and serum glutathione (GSH) depend on the action of sulfhydryl groups.

2.2.3.1 principle:-

5,5´-Dithiobis (2.nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of (GSH) to an intensly yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the (GSH) concentration.[52] (Figure 2-1)

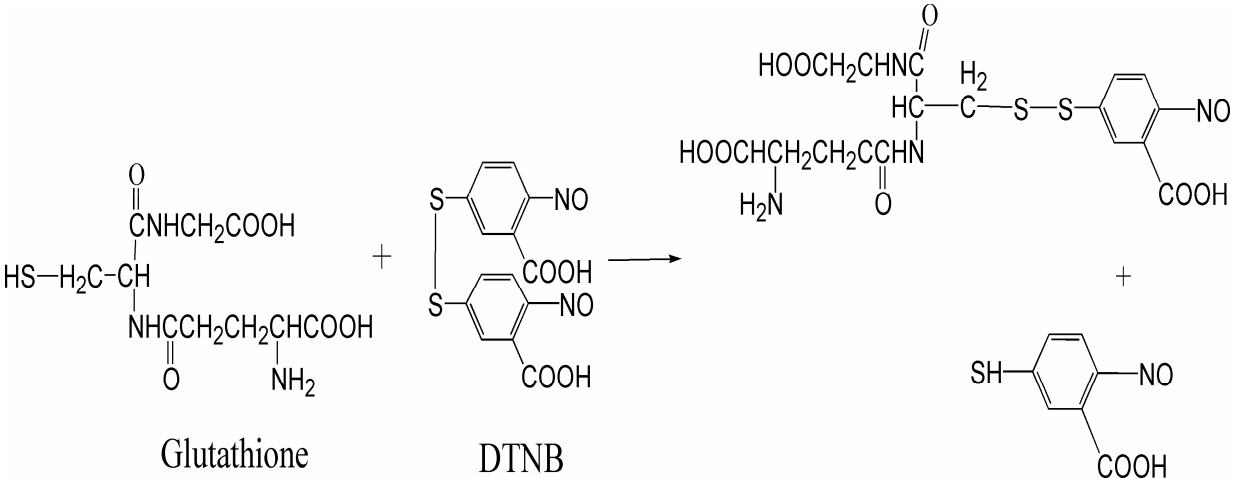


Figure 2-1 Reaction between GSH and DTNB[74]

2.2.3.2 preparation of reagents: -

1. Precipitating solution.Trichloroacetic acid (TCA) 50%
   50gm of TCA was dissolved in a final volume of 100 ml of DDW.
2. Ethylenediamine tetracetic acid – di sodium (EDTA Na_2)(0.4 M)
   148.9 gm of EDTA were dissolved in a final volume of 1 liter DDW.
3. Tris-EDTA buffer (0.4M) pH 8.9
   48.458 gm of Tris were dissolved in 800 ml of DDW. 100ml of (0.4M) EDTA solution were added and bring to a final volume of 1 litter with DDW. The pH was adjusted to 8.9 by the addition of 1M HCl. (stable for at least 10 days)
4. DTNB reagent (0.01M)
   0.099 gm of DTNB was dissolved in absolute methanol, and bring to a final volume of 25 ml, (this reagent is stable for at least 13 week at 4C°)
5. GSH standards
   Stock standard solution (0.001M) was prepared by dissolving 0.0307gm of GSH in a final volume of 100 ml of (0.4M) EDTA solution.

Dilutions were made in EDTA solution to 2,5,10,15,20, 30,40 and 50 µM. (working standard solution prepared daily).

2.2.3.3 procedure: -

Serum GSH was determined by using a modified procedure utilizing Elaman's reagent (DTNB), which is summarized as follows: -

Duplicates of each standard and sample test tubes are prepared then pipettes into test tubes:

Reagents	Sample µL	Reagent Blank µL	Standard µL
Serum	100	------	-----
Standard	------	------	100
DDW	800	900	800
TCA	100	100	100

Tubes were mixed in vortex mixture intermittently for 10 -15 minutes, and centrifuged for 15 minutes at 300xg then pipettes into test tubes

Reagents	Sample µL	Reagent Blank µL	Standard µL
Supernatant	400	400	400
Tris–EDTA buffer	800	800	800
DTNB reagent	20	20	20

Tubes were mixed in vortex mixture. The spectrophotometer was adjusted with reagent blank to read zero absorbency (A) at 412 nm,and

the absorbency of standards and sample was read within 5 minutes of the addition of DTNB.

2.2.3.4 Calculation of serum (GSH) concentration .

The concentration of serum GSH was obtained from calibration curve in µM. (Figure 2-2) .

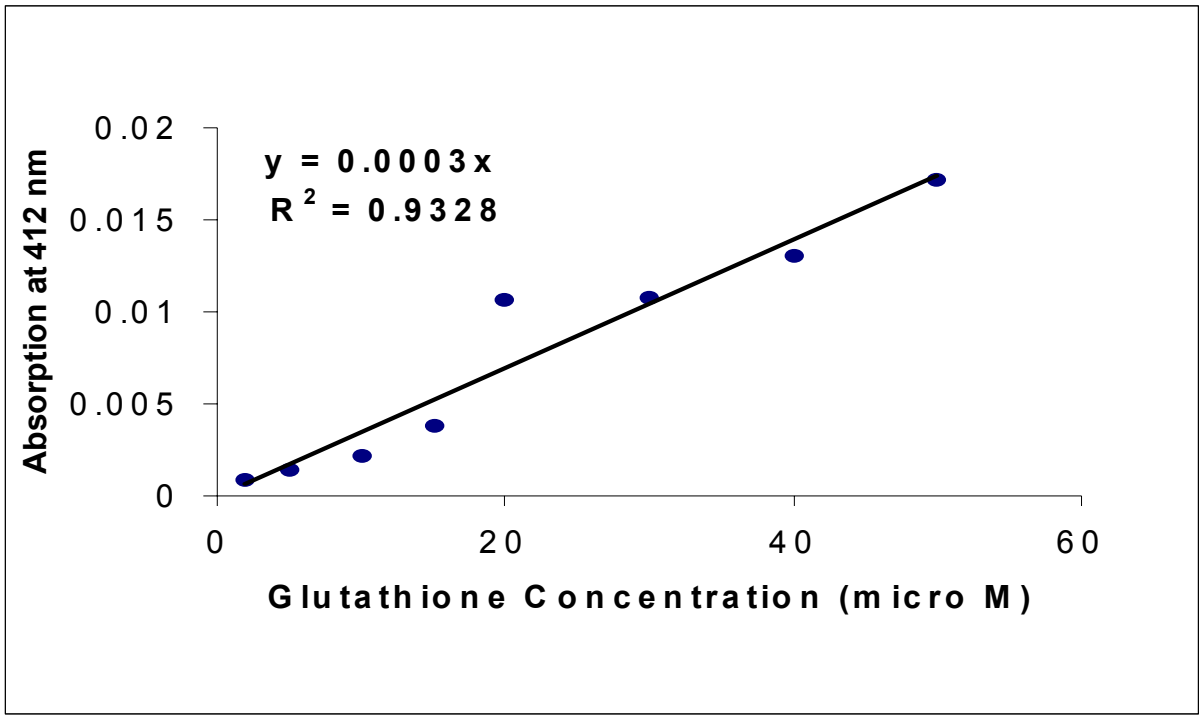


Figure 2-2 standard curve for GSH concentration determination.

2.2.4 Determination of vitamin C.

Numerous analytical methods are available for assessment of vit. C nutritional status. Chemical methods depend on either the reducing property of the 1,2–enediol group that leads to absorbency changes in indicator dyes,or formation of hydrozones. Other methods including fluorometric and HPLC techniques, have been developed. In most methods, protein is precipitated with metaphosphoric or perchloric acid before analysis[52,73].

2.2.4.1 Determination of serum total vitamin C: -

2.2.4.1.1 Principle: -

According to 2,4-dinitrophenylhydrazine (DNPH) methods, vit. C is oxidized by Cu^{+2} to DHA and diketogulonic acid[52] . When treated with 2,4-DNPH the hydrazones derivatives is produced , which, in the presence of sulfuric acid, forms an orange – red complex that absorbs at 520 nm (Figure 2-3).

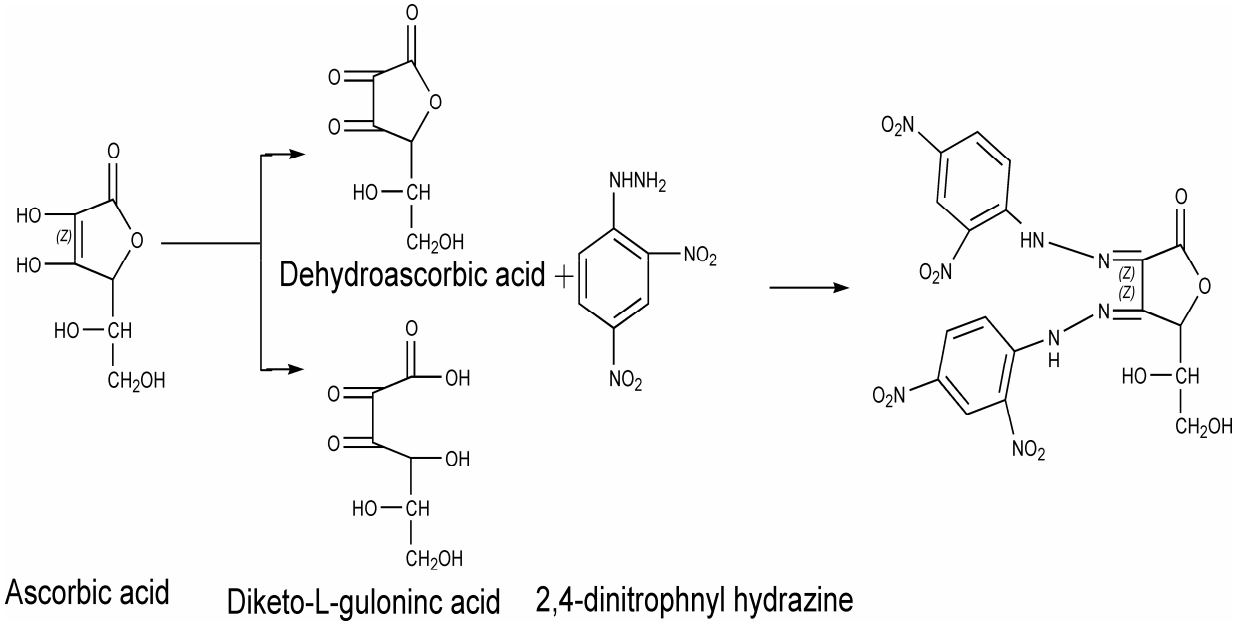


Figure 2-3 Reaction between Ascorbic Acid and 2,4-DNPH[74]

2.2.4.1.2 Preparation of reagents.

1. Metaphosphoric acid (m-HPO_3) (0.75 M)

   30 gm of m-HPO_3 were dissolved in a final volume of 500 ml of DDW. (Stable for 1 week).

2. Sulfuric acid (H_2SO_4) (4.5 M)

Carefully 250 ml of concentrated H_2SO_4 was added to 500 ml of cold DDW. When the solution cooled to room temperature , DDW. was added to 1 litter, with mixing (stable for 2 years) .

3. Sulfuric acid (H_2SO_4) (12M)

Carefully 650 ml of concentrated H_2SO_4 were added to 300 ml of cold DDW. and brought to a final volume of 1 litter (stable for 2 years).

4. 2,4- DNPH reagent (0.01 M)

10 gm of 2,4-DNPH were dissolved in 400 ml of (4.5 M) H_2SO_4 and brought to a final volume of 500 ml with(4.5 M) H_2SO_4, then refrigerated overnight, and filtered, (stable for at least 1 week at refrigerated temperature).

5. Thiourea (0.66 M)

5 gm of thiourea were dissolved in a final volume of 100 ml of DDW. (stable for 1 month at 4C°) .

6. Copper sulfate (0.027 M)

0.6 gm of anhydrous copper sulfate was dissolved in a final volume of 100 ml of DDW. (Stable for 1 year at room temperature).

7. DTCS reagent

5 ml of the thiourea, 5 ml of the copper sulfate, and 100 ml of the 2,4-DNPH reagent were combined. (Store in bottle at 4 C°for a maximum of 1 week)

8. Ascorbic acid standards

Stock standard solution (2.8 mM) is prepared by dissolving 50 mg of ascorbic acid in a final volume of 100ml of m-HPO_3. Dilution are made in m-HPO_3 to 2.5, 5 and 20 mg/L (0.014, 0.028, and 0.11mM) respectively. There are the working standards (All working standards should be prepared daily).

2.2.4.1.3 Procedure.

The procedure for the determination of total vitamin C in serum by 2,4-DNPH method is summarized as follows: -

Duplicates of each standards and sample test tubes are prepared then pipettes into test tubes.

Reagent	Sample (μL)	Reagent	Blank (μ L)	Standard (μL)
M-HPO_3	800	--------	-------	-------
Serum	200	--------	-------	-------

Tubes were mixed in vortex mixture, then centrifuged at 2500xg for 10 minutes

Reagent	Sample (μL)	Reagent Blank (μ L)	Standard (μL)
Supernatant	600	------	-------
Standard	-------	------	600
m-HPO_3	--------	600	-------
DTCS regent	200	200	200

Tubes were capped and mixed in vortex mixture, then incubated in a water bath at 37c℃for 3 hours.

The tubes were removed from the water both and chilled for 10 minutes in an ice bath, with slow mixing.

Reagent	Sample (μL)	Reagent Blank (μ L)	Standard (μL)
Cold H_2SO_4	1000	1000	1000

Tubes were mixed in vortex mixture and returned immediatlly to the ice bath. The spectrophotometer was adjusted with blank to read zero absorbency (A) at 520 nm, and the absorbance of standards and sample is read.

2.2.4.1.4 Calculation of serum total vit. C.

The concentration of the samples were obtained from the calibration curve (Figure 2-4) and multiplied by 5 (to correct for dilution of the serum by m- HPO_3) to give the concentration of vitamin C per liter of serum.

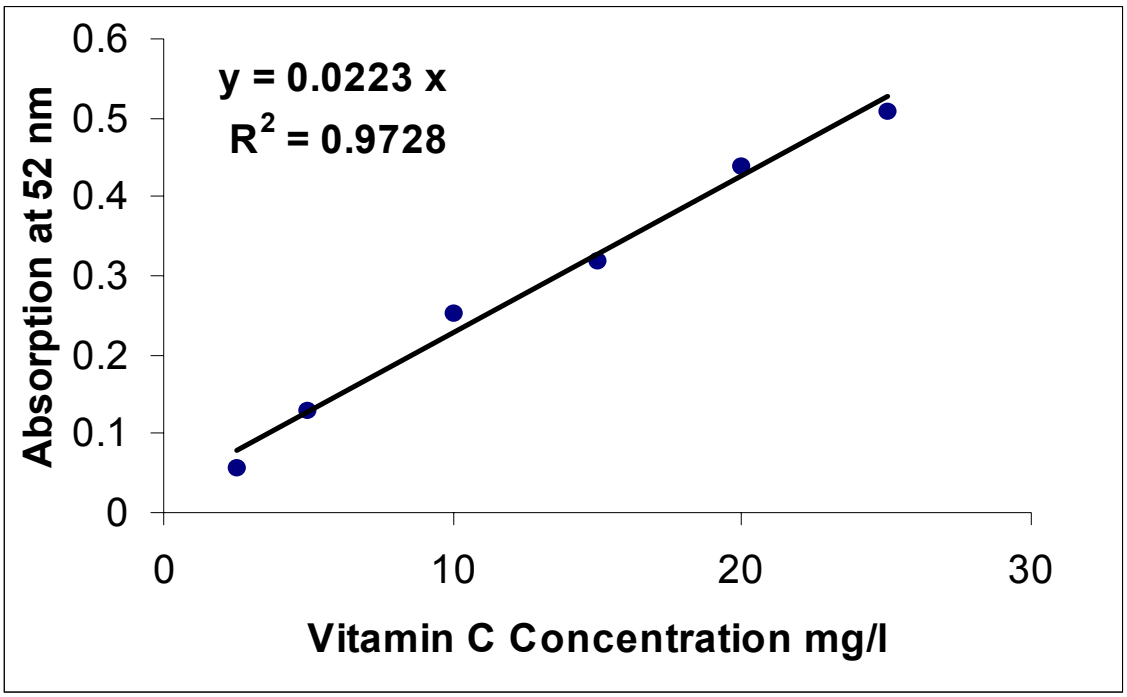


Figure 2-4 Standard curve for 2,4-DNFH method for ascorbic acid

2.2.4.2 Determination of serum Reduced Vitamin C.

2.2.4.2.1 Principle: -

The reducing properties of ascorbic acid are utilized in the 2,6-dichloro--phenolindophenol (2,6-DCIP) method[73]. AA causes a reduction in the dye from the oxidized to the colorless leuco form. The change in color can be monitored photo metrically at 505 or 520nm or titera-metrically. 2,6-DCIP is blue compound, being red in acid solution and on titration with a solution of ascorbic acid is reduced to the colorless leuco base, the AA being oxidized to DHA. It thus seen that if a mixture of AA an DHA is present, only the former reacts with the dye[69]. (Figure 2-5)

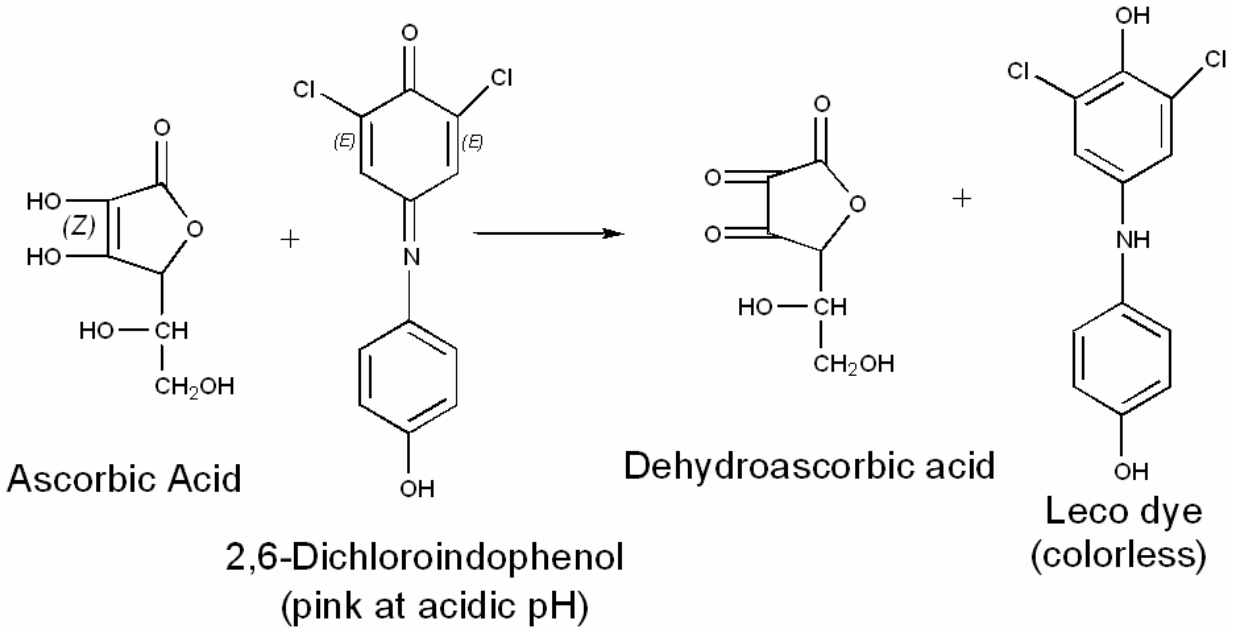


Figure 2-5 Reaction between Ascorbic Acid and 2,6 DCIP[74]

2.2.4.2.2 Calculation of serum reduced vitamin C: -

In the present study, reduced AA was calculated from a correlation study between the DNPH and DCIP methods conducted on 611 patient sample, and showed good agreement [74].

DNPH = 1.088(DCIP) + 0.12

There fore.

$$\text{Reduced AA} = \frac{\text{Total AA (DBPH method)} - 0.12}{1.088}$$

2.2.5 Determination of leukocytes vitamin C :-

Leukocytes can easily be isolated, and such preparations often the specimen of choice. A description of the method for the preparation of leukocytes as follows .[75]

2.2.5.1 Principle:-

Whole blood is allowed to stand at room temperature for 30 to 60 min. Erythrocytes settle first; leukocytes, whose buoyant density is less than that of red blood cells, sediment more slowly and form a " buffy coat "between the erythrocytes and the supernatant plasma. If the density of the medium through which sedmintation occurs is increased, for example by the addition of dextran , leukocytes remain suspended. Recovery of the supernatant and subsequent centrifugation at low centrifgal force result in a pellet made up largely of leukocytes. Contaminating erythrocytes can be eliminated by selective shock treatment, because they are less resistant than white blood celles to lysis in hypotonic solutions.

2.2.5.2 Specimen collection and storage:-

Ten milliliters of blood collected in acid-citrate-dextrose (ACD solution B). Leukocytes in such specimens remain viable for up to 72 h. of storage or shipment. A considerably lesser yield of leukocytes is obtained from heparinized blood unless the isolation is carried out within 24 h. after the speciment is drawn. For there fore, the inclusion of glucose (dextrose) in ACD solution B helps maintain leukocyte viability during prolonged storage or shipment times.

2.2.5.3 Reagents:-

1-Dextrean solution , 5 g / dL

immediately before use, 0.5 g of dextran was dissolved in 10 ml of sodium chloride solution , 0.7 g / dL

2-Sodium chloride , 0.7 g / dL

0.7 g of sodium chloride was placed into a 100 ml volumetric flask and diluted to mark with water. stable for 2 mon. At 8 Cº.

3-Sodium chloride 0.9 g / dL

0.9 g of sodium chloride was placed into a 100 ml volumetric flask and diluted to the mark with water. Stable for 2 mo. At 8 Cº.

4- Sodium chloride 1.8 g / dL

1.8 g of sodium chloride was placed into a 100 ml volumetric flask and diluted to the mark with water. Stable for 2 mo. At 8 Cº.

2.2.5.4 Procedure:-

1- 10 ml of anticoagulated blood was Placed into a 15 ml plastic centrifuge tube.

2- 2 ml of freshly prepared solution of dextran, 5g / dL was added in sodium chloride , 0.7 g / dL . Mixed gently by inversion.

3-allowed to stand for 45 min. for sedimentation of cells.

4-Drawer off supernatant with a plastic disposable pipet and discharged it into another plastic centrifuge tube.

5-At 4 to 10 ºC, centrifuged at 5000 xg for 10 min.

6-Drawer off supernatant and discarded.

7-Resuspended the button of white blood cells in 1 ml of cold sodium chloride 0.9 g / dL.

8-Shock treated the cells as follows: Added 3 ml of ice-cold DDW and mixed gently for 45 s. Immediately added 3 ml of cold sodium chloride 1.8 g / dL. And mixed

9-Centrifuged at 500 xg for 10 min.

10-followed steps 7 through 10 for second shock treatment.

Leukocytes were lysed by repeated freezing and thawing after addition of 0.2 % Triton X 100. The concentration of vit. C was determined by the same method that was explained above in determination vit. C in serum.

2.2.5.5 Determination of cells count :-

1- Resuspended cells in 1.0 mL of cold sodium chloride 0.9 g/dL . Then added an additional 4 ml of sodium chloride 0.9g/dL to give a total volume of 5 mL (= V).

2- Allowed to stand 30 to 45 min at 4 °C and resuspended the cells.

3- Made a 500 fold dilution (40 µL white blood cell suspension + 20 mL ISOTON) and cells were counted in this suspension on the Coulter counter at the white blood cells were seated . The count (= W) was recorded.

4- The white blood cells were centrifuged at 600 xg for 10 min.

5- Supernatant was discarded . cells were resuspended in 1 mL ice cold DDW , and the volume was recorded (= v)

6- The number of leukocytes per liter was calculated as follows:-

Leukocytes 10^{10} cells/L=W/0.5 x 500 x V/v x 10^{3}

2.2.6 Determination of malondialdehyde (MDA) :-

Lipid peroxide was estimated according to the method described by Rehncrona et al[76]. Measurement of MDA, a secondary product of lipid peroxidation, was based on the calorimetric reaction with thiobarbituric acid (TBA) . (Figure. 2-6.)

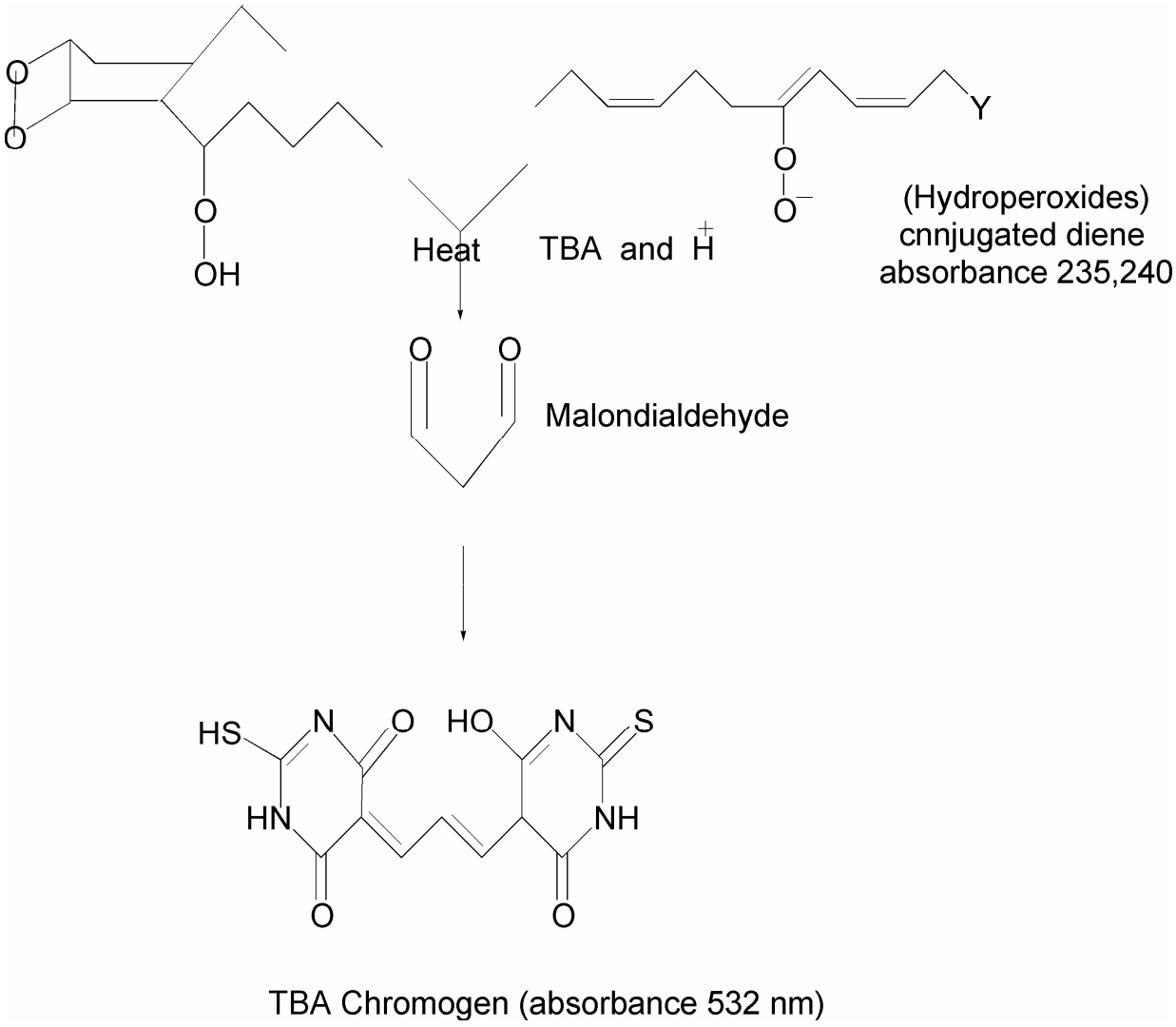


Figure 2-6 Shcematic diagram showed the principle of reaction for assessment lipid peroxidtion via determenation the byproduct; Malondialdehyde [77]

2.2.6.1 Procedure: -

The level of serum malondialdehyde is determined by a modified procedure described by Guidet and shah [78]. In brief; to (150 µl) serum sample add the followings : -

1. (1 ml) trichloroacetic acid (TCA) 17.5%.
2. (1 ml) of 0.6% thiobarbituric acid (TBA) mixed well by vortex, incubate it in boiling water bath for (15 minutes), then allowed to cool.
3. Then add (1 ml) of 70% TCA. And let the mixture to stand at room temperature for (20 minutes).
4. Centrifuged at 2000 rpm for (15 minutes), and take out the supernatant for measuring the absorbency at (532nm).

2.2.6.2 Calculation of MDA: -

$$\text{The concentration of MAD} = \frac{\text{absorbance at 532 nm}}{L \times E_o} \times D$$

L:- light bath (1 cm)

E_o:- extinction coefficient = 1.56×10^5 M cm^{-}

$$D\text{:- dilution factor} = \frac{\text{1 ml vol. used in Ref.}}{0.15} = 6.7$$

2.2.7 Determination of cholesterol: -

Serum level of cholesterol was determined by enzymatic method, according to instruction of the company (BIOLABO / FRANCE) within the Kit.

2.2.7.1 principle: -

Cholesterol was determined according to the following reactions: -

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol estreas}} \text{Cholesterol} + \text{Free Fatty Acid}$$

$$\text{Choesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten 4 one 3} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-Ameno-antipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine (pink)} + 4H_2O$$

The intensity of the pink\red color formed is proportional to the cholesterol concentration

Cholesterol

2.2.7.2 Reagents

1. Buffer solution :-

Phosphate buffer	0.100 mol\L
Chloro-4-phenol	0.5 mmol\L

2. Enzymes: -

Cholesterol oxidase	≥ 100 U\L

Cholesterol esterase	≥ 170 U\L
Peroxidase	≥ 1200 U\L
Cholic acid sodium salt	2mmol\L
4 Amino – antipyrine	0.3mmol \L

3. Standard

Cholesterol	200mg \dl

All reagents are stable for at least 360 days at +2 to +8℃.

2.2.7.3 Procedure: -

Pipette into test tube	Sample	Standard	Blank
Sample	10µl	----------	---------
Standard	--------	10µl	--------
Reagent	1ml	1ml	1ml

The tubes were mixed and incubated at 37 ℃ for 5 min. and measured at 500nm.

2.2.7.4 Calculation: -

The concentration of cholesterol was calculated as follows: -

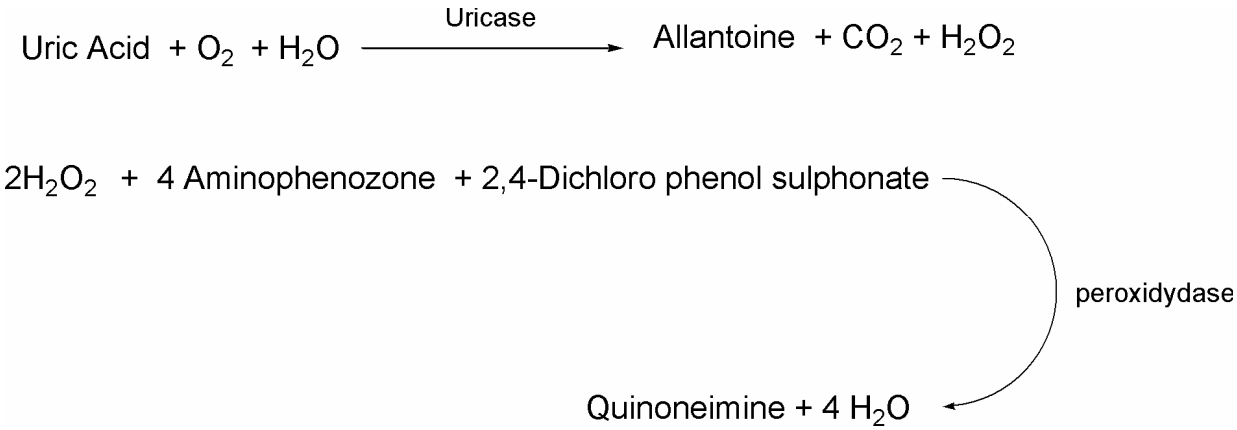
$$\text{Cholesterol concentration} = \frac{\text{A Sample}}{\text{A Standared}} \times \text{Standared concentration}$$

2.2.8 Determination of serum uric acid

Serum uric acid was determined by enzymatic method according to instruction of the company (BIO MAGHREB) within the Kit .

2.2.8.1 Principle:-

Uric acid was oxidized by uricase to allantoine and hydrogen peroxide according to the following equation :-



2.2.8.2 Reagents:-

1- Buffer solution

Phosphate buffer	pH 7.4	50 mmol / L
2-4 DHBS		4 mmol / L

2- Enzymes

Uricase	70 U / L
Peroxidase	660 U /L
4-Aminophenazone	1 mmol / L

3- Standared

Uric Acid	6 mg / dL

This working reagent is stable three weeks at 2 – 8 C°, 7 days at 20 – 25 °C.

2.2.8.3 Procedure:-

Pipettes into test tubes	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Standared	----------	20 µ l	----------
Sample	----------	----------	20 µ l

The tubes were mixed and incubated at 37 Cº for 5 min. and measured at 510 nm .

2.2.8.4 Calculation: -

The concentration of serum uric acid was calculated as follows:-

$$\text{Uric Acid conc.} = \frac{\text{A Sample}}{\text{A Standared}} \times n$$

n = 6

2.2.9 Determination of creatinine: -

2.2.9.1 Principle: -

Kinetic determination was without deproteinization; the complex was formed by creatinine and picric acid in an alkaline medium was measured. The speed of absorbency change is proportional to the creatinine concentration. (Kit, Biocon / Germany) .

2.2.9.2 Reagent: -

1. Picric acid solution

Picric acid	35 mmol /l

2. NaOH solution

Sodium hydroxide	0.32 mol/l

3. Standard

Creatinine	2 mg/dl

The solution mixture is stable for 10 day at +4 to +8 C˚ Or one day at +20 to +25C˚

2.2.9.3 Procedure.

Wave length 492nm, Temperature +37C˚

Working solution	1000 µl	1000 µl
Standard	100µl	---------
Sample	---------	100 µl

The tubes were mixed, poured immediately into cuvette. After exactly 20 seconds A_1, was read of sample and standard.

Exactly 80 seconds after the first reading, A_2 was readed for sample and standard.

2.2.9.4 Calculation: -

$$\text{Serum Creatinine conc.} = \frac{\triangle \text{ A Sample}}{\triangle \text{ A Standard}} \times 2$$

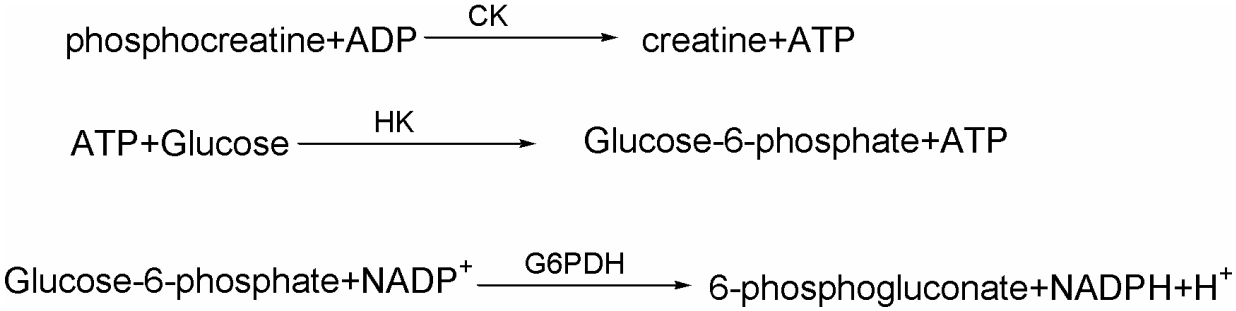
$$\triangle A = A_2 - A_1$$

2.2.10 Determination of CK: -

CK activity was measured by kinetic method, according to instruction of the company (LINER chemicals / Spain) within the Kit.

2.2.10.1 Principle: -

Creatine kinase catalyzes the transfer of phosphate group from phospho creatine to ADP. This reaction is coupled to those catalyzed by hexokinase and glucose –6- phosphate dehydrogenase. The rate of NADPH formation is proportional to the concentration of CK present in the sample.



2.2.10.2 Reagent

1. Reagent 1

Imidazol pH 6.7	100 mmol/L
Glucose	20 mmol/L

2. Reagent 2

Phosphocreatine	30 mmol/L
ADP	2 mmol/L
HK	2500 U/L
G6PDH	1500 U/L

Working reagent was prepared by dissolving a tablet of Reagent 2 in one bottle of Reagent 1.

The working reagent is stable for 5 days at 2-8C° Or 1 day at room temperature.

2.2.10.3 Procedure: -

Sample	50µl
Working reagent	2.5ml

The tubes were mixed and incubated for 2 minutes at 37 ℃ two minutes after and at 340nm, initial absorbency was recorded and at 1minute intervals There after for 3 minutes.

2.2.10.4 Calculation: -

$$\text{CK U/L} = \frac{\Delta A}{\text{min}} \quad X\ 8095$$

Min = 3

2.3 Statistical Analysis

All values were expressed as ± standard deviation (SD). Student's t-test was used to estimate differences between the groups, and the differences were considered significant when the probability (P) was $p < 0.05$.

CHAPTER THREE

3 Result and Discussion

3.1 GSH Concentration in serum of patients with DM type 2:-

Reduced glutathione (GSH) and activity of GSH related enzymes play a Key role in defense against oxygen free radicals , whose production was, raised in patients affected by diabetes mellitus[79]. Its function includes the detoxification of xenobiotics , carcinogens ,free radicals , and peroxides ; regulation of immune function ,and maintenance of protein structure , function , and turnover. [80]

Numerous laboratory studies have suggested that GSH is a critical factor in protecting organisms against toxicity and disease . Blood and GSH concentration may serve as an indicator of GSH status and, thus disease risk in human subjects. [80,72]

Compared with healthy control, GSH concentrations were found to be significantly decrease in serum of patients with DM, and GSH levels in females were less than in males patients.(Table (3-1), Figure(3-1)). show the mean ± SD of patients and controls groups , mean of IDOM was (16.836 ± 6.593) for males and (15.749 ± 7.829) for females , and the mean of control group was (27.856 ± 3.924) for males and (23.609 ± 3.18) for females .

Our results are in good agreement with the results obtained by ciuchi. [81] . , .Tessier[82] ,Erika[83] And lany .Lee . [84]

Table 3-1 GSH concentration (μM) in serum of diabetic patients type 2 compared with controls

Group	Sex	n	GSH (μM)	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	27.856	3.924	35.482	19.552	___	
H. Control	F	20	23.609	3.18	30.119	18.771	___	
Diabetes P.	M	24	16.836	6.593	29.08	9.451	P<0.05	S
Diabetes P.	F	26	15.749	7.829	23.593	7.689	P<0.05	S

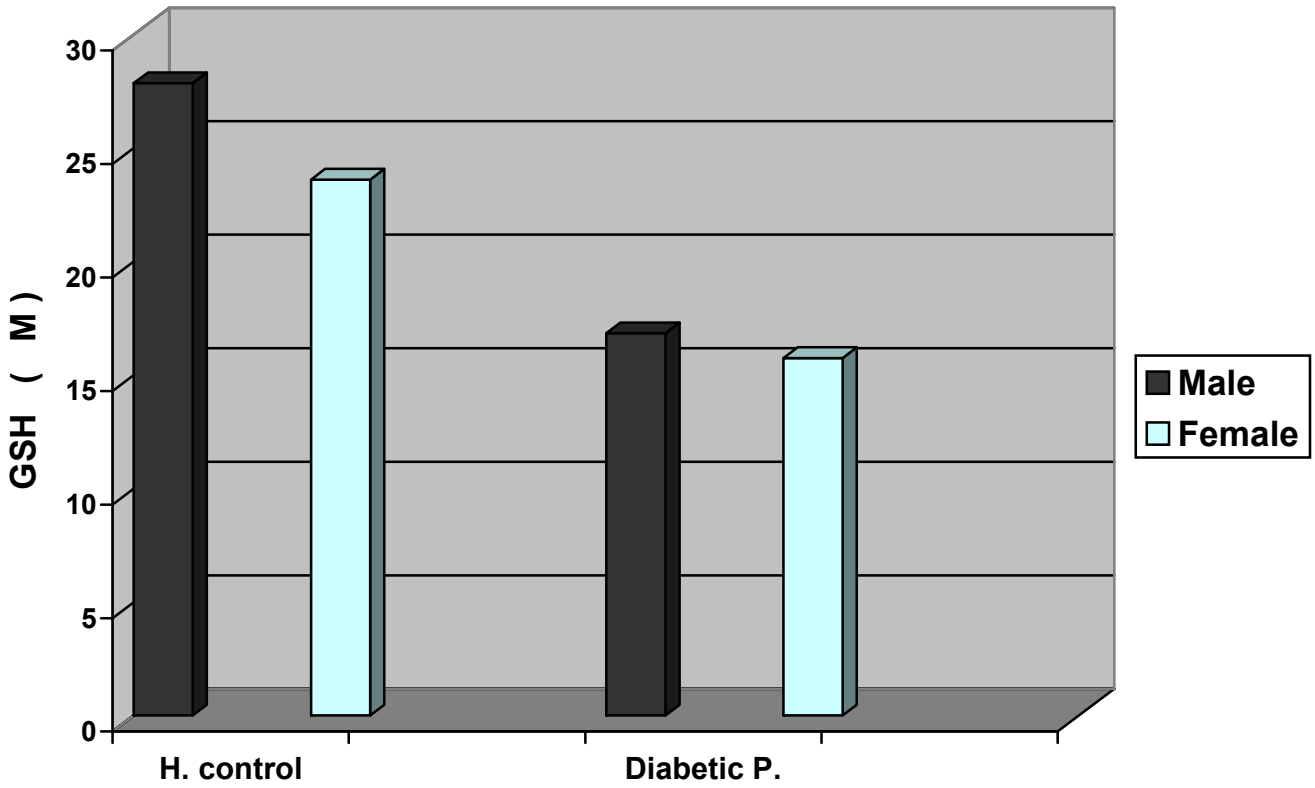


Figure 3-1 GSH concentration in serum of diabetic patients type 2 compared with controls

Another studies have reported that GSH concentration are 8-10% greater in smoker that in nonsmokers and no sex differences. [80]

Intracellular GSH, which is normally present at millimole per liter concentration, has a protective role against oxidative damage by neutralizing vitamin C or by action of GSH peroxidase . GSH peroxidase is one of the main enzyme neutralizing H_2O_2 , it catalyses the reaction :-

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

The conversion of GSSG to GSH is catalyses by glutathione reductase.this enzyme contains one or more sulphydryl group residues which are essential for its catalytic activity[85]. In such a situation, free radicals as well as aldehydes may cause the inactivation of glutathione reductase. [86] .

3.2 Vitamin C concentration in serum of patients with DM type 2:-

Vitamin C is an important vitamin which participates in a great variety of biological events concerning electron – transport reaction, the oxidative catabolism of aromatic amino acid, and scavenging ROS and RNS and may there by prevent oxidative damage to important biological macromolecules such as DNA, lipid , and protein. [39,87]

Vitamin C functions as an antioxidant by direct reaction with ROS or

regenerating vitamin E from α-tocopheroxyl radical (Figure 1-7). Thus it protects cell membranes from external oxidants and has a GSH-sparing effect. However, vitamin C cannot be efficiently transported into blood cells. The existence of DHA has long been considered evidence of ascorbate oxidation. [37]

Compared with healthy control , total and reduced vitamin C was found to decreased significantly in serum and leukocyte patients with type II diabetes mellitus (Table 3-2, Figure 3-2 , Table 3-3 , Figure 3-3 Table 3-4 Figure 3-4 , Table 3-5 and Figure 3-5).

Depleted serum vitamin C levels are shown in several conditions related to oxidative stress, such as diabetes mellitus. [87]But no report is available on leukocyte vitamin C levels in diabetics.

However, the results show that leukocytes are also affected by oxidative stress in these patients. [88]

The findings concerning plasma and leukocyte vitamin C levels of diabetics are somewhat controversial. Stankova et al.[89] found that plasma ascorbic acid concentration in non-insulin –dependent diabetes mellitus was significantly reduced. Cunningham et . al [90] and Chen et al. [91] showed that mononuclear leukocyte level of ascorbic acid was significantly lower than that of controls in both insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus. These results are in accordance with our results.

Table 3-2 Total Vitamin C concentration (mg/dl) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	total Vit C. mg/dl	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	1.124	0.258	1.607	0.615	___	
H. Control	F	20	0.976	0.2	1.432	0.663	___	
Diabetes P.	M	24	0.999	0.173	1.359	0.663	P<0.05	S
Diabetes P.	F	26	0.893	0.166	1.312	0.621	P>0.05	NS

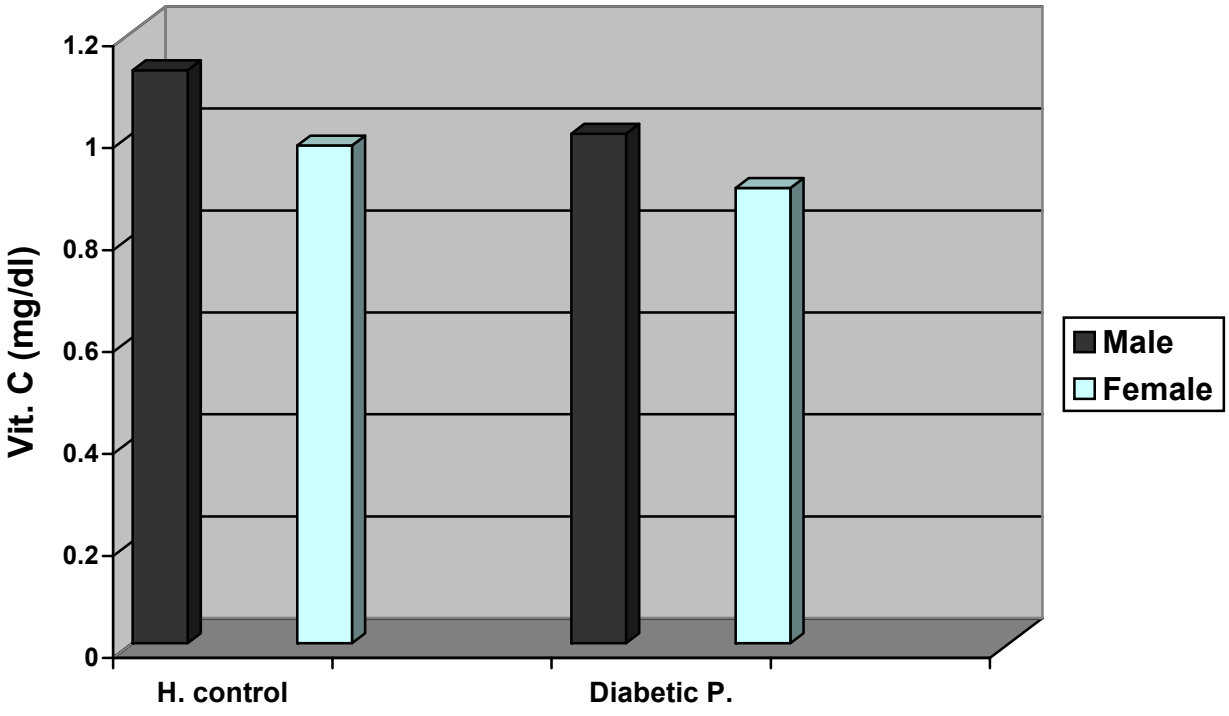


Figure 3-2 Total Vit. C concentration in serum of diabetic patients type 2 compared with controls

Table 3-3 leukocytes total Vit. C concentration (µg/10^8 leuk) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	Leukocytes total Vit. C µg/10^8 leuk	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	41.04	6.755	61.029	28.293	___	
H. Control	F	20	40.853	7.571	57.051	28.982	___	
Diabetes P.	M	24	36.359	6.688	49.285	25.291	P<0.05	S
Diabetes P.	F	26	35.542	6.525	48.125	21.215	P<0.05	S

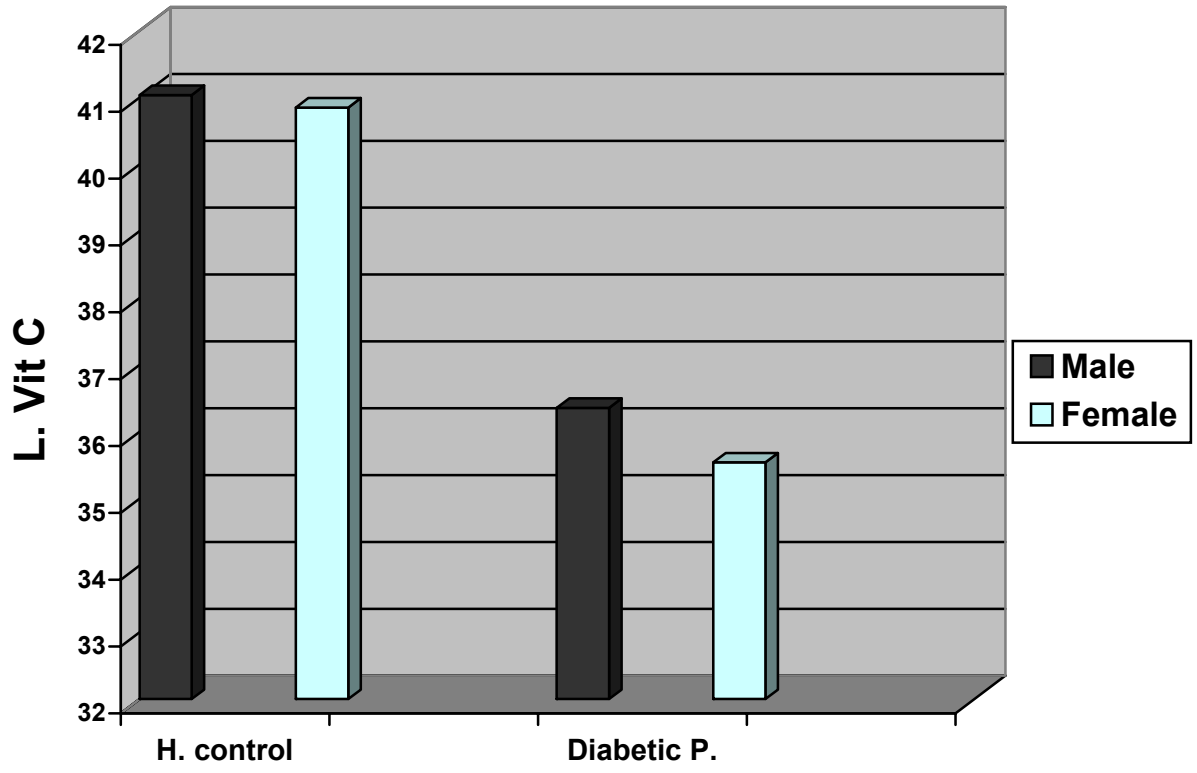


Figure 3-3 Leukocytes total Vit. C concentration in leukocyte of diabetic patients type 2 compared with controls

Table 3-4 Reduced Vitamin C concentration (mg/dl) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	R. Vit C. mg/dl	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	0.931	0.236	1.366	0.454	___	
H. Control	F	20	0.800	0.178	1.205	0.499	___	
Diabetes P.	M	24	0.812	0.161	1.138	0.499	P<0.05	S
Diabetes P.	F	26	0.741	0.153	1.095	0.46	P<0.05	S

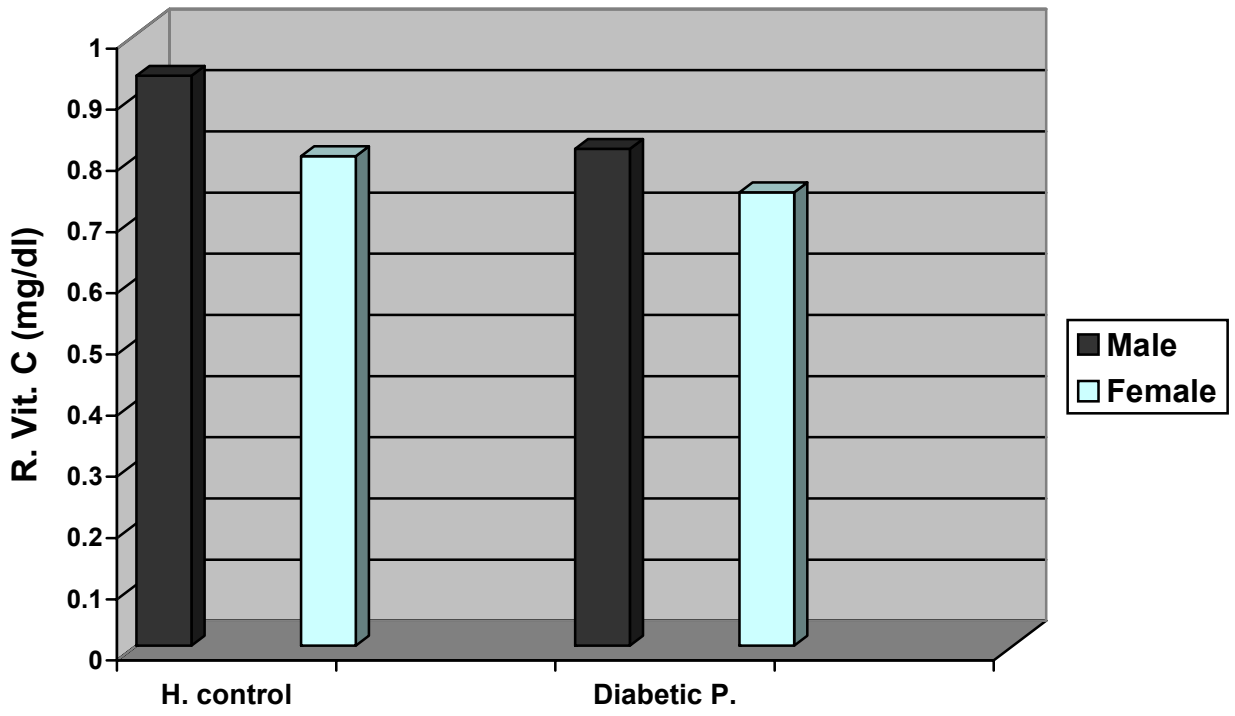


Figure 3-4 Reduced Vit. C concentration in sera of diabetic patients type 2 compared with controls

Table 3-5 Leukocytes. Reduced Vit. C concentration (µg/10^8 leuk) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	Leukocytes R.Vit. C µg/10^8 leuk	±SD	Upper Value	Lower Value	P	Sig
H. Control	M	22	37.542	6.354	55.982	25.894	___	
H. Control	F	20	37.828	6.92	52.326	26.527	___	
Diabetes P.	M	24	33.229	6.27	45.188	23.135	P<0.05	S
Diabetes P.	F	26	33.239	6.216	44.122	19.388	P<0.05	S

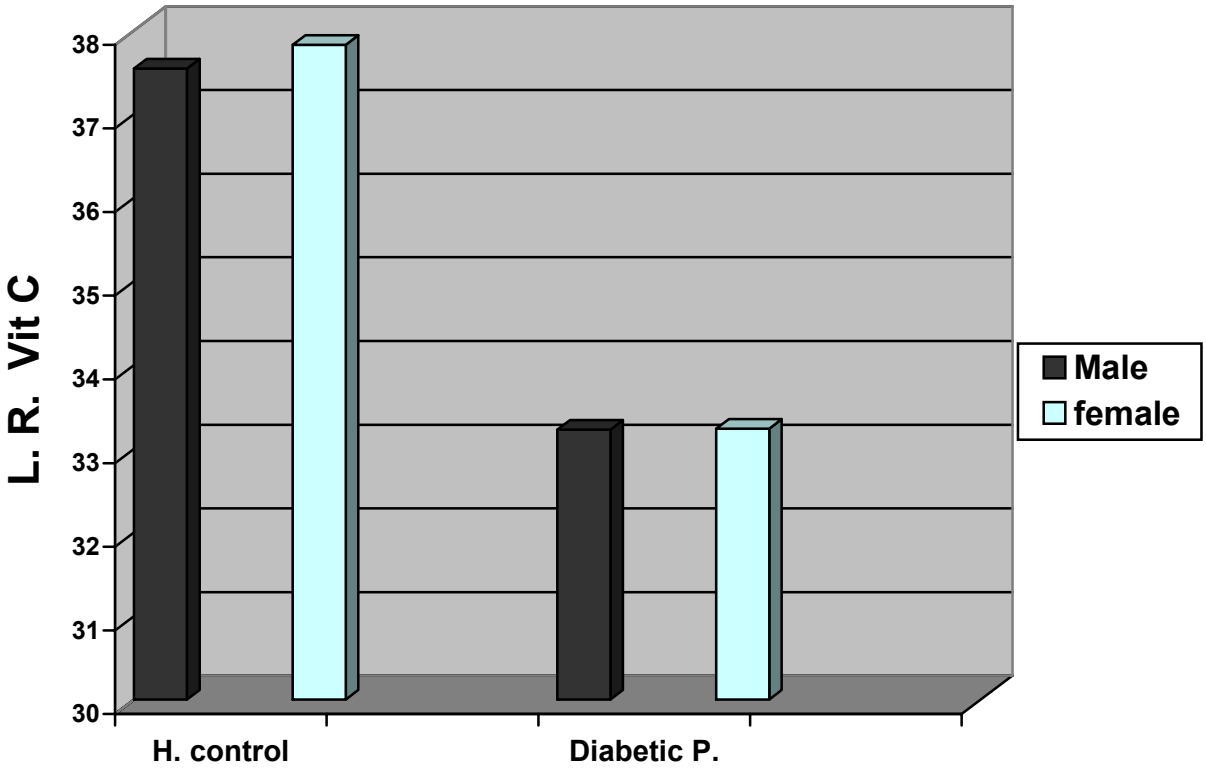


Figure 3-5 Leukocytes reduced Vit. C concentration in leukocyte of diabetic patients type 2 compared with controls

The correlation between glutathione and serum ascorbic acid and leukocytes ascorbic acid was highly significant. The correlation coefficients: r = 0.63, 0.68, for women and r =0.65, 0.65, for men in the serum and leukocytes ascorbic acid, respectively.

Several studies with rodents have pointed to an association between glutathione and ascorbate. The concentration of ascorbate in the tissues of newborn rats or guinea pigs that are not able to synthesize ascorbate decreases significantly after treatment with buthionine sulfoximine, an agent that inactivates glutamate–cysteine ligase and reduces tissue glutathione[126]. In these animals, the administration of ascorbate increases tissue glutathione, indicating that ascorbate spares glutathione. Conversely, the effects of glutathione esters in ascorbate-deficient guinea pigs suggest that glutathione spares ascorbate [126]. Interestingly, a correlation similar to that was observed between total glutathione and ascorbate in rat heart, to which oxidative stress was induced by ischemia reperfusion [127].

In another study, the concentration of ascorbate in rat hepatic cells is show to double after administration of lipoic acid, a compound that is known to increase the concentration of intracellular glutathione [128]. However, in a recent study with ascorbate requiring Shionogi rats with osteogenic disorder, the concentrations of glutathione in liver and kidney are unaffected by a 60–70% diet-induced decrease of ascorbate in these tissues [129]. The study of glutathione and ascorbate synergy in humans is limited to the measurement of glutathione in plasma or erythrocytes. Henning et al [130] reported that a reduction in the

concentration of vitamin C in plasma to 6 mmol/L led to a significant decrease in the concentration of total plasma glutathione as well as in the ratio of glutathione to glutathione disulfide.

Conversely, 2 studies reported significant increases in erythrocyte glutathione after supplementation with vitamin C. A 4 weeks regimen of 1 g vitamin C/day is reported to increase erythrocyte glutathione by 28% [131]. Similarly, erythrocyte glutathione is shown to increase nearly 50% after supplementation with 0.5 or 2 g vitamin C/d for 1 week, although there was a large variation in individual responses [132].

3.3 Llipid peroxidation in serum of patients with DM type 2:-

From the Table (3-6) and Figure (3-6), lipid peroxidation of patients was significantly (P> 0.05) increased.

Malondialdehyde is one of the lipid peroxidation products frequently used to determine the oxidant / antioxidant balance in diabetic patients. [94,95] However, there are contradictory results in literature about serum MDA levels in patients with diabetes . Sundaram et al.[96], and Hatice P. [97] found that lipid peroxidation was significantly raised in serum of patients with type II diabetes, and these results are in accordance with our results .

The exact mechanism by which elevated blood glucose leads to lipid peroxidation in plasma of diabetic patients is still unknown. however , in vitro studies have shown that glucose can enolize and thereby

reduce molecular oxygen under physiological conditions , yielding α–keto aldehydes , hydrogen peroxide and free radical intermediates . [92,93]

High levels of glucose can produce permanent chemical alterations in proteins and increase lipid peroxidation in a variety of experimental models of hyperglycemia[98,99] . Hyperglycemia, itself, may stimulate platelet aggregation[100,101] , and autooxidation of glucose may also lead to free radical production in diabetics . [45]

Negative correlation was found between the levels of serum MDA and vitamin C (r=-0.517, -0.501) for male in serum and leukocytes respectively and (r=-0.611, -0.631) for female in serum and leukocytes respectively, and this result are in agreement with the results obtained by Noyan [133]

Table 3-6 MDA concentration (nmol/ml) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	MDA nmol/ml	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	2.884	0.682	4.171	1.382	___	
H. Control	F	20	2.409	0.61	3.91	1.451	___	
Diabetes P.	M	24	5.151	1.135	7.135	3.101	P<0.05	S
Diabetes P.	F	26	4.941	1.064	6.971	2.809	P<0.05	S

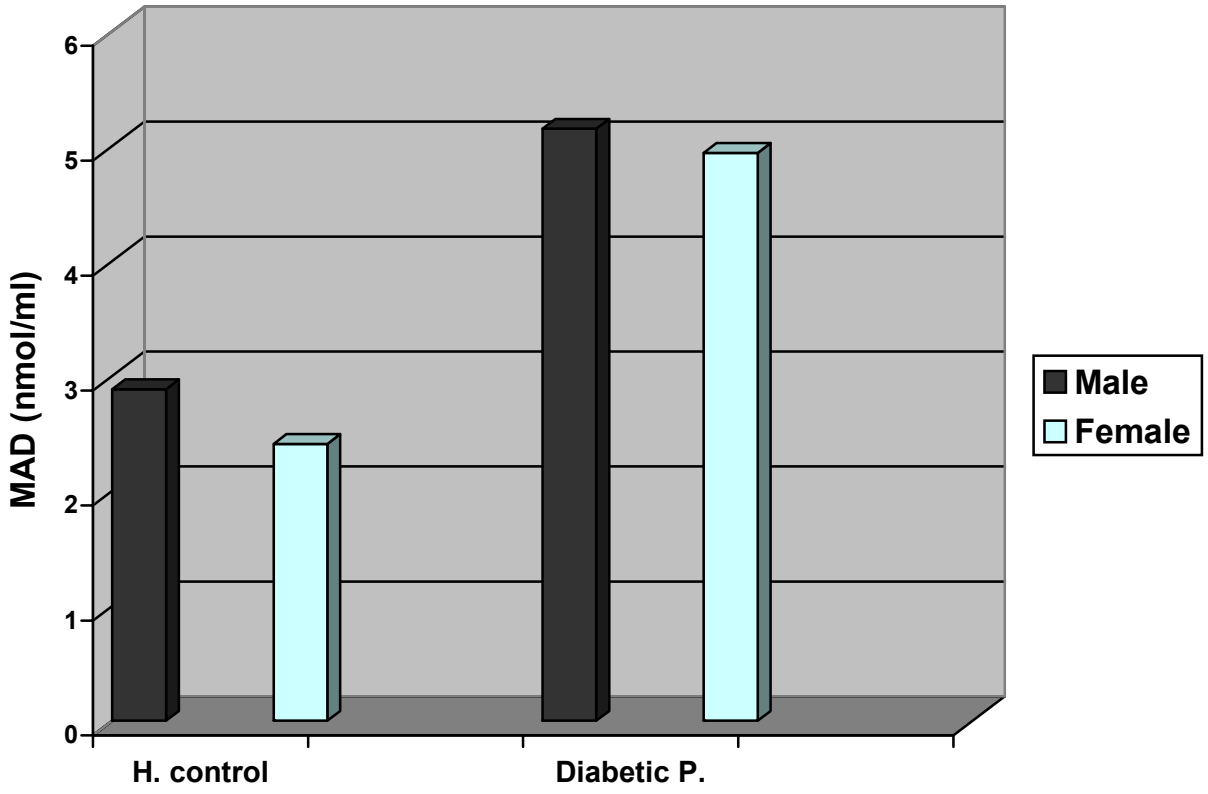


Figure 3-6 MDA concentration in sera of diabetic patients type 2 compared with controls

3.4 Total cholesterol concentration in serum of patients with DM type 2:-

In DM . patients the increase in the cholesterol level of the blood plasma was noted more frequently than in persons without disorders in carbohydrate metabolism . Insulin stimulates the synthesis of cholesterol in liver tissues[102].While In D.M patients formation of cholesterol and ketone bodies will increase. [103]

Increase amount of free fatty acid (FFA) dose not have time to be oxidized in the krebs cycle and in D.M patients with a lack of insulin, the content of blood FFA, triglycerides and cholesterol increase [14]. In this work , the lower values of serum total cholesterol (STC) in NIDDM , were (132.4) mg/dl and (108.1) mg/dl while the highest values were (395.7) mg/dl and (379.5) mg/dl for males and females respectively . Figure (3-7) represents the (mean) of the control and patient groups of serum total cholesterol , Table (3-7) shows the mean , ± SD of STC in NIDDM, it was (230.6 ± 75.942) for males patients and (228.5 ± 56.786) for females patients , while for healthy controls was (197.3 ± 23.124) and (199.2 ± 36.718)for males and females respectively ,and these result were significant (P> 0.05) .

These results were in agreement with the results obtained by polumbo[105]. Who showed that DM patients had an increase in the synthesis of endogenic cholesterol and retardation of its excretion .
Stout[112], postulated the influence of uncontrolled hyperglycemia in DM patients on the increase in the production of cholesterol and also in relation between the level of triglycerides and the excretion of bild acids

Table 3-7 Total cholesterol concentration (mg/dl) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	Total Cholesterol mg/dl	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	197.3	23.124	274	183	___	
H. Control	F	20	199.2	36.718	325.5	138.9	___	
Diabetes P.	M	24	230.6	75.942	395.7	132.4	P<0.05	S
Diabetes P.	F	26	228.5	56.786	379.5	108.1	P<0.05	S

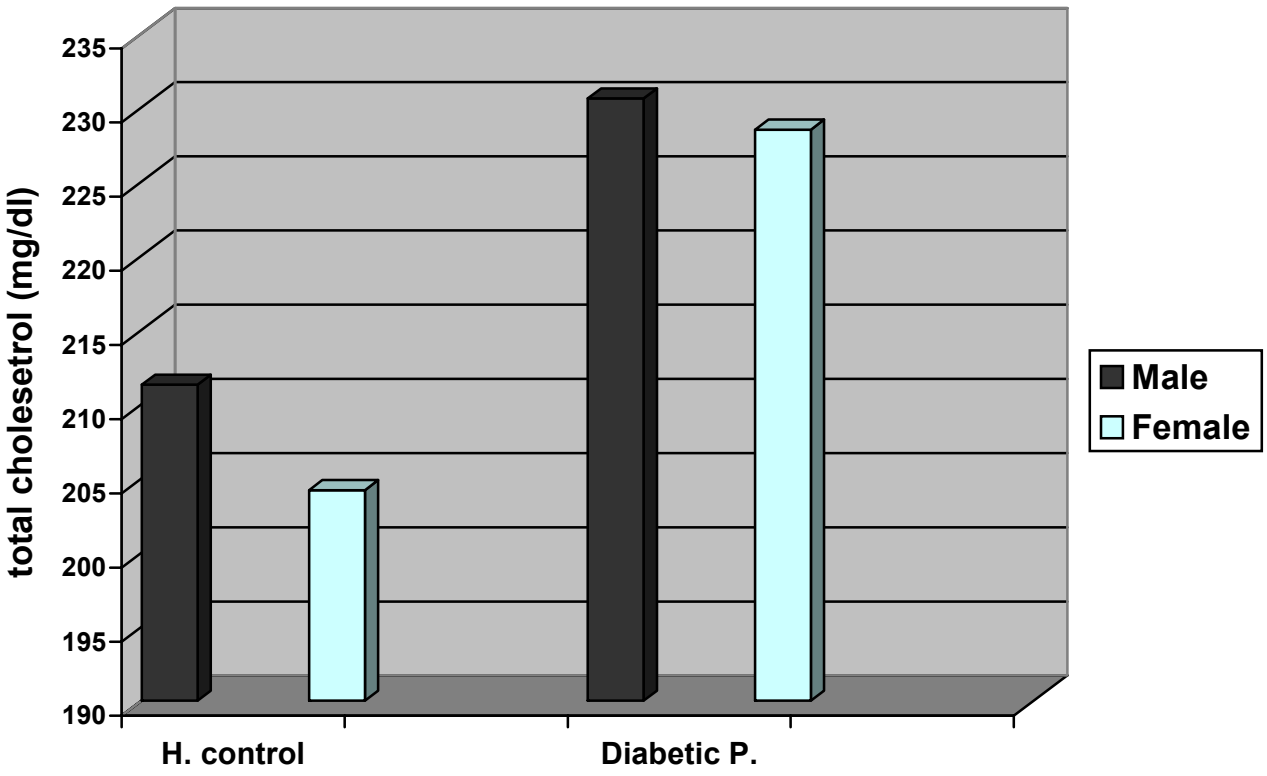


Figure 3-7 Total cholesterol concentration in sera of diabetic patients type 2 compared with controls

3.5 Serum uric acid in serum of patients with DM type 2:-

The evidence suggests that hyperuricemia is linked to obesity[107], hypertension[108], reduced HDL cholesterol , [109] hypertriglyceridemia[110], hyperinsulinemia and reduced insulin sensitivity [111,112].

Figure (3-8) represents the (mean) value of serum uric acid , Table(3-8) shows the mean , ± value of serum uric acid in NIDDM, which was (8.89± 2.288) mg/dl for male patients , and (8.61± 1.767) mg/dl for females patients , while for healthy controls was(4.27 ± 0.621) mg/dl (3.89± 0.57) mg/dl for males and females respectively , and these result are significant (P> 0.05) .

Elevated levels of serum uric acid are due to either an increase in uric acid production or a decrease in its excretion . [112].

Uric acid is one of the major endogenous water – soluble antioxidants of the body [113]. There is accumulating evidence that increased oxidative stress is closely related to diabetes and its vascular complications . [114] Thus , high circulating uric acid levels may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants , eg , uric acid. Interestingly , uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress . [113]

Table 3-8 Uric acid concentration (mg/dl) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	Uric Acid mg/dl	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	4.27	0.621	5.3	3.2	__	
H. Control	F	20	3.89	0.57	4.8	2.9	__	
Diabetes P.	M	24	8.89	2.288	13.1	5.8	P<0.05	S
Diabetes P.	F	26	8.61	1.767	13	5.8	P<0.05	S

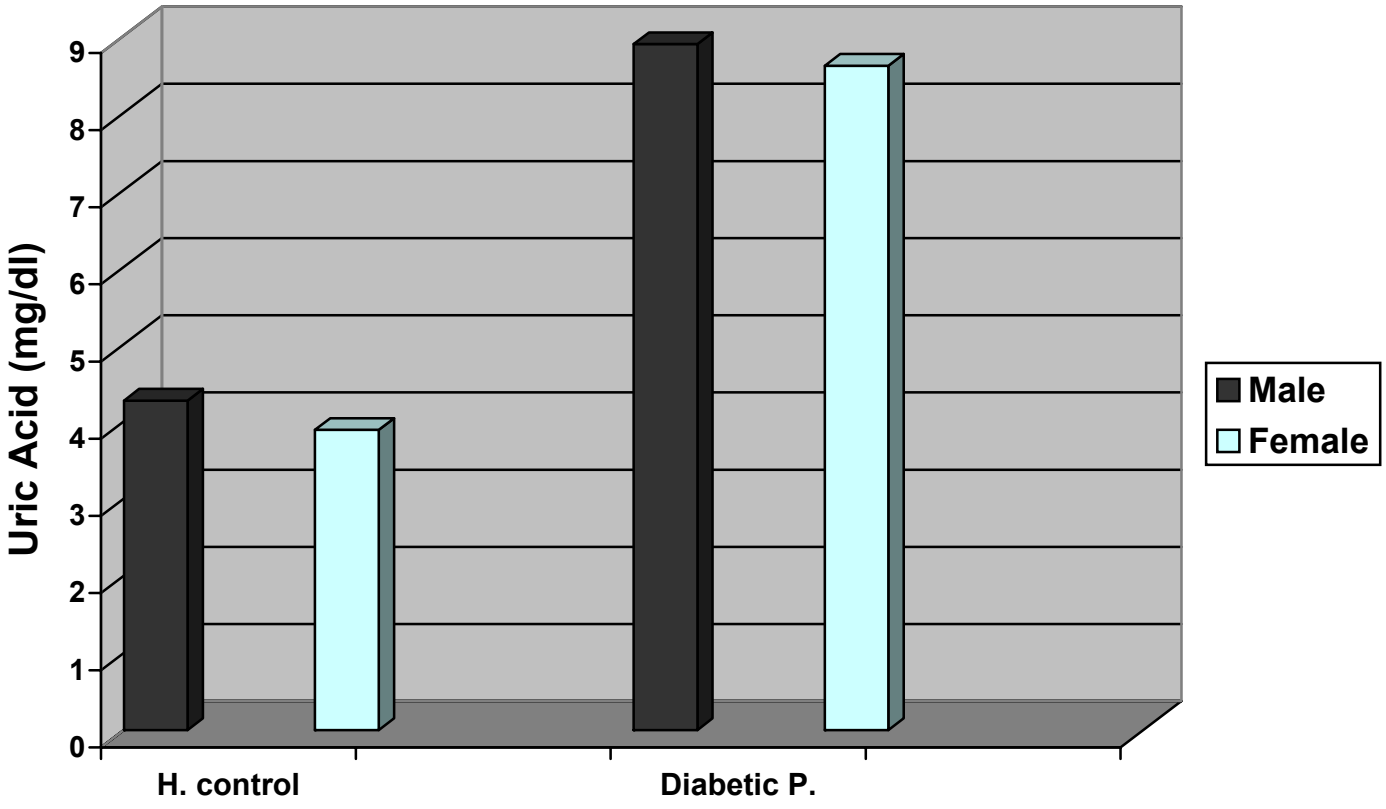


Figure 3-8 Uric acid concentration in sera of diabetic patients type 2 compared with controls

The results obtained in this study are in agreement with the results obtained by perry I [115]., Bonora . [116],, and Agamah . [117] ,

There were negative correlation between serum uric acid and vitamin c (r=-0.346 , -0.432) for male in serum and leukocytes respectively and (r=- 0.41, -0.461) for female in serum and leukocytes respectively , and this result is in agreement with the results obtained by Sinha . [134]

3.5.1 Uric acid as a marker of insulin resistance:-

Insulin resistance syndromes result in attenuation of insulin-mediated glucose utilization and confer a substantial increase in cardiovascular risk, through activation of several pathways including the sympathetic nervous system. Elevated serum uric acid is a consistent feature of the insulin resistance syndromes, which are also charcterized by elevated plasma insulin level, blood glucose concentration, and serum triglyceride concentration, and raised body mass index and waist-hip ratio. Insulin has aphysiological action on renal tubules, causing reduced sodium and uric acid clearance. Despite blunting of the action of insulin on glucose metabolism, sensitivity to the renal effects persists. Because plasma insulin concentration is characteristically elevated, hyperuricaemia may a rise as a consequence of enhanced renal insulin activity. Elevated serum uric concentrations predict subsequent development of diabetes mellitus, and hypertension, even in the presence of normal creatinine clearance and plasma glucose concentration, and therefore may be a subtle, early marker of peripheral insulin resistance syndromes. [54]

3.6 Creatinine concentration in serum of patients with DM type 2:-

In conclusion , crn degradation seems to be virtually irrelevant under conditions when crn migth even have a beneficial effect by acting as a hydroxyl radical scavener . At greatly reduced glomerular filtration rate (GFR), on the other hand , when the serum concentration of crn and oxidative stress are considerably increased , the formation of toxic crn degradation products is favored and may contribute significantly to further disease progression . Because Crn degradation is stimulated by ROS and in practicular by the hydroxyl radical , the serum concentration of creatol and methylguanidine as well as creatol /crn and methylguanidine /crn ratios may not only serve as diagnostic indeces for the degree of chronic renal failure (CRF) , but also be used as measures of oxidative stress in patients .[117,60]

Figure (3-9) represents the (mean) of the control and patients groups of serum cratinine concentration (SCC), table (3-9) shows the mean , ± SD and P of (SCC) in NIDDM , it was (1.144± 0.389) mg/dl for males patients , and (0.936± 0.306) mg/dl for females patients , as compared with that found in healthy controls (0.831 ± 0.337) mg/dl and (0.784± 0.253) mg/dl for meals and females respectively , and these result are significant (P> 0.05) .

These results are in agreement with the results obtained by Beatriz [118], and christensson .[119]

Table 3-9 Creatinin concentration (mg/dl) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	Creatinin mg/dl	±SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	0.831	0.337	1.631	0.413	___	
H. Control	F	20	0.784	0.253	1.251	0.411	___	
Diabetes P.	M	24	1.144	0.389	2.017	0.509	P<0.05	S
Diabetes P.	F	26	0.936	0.306	1.691	0.491	P<0.05	S

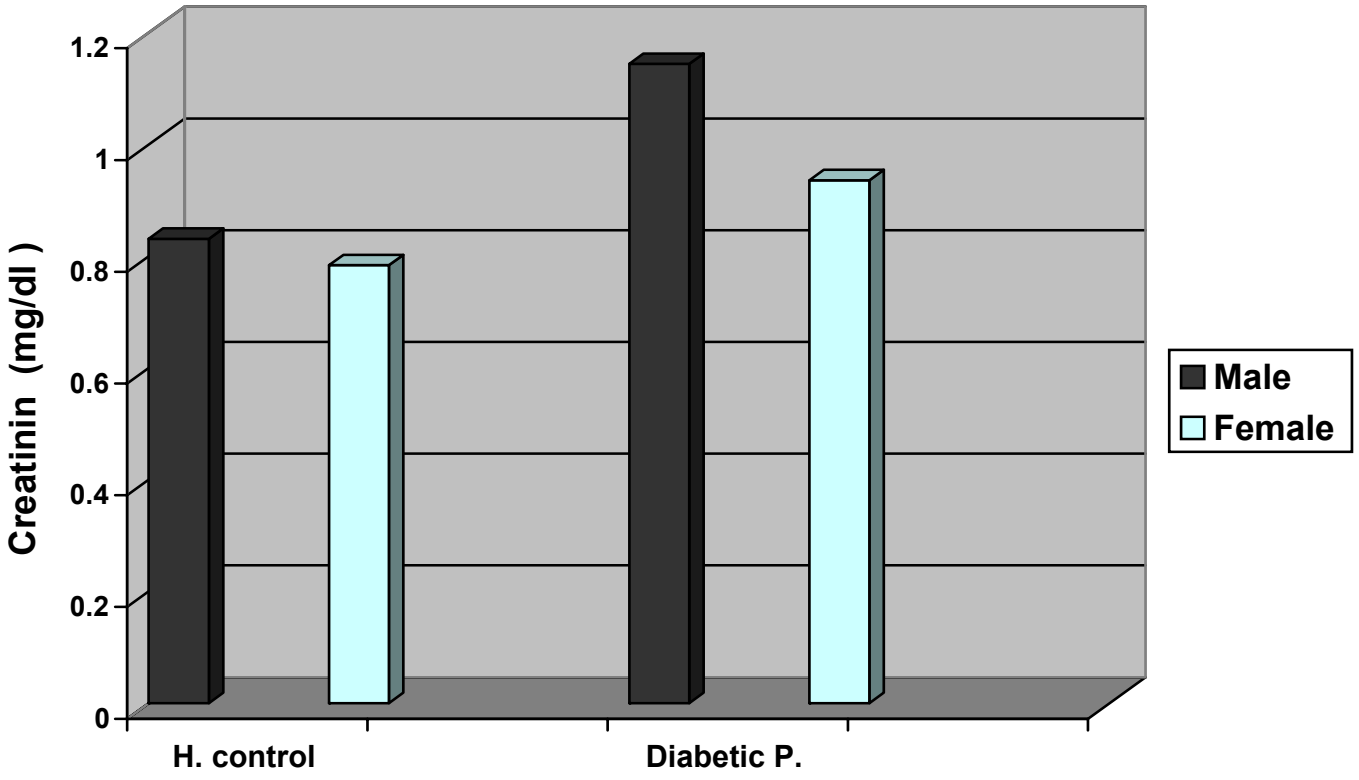


Figure 3-9 Creatinine Concentration in Sera of diabetic patients type 2 compared with controls

3.7 Creatine kinase activity in serum ofpatients with DM type 2:-

The serum total CK level in healthy individuals depends on age, race, lean body mass and physical activity.[120,121] Contradictory reports can be found in the literature on the relationship between diabetes and serum creatine Kinase activity, which has been variously described as increased or decreased in diabetes. [122,123,124,125]

Figure (3-10) represents the (mean) of the control and patients groups of serum total CK activity.

Table (3-10) shows the mean, ± SD and P of serum CK activity in NIDDM, it was (217.89 ± 88.663) U/L for male patients , and (196.412 ± 72.784) U/L for female patients , while for healthy control was (89.567 ± 11.267)U/L and (87.188 ± 11.748)U/L for male and female respectively, and these result are significant (P< 0.05).

The result above are agreement with Lazarov [123]., and Scott .[124]

Table 3-10 Total CK activity level (U/L) in sera of diabetic patients type 2 compared with controls

Group	Sex	n	CK U/L	+SD	Upper Value	Lower Value	P	Sig.
H. Control	M	22	89.567	11.267	113.415	73.145	___	
H. Control	F	20	87.188	11.748	112.079	70.367	___	
Diabetes P.	M	24	217.89	88.663	420.621	43.173	P<0.05	s
Diabetes P.	F	26	196.412	72.784	412.854	40.475	P<0.05	s

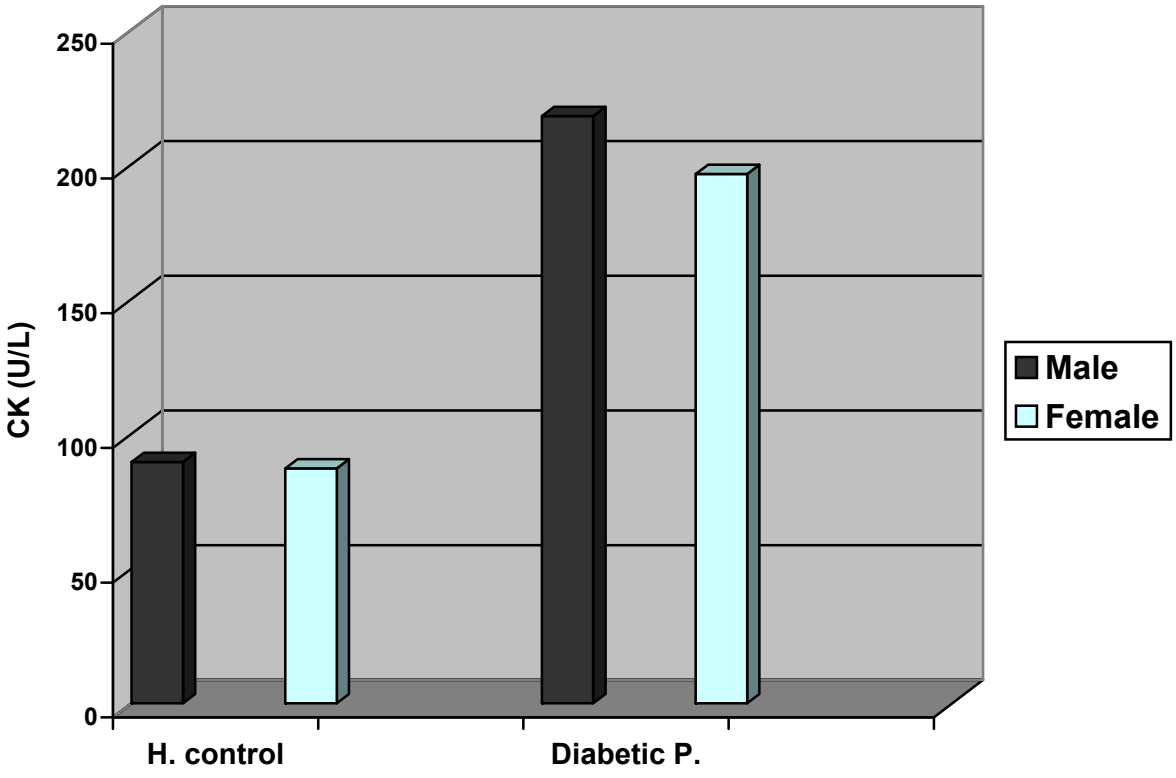


Figure 3-10 total CK activity in Sera of diabetic patients type 2 compared with controls

Conclusions

1- A new finding that Vitamin C (ascorbic acid and dehydroascorbic acid) significantly decreased in serum and leukocytes of patients of NIDDM, except in female , the decreasing was not significant, as a response to oxidative stress.

2- Glutathione (GSH) significantly decreased in serum of patients of NIDDM, as a response to oxidative stress.

3- MDA significantly increased as a response to increase Lipid peroxidation.

4- Creatinine significantly increased due to greatly reduced glomerular filtration rate (GFR), on the other hand , when the serum concentration of crn as well as oxidative stress are considerably increased.

5- Uric acid significantly increased.It may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants, like uric acid, but within its normal physiological value (3-7 mg/dl).

6- Total cholesterol concentration was significantly increased. Increase amount of free fatty acid (FFA) could have no time to be oxidized in the krebs cycle and in D.M patients with a lack of insulin, the content of blood FFA, triglycerides and cholesterol increased.

7- CK significantly increased and it may be due to oxidative stress.

LIST OF TABLES

Futures studies

1-determine lipid peroxidation in leukocytes of patients with NIDDM and IDDM.

2-Study the kinetic of CK and its isoenzymes in serum of NIDDM and IDDM.

3-Study the activity of glutatione reductase in patients with NIDDM and IDDM.

حالة مضادات الاكسدة في مصل الدم وكريات الدم البيض للمرضى المصابين باء السكري غير المعتمد على الانسولين

رسالة مقدمة الى

مجلس كلية العلوم-جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير في

علوم الكيمياء الحياتية

من قبل

احمد يونس عبد الجنابي

بكالوريوس علوم كيمياء

جامعة بغداد 1999 م

الخلاصة

أجريت هذه الدراسة على 50 مريضا (24 ذكور , 26 إناث) يعانون من الإصابة بداء السكري النوع الثاني (غير المعتمد على الأنسولين) و 42 من الأصحاء (22 ذكور, 20 إناث) راجعوا مستشفى المحمودية العام في مدينة المحمودية للفترة من نيسان 2004 إلى آب 2004 .

تم قياس تركيز فيتامين c في مصل الدم وفي كريات الدم البيض , وبعض مضادات الأكسدة مثل الكلوتاثايون وحامض اليورك و الكرياتينين , كذلك اشتملت الدراسة على قياس فعالية أنزيم الكرياتين كاينيز , وقياس تركيز المالون داي الديهايد و الكوليستيرول الكلي في الدم.

بالمقارنة مع الأصحاء , فان تركيز فيتامين c في مرضى السكري النوع الثاني ينخفض بصورة معنوية في مصل الدم وفي كريات الدم البيض عدا عند الإناث المصابات بالمرض فان مقدار الانخفاض في مستوى الفيتامين في مصل الدم غير معنوي.

كذلك أظهرت النتائج انخفاض مستوى الكلوتاثايون في مصل الدم بصورة معنوية فيما ارتفع تركيزحامض اليورك و الكرياتينين في مصل الدم بصورة معنوية .

و أظهرت الدراسة أيضا زيادة فعالية أنزيم الكرياتين كاينيز في مصل الدم بصورة معنوية وكذلك تركيز المالون داي الديهايد و الكوليستيرول في مصل الدم مقارنة بالأصحاء.

وبذلك تكون هذه الدراسة هي اول دراسة تعنى بقياس مستويات فيتامين (C) في كريات الدم البيضاء لمرض السكري من النوع غير المعتمد على الانسولين.

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