تقييم الحالة التأكسدية لمرضى داء السكري بأستخدام بعض المؤشرات الأنزيمية

أطروحة مقدمة الى
مجلس كلية العلوم ـ جامعة بابل
وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة علوم
في علوم الكيمياء / الكيمياء الحياتية السريرية

من
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تشرين الأول ـ ٢٠٠٥ رمضان - ١٤٢٦

الخلاصة

يعتبر داء السكري من المشاكل الصحية الشائعة، والذي يتميز بارتفاع مستوى السكر الناجم عن الفقدان أو النقص النسبي للأنسولين الذي تفرزه خلايا بيتا في جزر لانكرهانس في البنكرياس.

يعد مرض السكري من العناصر المتلازمة Syndrome لضغط المؤكسدات ذات الصلة بالتوازن بين أنواع الأوكسجين الفعال ونظام الكاسحات Scavengers.
تهدف هذه الدراسة إلى إظهار مستوى ضغط المؤكسدات للمصابين بالنوع الأول والنوع الثاني من مرض السكري وكشف أقوى مؤشر للجذور الحرة في حالة هذا المرض.
تم إجراء هذه الدراسة في مستشفى الديوانية العام وفي قسم الكيمياء / كلية العلوم / جامعة بابل وفي قسم الكيمياء / كلية الطب / جامعة النهرين, العراق. صممت هذه الدراسة لغرض الكشف عن بعض المتغيرات الكيميائية الحياتية لمصل الدم. خضع ٥٠ مريضا بالسكري من النوع الأول، و٥٠ مريضا بالسكري من النوع الثاني، و٥٠ شخصا سويا لفحوصات هذه الدراسة.

القياسات الرئيسية في هذه الدراسة هي قياس التغيرات البايولوجية النوعية لمتلازمات الحالة التأكسدية (أكسدة الدهون وارتفاع مستوى الهوموسستين) وقياس فعالية بعض مضادات الأكسدة الإنزيمية Enzymatic Antioxidants: إنزيم Superoxide Dismutase وإنزيم Catalase وإنزيم Glutathione S-transferase و إنزيم Creatine Kinase وقياس بعض مضادات الأكسدة غير الإنزيمية Non-enzymatic Antioxidants: الكلوتاثايون Glutathione وفيتامين E وفيتامين C وفيتامين A وقياس العناصر التالية: الحديد والنحاس والزنك والكروم والسيلينيوم.

سجلت نتائج هذه الدراسة ظهور ارتفاع ملحوظ في مستوى المالون داي ألدهايد (الناتج النهائي لأكسدة الدهون) والهوموسستين الكلي في مصول دم المرضى المصابين بالسكري من النوع الأول والثاني عن ذلك المستوى الذي ظهر في المجموعة الضابطة. أظهرت مستويات أكسدة الدهون ارتباط معنوي سالب جدير بالملاحظة مع مستويات فيتامين E ($r = -0.345$, $P \leq 0.05$) ولم يظهر أي ارتباط مع فيتامين C و A في مصول المرضى المصابين بالنوع الأول من مرض السكري، بينما في المرضى المصابين بالنوع الثاني من مرض

السكري، فقد أظهرت ارتباطا معنويا سالبا مع مستويات فيتامين C ($r = -0.284, P \leq 0.05$) ولم يظهر أي ارتباط معنوي مع فيتامين E و A.

أشارت هذه الدراسة إلى أن الإنزيمين Superoxide Dismutase و Glutathione S-transferase يرتفع مستواهما بشكل ملحوظ في مصول دم المرضى المصابين بالنوع الأول والثاني من مرض السكري عن مستوياتهما في المجموعة الضابطة ($P \leq 0.05$). ولكن الدراسة أظهر انخفاضا ملحوظا في مستويات إنزيمي Creatine Kinase و Catalase في مرضى كلا نوعي مرض السكري. على الرغم من ذلك فإن مستوى فعالية إنزيم Superoxide Dismutase في كلا نوعي مرض السكري يميل إلى أن يكون متفاوتا ولكنه لم يظهر ارتباطا معنويا مع مستويات النحاس وإنزيم Catalase. بينما أظهر ارتباطا معنويا سالبا مع مستوى الزنك ($P \leq 0.05$).

أظهرت الدراسة أيضا انخفاضا معنويا في مستويات الكلوتاثايون وفيتامين E وفيتامين C وفيتامين A في مصول المرضى المصابين بمرض السكري من النوع الأول والثاني بالمقارنة مع المجموعة الضابطة ($P \leq 0.05$). فضلا عن أن الكلوتاثايون قد أظهر علاقات غير معنوية مختلفة مع مستويات المالون داي ألدهايد و الهوموسستين الكلي و فعالية إنزيم Creatine Kinase في مصول المرضى المصابين بمرض السكري من النوع الأول والثاني. إضافة إلى ذلك، أظهر الكلوتاثايون ارتباط معنوي سالب مع مستوى فعالية إنزيم Glutathione S-transferase في مصول دم المرضى المصابين بالنوع الثاني من مرض السكري ($r = -0.309, P \leq 0.05$)، في حين كان هذا الارتباط غير معنوي لدى المرضى المصابين بالنوع الأول من مرض السكري.

دلت هذه الدراسة على أن مرض السكري يسبب اضطراب في مستويات العناصر النزرة، لذا كان هناك انخفاض معنوي في مستوى الحديد والزنك والكروم والسيلينيوم في مصول دم المرضى المصابين بالنوع الأول من مرض السكري عن مستواها في المجموعة الضابطة ($P \leq 0.05$). بينما لوحظ انخفاض معنوي لدى مرضى النوع الثاني من مرض السكري عن تلك التي ظهرت في المجموعة الضابطة. كما أن هنالك ارتفاع معنوي في مستويات النحاس في مصول دم المرضى المصابين بمرض السكري من النوع الأول والثاني عن مستوياته في المجموعات الضابطة ($P \leq 0.05$).

في هذه الدراسة، لوحظت زيادة غير معنوية، في مستويات كل من المالون داي ألدهايد ، الهوموسستين الكلي،النحاس، الحديد وفعالية إنزيم Glutathione S-transferase في

حين ظهر انخفاض طفيف، ولكن غير معنوي أيضا، في مستويات كل من الكلوتاثايون، فيتامين C، الزنك، السيلينيوم، الكروم، وفعالية الإنزيمات Superoxide Dismutase ، Creatine Kinase، Catalase في مصول دم المرضى المدخنين المصابين بالنوع الأول والثاني من مرض السكري عن مستوياتها التي ظهرت لدى المرضى غير المدخنين. كذلك، لوحظ ارتفاع طفيف ولكن غير معنوي في مستوى فيتامين E في مصول دم المرضى المدخنين المصابين بالنوع الأول من مرض السكري ومستوى فيتامين A في مصول دم المرضى المدخنين المصابين بالنوع الثاني من مرض السكري عن مستوياتها في المرضى غير المدخنين ($P \leq ٠.٠٥$). لكن كان هنالك انخفاضا معنويا في مستويات فيتامين A في مصول دم المرضى المدخنين المصابين بالنوع الأول من مرض السكري وفيتامين E في مصول دم المرضى المدخنين المصابين بالنوع الثاني من هذا المرض بالمقارنة مع المجموعات الضابطة ($P \leq ٠.٠٥$).

توصلت الدراسة إلى أن مرض السكري هو أحد عناصر المتلازمة لضغط المؤكسدات، وهو يختزل بعض مضادات الأكسدة الإنزيمية وغير الإنزيمية. كما استنتجت الدراسة أن انخفاض مستويات مضادات الأكسدة (الكلوتاثايون و Catalase) يعتبر من افضل الدلائل على ارتفاع المتلازمة لضغط المؤكسدات في مرض السكري. ويمكن استخدام جميع مؤشرات الجذور الحرة في هذه الدراسة للكشف المبكر عن مضاعفات مرض السكري. يعمل التدخين على اختزال مستويات مضادات الأكسدة، لذا تزداد المتلازمة لضغط المؤكسدات ومضاعفات مرض السكري لدى المرضى المدخنين المصابين بهذا المرض عن مستوياتها لدى المرضى غير المدخنين.

EVALUATION OF THE OXIDATIVE STRESS IN PATIENTS WITH DIABETES MILLITUS USING SOME ENZYMATIC ACTIVITIES

A THESIS

SUBMITTED TO COUNCIL OF COLLEGE OF SCIENCE
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY-BIOCHEMISTRY

BY
FERDOUS JABIR

October-٢٠٠٥ Ramadan- ١٤٢٦

Certification

We certify that this thesis was prepared under our supervision at the Department of chemistry, College of science, University of Babylon as partial requirements for the degree of Doctor of philosophy of Science in Biochemistry at this work.

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Dedication

To my husband……

My Family …… and ……

Diabetic Patients especially,

Hussam F. Abbas

Ferdous

Summary

Diabetes mellitus is a common health problem characterized by hyperglycemia resulted from absolute or relative decrease in insulin secretion from β-cells of the islets of Langerhans in pancreas. Diabetes mellitus is considered as a member of oxidative stress syndrome. It is associated with an imbalance between types of free radicals and scavengers system. This study is aimed to assess the oxidative stress status in patients with type ١ or ٢ diabetes mellitus disease, and to explore the good free radical marker in this state.

This research was conducted in Al-Diwaniya General Hospital; Department of Chemistry, College of Sciences, Babylon University; Department of Chemistry, College of Medicine, Al-Nahrain University; Iraq. This study is designed to screen the serum for certain biochemical variables.

The subjects of this study were ٥٠ patients with type ١ diabetes mellitus, ٥٠ patients with type ٢ diabetes mellitus, and ٥٠ apparent healthy subjects were enrolled in this study.

The main outcome measurement were assessment of specific biological alterations in oxidative stress syndrome: Lipid peroxidation, and hyperhomocystienemia, assessment of some enzymatic antioxidants: Extracellular superoxide dismutase, catalase, glutathione S-transferase, and creatine kinase, assessment of some non-enzymatic antioxidants: Glutathione, vitamin E, vitamin C, and vitamin A, and assessment of minerals: Iron, Copper, Zinc, Chromium, and Selenium.

The results pointed out elevated levels of malondialdehyde, and total homocystiene significantly in serum of patients with type ١ and ٢ diabetes mellitus (DM) disease compared to control, $P \leq$ ٠.٠٥. Levels of lipid peroxidation showed a significant negative correlation with levels of vitamin E ($r =$ -٠.٣٤٥, $P \leq$ ٠.٠٥), whereas there is not significant negative

correlation with vitamin C and A in serum of patients with type ١ DM. While in patients with type ٢ DM, the results showed a significant negative correlation with levels of vitamin C (r = -٠.٢٨٤, P≤ ٠.٠٥), and a negative correlation but not significant with vitamins E and A.

This study reported that extracellular superoxide dismutase, and glutathione S-transferase activities are significantly higher in serum of patients with type ١ and ٢ diabetes mellitus disease than that of controls, P≤ ٠.٠٥. But there is a significant decrease in levels of catalase and creatine kinase enzyme activities in both types of diabetic patients, P≤ ٠.٠٥. However extracellular superoxide dismutase enzyme activity in both types of diabetic patients tend to be altered and did not show a significant correlation with levels of copper or catalase enzyme activity, while there is a significant negative correlation with level of zinc, P≤ ٠.٠٥.

This study showed that serum glutathione, vitamin E, vitamin C, and vitamin A are significantly decreased in patients with type ١ and ٢ DM when they are compared with controls, P≤ ٠.٠٥. The levels of glutathione showed different correlation, but not significant with levels of malondialdehyde, total homocystiene, and creatine kinase enzyme activity in serum of diabetic patients (with type ١ and ٢ DM). Furthermore, glutathione shows a significant negative correlation with level of glutathione S-transferase activity in serum of patients with type ٢ DM (r= -٠.٣٠٩, P≤ ٠.٠٥), but this correlation is not significant in patients with type ١ DM.

The results of this study also indicated that diabetes results in disturbance of trace elements. Thus there is a significant decrease in levels of serum iron, zinc, chromium, and selenium in patients with type ١ and ٢ DM from control, P≤ ٠.٠٥. As well as there is a significant increase in levels of copper in serum of patients with type ١ and ٢ DM from control, P≤ ٠.٠٥.

In this study, the results reported no significant difference in levels of Malondialdehyde, total homocystiene, glutathione, vitamin C, copper, iron, zinc, selenium, chromium and glutathione S-transferase, extracellular superoxide dismutase, creatine kinase, catalase activities in serum of smokers diabetic patients with type ١ and ٢ DM than that of non-smokers diabetic patients, as well as levels of vitamin E in serum of smokers with type ١ DM, and levels of vitamin A in serum of smokers with type ٢ DM than that of non-smokers diabetic patients. But there is a significant difference in levels of vitamin A in serum of smokers with type ١ DM, and vitamin E in serum of smokers with type ٢ DM, $P \leq$ ٠.٠٥ than that of control.

This study concluded that diabetes mellitus is a member of oxidative stress syndrome, it reduces some enzymatic and non-enzymatic antioxidants. As well as decreased levels of the antioxidants (catalase, and glutathione) are the good indicators to evaluate the oxidative stress syndrome in diabetic patients, and all of free radical markers in this study can be used for early detection of diabetic complications. Smoking reduces the levels of antioxidants, thus increases oxidative stress syndrome, and diabetic complications in diabetic smokers are more than diabetic non-smokers.

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Contents

Conclusions

The results found in this study enable for concluding the following points:

١. Diabetes Mellitus disease is associated with increased free radicals generation, which has been observed through increased MDA, tHcy, and Cu, and decreased GSH, CAT, CK, antioxidant vitamins (E, A, C), zinc, chromium, and selenium.

٢. We could use the above markers for early detection of diabetic complications.

٣. Decreased levels of CAT activity and glutathione are the good indicators to evaluate the oxidative stress syndrome in diabetics than non-diabetics.

٤. Increased level of Ec-Cu/Zn SOD activity in diabetic patients more than the other antioxidants is indicating to presence high level of superoxide radical ($O_2^{\cdot-}$) that generating in DM.

٥. Smoke reduces levels of total antioxidant especially fat soluble vitamins (E and A), thus smoke increases oxidative stress syndrome and diabetic complications in diabetic smokers than diabetic non-smokers.

Recommendations

١. Hyperhomocysteinemia is one of the important biochemical markers altered to diabetic complications and death, thus this marker requires more study, especially its correlation with vitamin B_6, B_{12}, and folic acid to reduce and manage diabetic complications.

٢. Supplementation of antioxidant vitamins and zinc are necessary to reduce oxidative stress syndrome and lipid peroxidation degree, thus reduced atherosclerotic complications in diabetics.

٣. Iron require more study in Iraq country to found the reasons for decreased its level in Iraqi diabetic patients especially when all research found elevated level of iron in diabetic patients in other countries.

Abbreviations

ADP	Adinosin Diphosphate
ATP	Adinosin Triphosphate
AGEs	Advanced Glycosylation End-products
AR	Aldose Reductase
ROOH	Alkyl hydroperoxide
ADA	American Diabetes Association
AAS	Atomic Absorption Spectrophotometer
CVD	Cardiovascular disease
CAT	Catalase
CDNB	١-Chloro-٢,٤-dinitrobenzene
CK	Creatine Kinase
CK-BB	Creatine Kinase-Brain Type
CK-MB	Creatine Kinase-Hybrid Type
CK-MM	Creatine Kinase-Muscle Type
DNA	Deoxyribose Nucleic Acid
DM	Diabetes Mellitus
DTNB	٥,٥-Dithiobis (٢-nitrobenzoic acid)
DDT	Dithiotheritol
EDRF	Endothelial Derived Relaxation Factor
ENOS	Endothelial Nitric Oxide Synthase
Ec-Cu/Zn SOD	Extracellular Superoxide Dismutase
FBS	Fasting Blood Sugar
FPG	Fasting Plasma Glucose
FAAS	Flamless Atomic Absorption Spectrophotometer
GDM	Gestational Diabetes Mellitus
GPx	Glutathione Peroxidase

$GS^{\bullet}$	Glutathione radical
GR	Glutathione Reductase
GST	Glutathione S-Transferase
GHb	Glycosylated Hemoglobin
$HbA_{١c}$	Hemoglobin $A_{١c}$
COHb	Hemoglobin carbon monoxide
HK	Hexokinase
HDL-Ch	High-Density Lipoprotein- Cholesterol
HPLC	High Performance Liquid Chromatography
Hcy	Homocysteine
٨-OHdG	٨-hydroxydeoxyguanine
$OH^{\bullet}$	Hydroxyl radical
HHcy	Hyperhomocysteinemia
IDDM	Insulin Dependent Diabetes Mellitus
ITF γ	Interferron γ
IL-١	Interleukin ١
LOOH	Lipid hydroperoxide
LO	Lipoxygenase
LDL	Low-Density Lipoprotein
LDL-Ch	Low Density Lipoprotein- Cholesterol
MDA	Malondialdehyde
MODY	Maturity-Onset Diabetes of Youth
NDDG	National Diabetes Data Group
$NO^{\bullet}$	Nitric Oxide radical
NOS	Nitric Oxide Synthase
NBT	Nitroblue Tetrazolium
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test

GSSG	Oxidized glutathione
Ox-LDL	Oxidized low-density lipoprotein
٨-oxoG	٨-oxo-٧,٨-dihydroguanine
$ONOO^-$	Peroxynitrite
PMN	Polymorphonuclear
PUFA	Polyunsaturated fatty acid
$PGG_٢$	Prostaglandin $G_٢$
ROS	Reactive oxygen species
NADPH NADP	Reduced, and Oxidized Nicotinamide Adnine Dinucleotide Phosphate
GSH	Reduced glutathione
$^١O_٢$	Singlet Oxygen
SDH	Sorbitol Dehydrogenase
SOD	Superoxide Dismutase
$O_٢^{\bullet -}$	Superoxide radical
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric acid reactive substance
α-TQ	α-Tochopherol
TCA	Trichloroacetic Acid
$^٣O_٢$	Triplet Oxygen
TNF	Tumor Necrosis Factor
VSMC	Vascular Smooth Muscle Cell
WHO	World Health Organization

Normal Values of Variables from References

Serum Malodialdehyde (MDA)	١.٧٥± ٠.٣ nmol/L
Serum Total Homocystiene (tHcy)	٩.١٧١ ± ١.٤٩ µmol/L
Serum Superoxide Dismutase (Ec-Cu/Zn-SOD)	٥٤٨ ± ٢٠ µg/dL
Serum Catalase (CAT)	٦.١٤± ٠.٠٢ kU/ml
Serum Creatine Kinase (CK)	١٨١.١ ± ٢.٨٦ U/L
Serum Glutathione S-Transferase (GST)	١.٩٦ ± ٠.٤١ U/L
Serum Glutathione (GSH)	١٢.٣٩ ± ١.٢٢ µmol/L
Serum Vitamin E	٨-١٠ mg/dL
Serum Vitamin A	٨٠٠-١٠٠٠ µg/dL
Serum Vitamin C	٠.٤-٠.٦ mg/dL
Serum Copper	
Male	١١.٠-٢٤.٠ µmol/L
Female	١٢.٠-٢٤.٤ µmol/L
Serum Iron	٧.٣٤-٢٣.٦ µmol/L
Serum Zinc	١١.٠-٢٤ µmol/L
Serum Chromium	٠.١-٠.٢ µg/L
Serum Selenium	٨٧-١٥٠ µg/L

١.١. Diabetes Mellitus (DM)

Diabetes mellitus is a very complex chronic disease with syndrome of hyperglycemia (Zhao, ٢٠٠١). It is result from absolute or relative decrease in insulin secretion from β-cell of the islets of Langerhans. Insulin is a polypeptide hormone that consists of a total of ٥١ amino acids in two chains connected by two disulfide bridges; figure (١-١a). Insulin is synthesized as a large single chain preproinsulin that is cleaved to a more immediate precursor proinsulin in the rough endoplasmic reticulum. Proinsulin is then packed into secretory granules, where it is broken down into equimolar amounts of insulin and an inactive C-peptide (Bishop *et al.*, ٢٠٠٠), figure (١-١b).

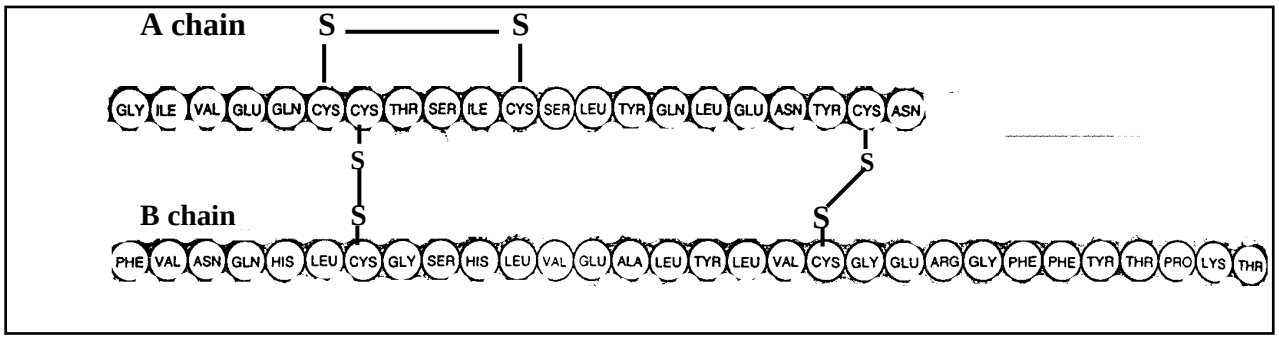
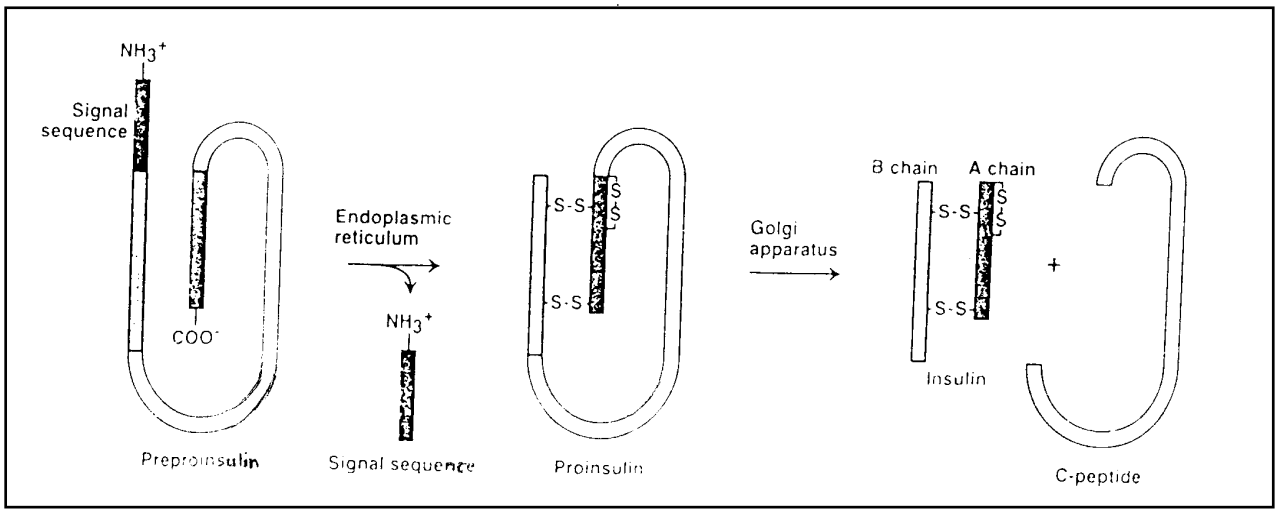


Figure (1-1a): Structure of human insulin (Champe & Harvey,



Figure(1-1b): Formatoin of human insulin from preproinsulin
(Champe & Harvey, .

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, high glucose in urine, increased appetite, ketoacidosis (ketone bodies in blood), acid/base imbalance, and diabetic coma.

Long-term complications of diabetes include blindness, neurophathy, kidney failure, lower extremity amputations, cardiovascular complication and pregnancy complication (Bishop *et al*., ٢٠٠٠).

١.٢. Criteria for the Diagnosis of DM

The old criteria for diagnosis of DM recommended by the World Health Organization (WHO) (١٩٨٥) were:

١. Fasting plasma glucose (FPG) ≥ ١٤٠ mg/dL (٧.٨ mmol/L).

٢. ٢-hours postlood glucose (٢-hPG) ≥ ٢٠٠ mg/dL (١١.١ mmol/L).

According to the new American Diabetes Association (ADA) recommendations to allow for earlier detection of the disease, all adults older than age ٤٥ years should have a measurement of fasting blood sugar every ٣ years unless the individual is otherwise diagnosed with diabetes (Bishop *et al*., ٢٠٠٠).

The modified criteria for diagnosis of DM recommended by an expert committee to allow earlier detection of the disease were:

١. FPG (Fasting Plasma Glucose) < ١١٠ mg/dL (٦.١ mmol/L) = normal fasting glucose.

٢. FPG ≥ ١١٠ mg/dL (٦.١ mmol/L) and < ١٢٦ mg/dL (٧.٠ mmol/L) = impaired fasting glucose (IFG).

٣. FPG ≥ ١٢٦ mg/dL (٧.٠ mmol/L) = provisional diagnosis of diabetes.

The corresponding criteria when the oral glucose tolerance test (OGTT) is used are the following:

١. ٢-h PG < ١٤٠ mg/dL (٧.٨ mmol/L) = normal glucose tolerance.

٢. ٢-h PG ≥ ١٤٠ mg/dL (٧.٨ mmol/L) and < ٢٠٠ mg/dL (١١.١ mmol/L) = impaired glucose tolerance.

٣. ٢-h ≥ ٢٠٠ mg/dL (١١.١ mmol/L) = provisional diagnosis of diabetes.

Glycosylated hemoglobin (GHb) is the term used to describe the formation of a glucose reacts with the amino group of hemoglobin (Bishop *et al.*, ٢٠٠٠), figure (١-٢).

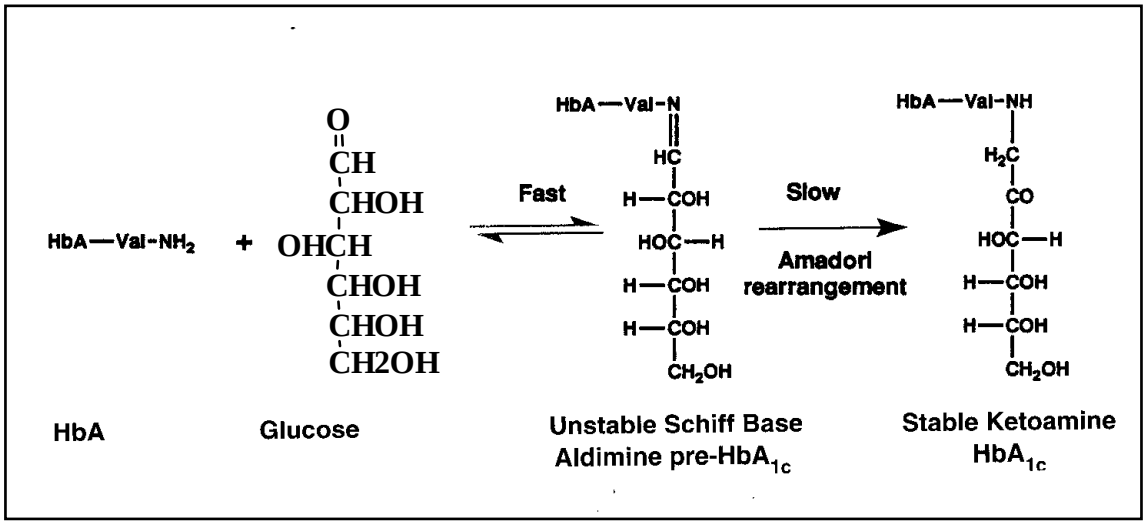


Figure (١-٢): The non-enzymatic glycosylation of HbA to $HbA_{١c}$ (Higgis & Bunn, ١٩٨١)

Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-average picture of the patient's blood glucose concentration over the past three months. Hemoglobin $A_{١c}$ ($HbA_{١c}$) is a reliable method of monitoring long-term diabetic control rather than

fasting plasma glucose (FPG). Normal values range from ٤.٥-٨.٠; and two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the red blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin will have less time to become glycosylated and the glycosylated hemoglobin level will be lower (Lehninger, ١٩٧٥).

١.٣. Classification and Eitology of DM

In ١٩٧٩, the National Diabetes Data Group (NDDG) developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: type ١, insulin-dependent diabetes mellitus (IDDM), and type ٢, non-insulin-dependent diabetes mellitus (NIDDM).

Established in ١٩٩٥, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the sponsorship of the American Diabetes Association (ADA), was given the task of updating the ١٩٧٩ classification system. The changes they proposed included eliminating the older terms of IDDM and NIDDM. The categories of type ١ and type ٢ were retained with the adoption of Arabic numerals instead of Roman numerals.

Therefore, the ADA/WHO guidelines recommend the following categories of diabetes (Bishop *et al*., ٢٠٠٠):

- Type ١ diabetes; is a result of cellular-mediated autoimmune destruction of the β-cells of the pancreas, causing an absolute deficiency of insulin secretion. Type ١ constitutes only ١٠٪ to ٢٠٪ of all diabetes and commonly occurs in childhood and adolescence. This disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with a genetic predisposition and

causes the immune destruction of the β-cells of the pancreas and therefore, a decreased production of insulin.

- Type ٢ diabetes; is characterized by hyperglycemia due to an individual's resistance to insulin with an insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type ٢ constitutes the majority of the diabetes cases. Most patients in this type are obes or have an increased percentage of body fat distribution in the abdominal region. This type of diabetes is associated with a strong genetic predisposition with patients at an increased risk with an increase in age, obesity and lack of physical exercise, and often goes undiagnosed for many years. Characteristics usually include an adult onset of the disease and milder symptoms than in type ١. Type ٢ commonly occurs after age ٣٥ years.

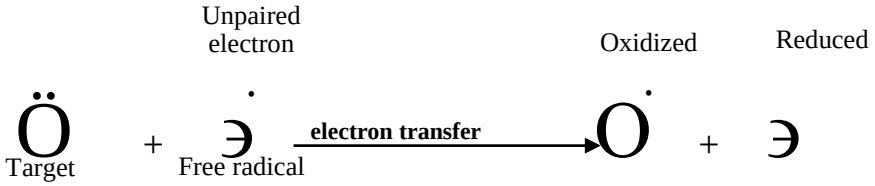
- Other specific types of diabetes are associated with certain conditions (secondary) including genetic defects of β-cell function or insulin action, pancreatic disease, disease of endocrine origin, drug or chemical included insulin receptor abnormalities, and certain genetic syndromes. Maturity-onset diabetes of youth (MODY) is a very rare form of diabetes that is inherited in an autosomal dominant fashion (Tripathi, ١٩٨٨; Malchoff, ١٩٩١).

- Gestational diabetes mellitus (GDM) is any degree of glucose intolerance with onset or first recognition during pregnancy. Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. However, this disease is associated with increased perinatal complications and an increased risk for development of diabetes in later years (Bishop *et al*., ٢٠٠٠).

١.٤. Free Radicals

Oxidation is a constant natural process that produces atoms or molecules with unpaired electrons, known as free radicals. A free radical can be defined as an atom or molecule that contains one or more unpaired electrons in its outer orbit. If an electron is lost from the outer orbit, the molecule becomes a free radical. Free radicals are unstable chemicals, which are produced during the many oxidative biochemical reactions in the body (Howard, ١٩٧٢; Oberley, ١٩٨٨).

A compound becomes a free radical through either oxidation i.e., the loss of an electron or through reduction i.e., the gain of an electron, however, a free radical is unstable and highly reactive in nature and may react with other nearby molecule also converting that molecule to a free radical, which can then initiate another reaction (Sinclair, ١٩٩٠).



Theoretically, a single free radical can ultimately causes an endless number of reactions. This chain reaction is terminated either by the free radicals reaction with another free radical or by the free radicals reaction with an antioxidant (Oberley, ١٩٨٨).

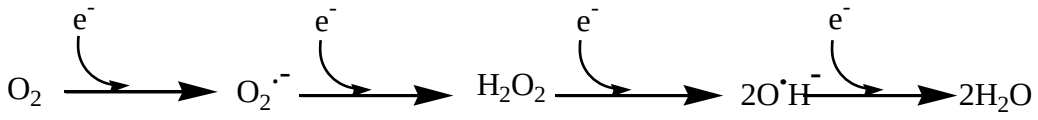
١.٤.١. The Source of Free Radicals in Human Body

Free radicals are continuously produced in our body as a byproduct of metabolism. Oxygen is the most important source of free radicals in the body. Most of the oxygen taken into the body is excreted as carbon dioxide; however, some oxygen is converted to water. To convert oxygen

to water, four electrons must be added to it at once (Murray *et al*., ٢٠٠٠; Kelly *et al*., ٢٠٠٥).

$$O_2 + 4e^- \longrightarrow 2H_2O$$

Approximately ٥٪ of the oxygen are reduced to water by adding one electron at a time. When a single electron is added, an unstable free radical is formed. Superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet -}$) are produced from oxygen by addition of one, two, or three electrons respectively (Champe *et al*., ١٩٩٤).

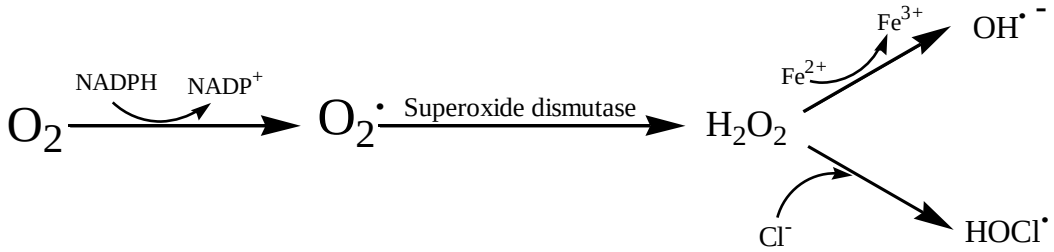


The sources of the free radicals are:

A- Internally generated sources of free radicals are (Bagchi & Puri, ١٩٩٨):

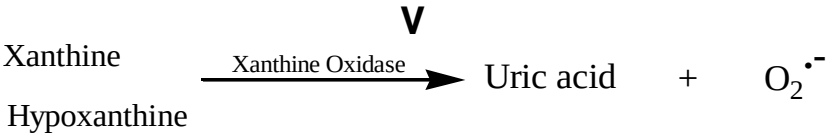
١. Mitochondrial electron transport

$$\text{Reduced flavin enzyme} + O_2 \rightarrow \text{Flavin – enzyme} + O_2^{\bullet -}$$



٢. Phagocytes

٣. Xanthine oxidase



$$NADPH + 2O_2 \xrightarrow{\text{NADP oxidase}} NADP^+ + 2O_2^{\bullet -} + H^+$$

٤. NADP oxidase

٥. Arachidonate Pathways

٦. Auto-oxidation of several biological important molecules such as adrenaline, cysteine, and homocysteine.

٧. Peroxisomes

٨. Exercise: It is induces oxidative stress as measured by oxidative damage of lipid, proteins, and even the genitic material (Sen, ١٩٩٩; Khanna et al., ١٩٩٩; Atalay, ٢٠٠٠; Atalay et al., ٢٠٠٠). On the other hand, exercise training-both endurance and interval type-appears to induce antioxidant protection and decrease oxidative insult. Thus regular physical exercise protects against exercise induced oxidative stress (Khanna et al., ١٩٩٩; Sen, ١٩٩٩).

٩. Inflammation

١٠. Ischaemia / reperfusion

١١. Reactions are involving iron, copper, and other transition metals such as Fenton and Haber-Weiss reactions

$$Fe^{٢+} + H_٢O_٢ \longrightarrow Fe^{٣+} + OH^{\bullet} + \bar{O}H \quad \text{(Fenton reaction)}$$

$$O_2^{\bullet -} + Fe^{٣+} \longrightarrow Fe^{٢+} + O_٢ \quad \text{(Iron catalyzed)}$$

$$H_٢O_٢ + Fe^{٢+} \longrightarrow Fe^{٣+} + OH^{\bullet} + \bar{O}H \quad \text{(Haber-Weiss reaction)}$$

B- Externally generated sources of free radicals are (Bagchi & Puri, ١٩٩٨):

١. Cigarette smoke.

٢. Environmental pollutant.

٣. Radiation.

٤. Ultraviolet light.

٥. Certain drugs, pesticides, anesthetics, and industrial solvents.

٦. Ozone.

١.٤.٢. Effects of Free Radicals

The effects of free radicals are:

١. The most common and most hazardous reaction encountered as a result of free radical oxidation is lipid peroxidation (Domingues *et al.*, ١٩٩٨). Free radical such as hydroxyl radical react with lipids of the cell membrane to form intermediate free radicals and peroxides. The latter initiator leads to subsequent free radical reactions that result in membrane damage (Halliwell & Gutteridge, ١٩٩٩).

$$LH \text{ (membrane unsaturated lipid)} + {}^{\bullet}OH \longrightarrow \underset{\text{Lipid alkyl radical}}{L^{\bullet}} + H_2O$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$$

٢. Protein exposed to free radicals may fragment, crosslink or aggregate. This leads to interference with ion channels, failure of cell receptors

and failure of oxidative phosphorylation (Stadtman & Berlett, ١٩٩٧).

٣. DNA free radical damages DNA and causes destruction of base deoxyribose sugars or single and double strand breaks (Cerutti, ١٩٩٤; Imlay & Linn, ١٩٨٨). The most frequent oxidative damage to DNA is believed to be the ٨-hydroxylation/oxidation of the guanine base to ٨-hydroxydeoxyguanosine (٨-OHdG), a molecule, which is equivalent to ٨-oxo-٧,٨-dihydroguanine (٨-oxoG) because the hydroxy hydrogen can easily move to the position-٧ leaving a double-bonded oxygen at the position-٨ (a resonance form of the two structures). ٨-OHdG/٨-oxoG is the most commonly studied biomarker of DNA oxidation, figure (١-٣). These events lead to mutagenesis, carcinogenesis, and cell death.

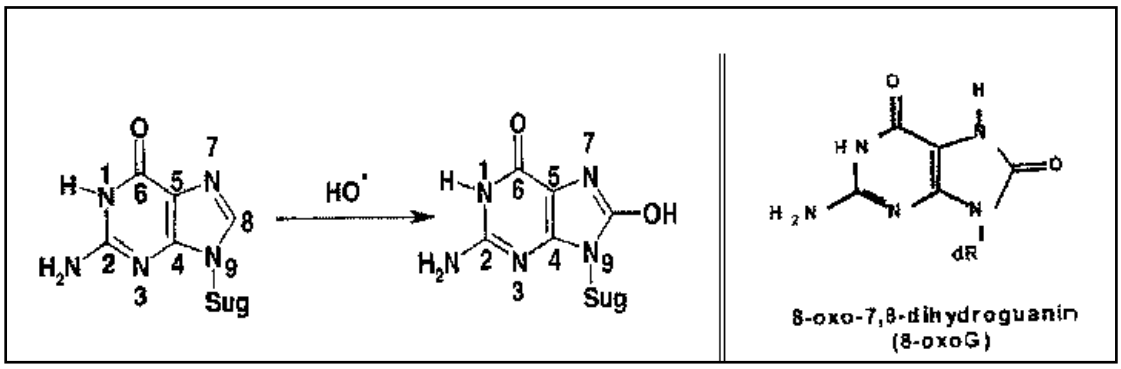


Figure (١-٣): Oxidative Damage to DNA: Formation of ٨-OHdG/٨-oxoG,dR: deoxyribose (Cerutti, ١٩٩٤).

١.٤.٣. Free Radicals and Pathogenesis of DM

The cause of diabetes mellitus is not fully understood. Recently, increasing evidence suggest that free radicals formation are involved in the pathogenesis of diabetes and the development of diabetic complications (Colman *et al*., ١٩٨٩; Giugliano *et al*., ١٩٩٥).

In type ١; β-cells are prone to be destroyed by free radicals because of the low antioxidant enzyme nature. Immune-effect cells such as macrophages, T-cells, nature killer cells and B-cells are believed to produce free radicals that cause damage to β-cells. There are two mechanisms of free radicals in β-cells destruction (Zhao, ٢٠٠١):

١. Infiltration macrophages produce superoxide as primary source of free radicals. The superoxide can be further converted to more active radical, hydroxyl radical; which attacks cellular membrane and cause DNA breaks, the consequence of DNA breaks leads to cells death if cells fail to repair the damage. Also, the activation of DNA repair enzymes deplete the DNA levels in cells, inhibiting proinsulin synthesis and causing cells more sensitive to free radicals (Colman *et al*., ١٩٨٩).

٢. Cytokines are released by T-cells, macrophages, NK cells in the insulitis and induce the formation of intracellular free radicals causing selective damage to β-cells. Interleukin ١ (IL-١) is the major factor in the damage of β-cells. In addition, interferon γ(ITF γ) and tumor necrosis factor (TNF) are released macrophages during the insulitis (Gerbitz, ١٩٩٢). These cytokines induce intracellular free radicals in endothelial cells, fibroblasts, and β-cells. Three types of free radicals are induced from these cells,

$O_2^{\cdot -}$, $OH^{\cdot}$, and $NO^{\cdot}$ radicals (Corbett & McDaniel, ١٩٩٥). IL-١ can induce the production of nitric oxide synthase (NOS), which is the enzyme in charge of the synthesis of $NO^{\cdot}$, therefore, IL-١ induces $NO^{\cdot}$ formation. Many evidence have suggested that $NO^{\cdot}$ results indirectly inhibitory effect on β-cells mitochondria function (Gerbitz, ١٩٩٢), thus $NO^{\cdot}$ can directly destroy β-cells.

Type ٢ DM is a heterogeneous disorder, that is, very different pathologic events result in the same clinical symptom (Leahy & Weir, ١٩٨٩). Genetic abnormalities, or environmental factors, or obesity, which may induce β-cells malfunction and/or insulin resistance, can cause mild hyperglycemia which further develops to type ٢ DM. Some studies indicate that there are alterations in free radicals generation and antioxidant enzymes. The free radicals activity in type ٢ DM patients was increased as measured by the markers of free radicals activity (Collier *et al*., ١٩٩٢).

١.٤.٤. Free Radicals and Diabetic Complications

Free radicals perform beneficial tasks such as aiding in the destruction of microorganisms and cancer cells. Excessive production of free radicals or inadequate antioxidant defense mechanisms, however, can lead to damage of cellular structure and enzymes (Sinclair *et al*., ١٩٩٠).

Damage to entire tissues can result from free radical-mediated oxidative alteration of fatty acids, also known as lipid peroxidation. There are well-characterized reaction that leads to the formation of the $O_2^{\bullet -}$, H_2O_2, and the highly toxic $OH^{\bullet}$. The cytotoxic potential of the $O_2^{\bullet -}$ is mainly from its ability to be converted to the $OH^{\bullet}$ directly or via interaction with H_2O_2. The $O_2^{\bullet -}$ can also interact with $NO^{\bullet}$ to form peroxinitrite ($ONOO^-$) which can degrade to form the $OH^{\bullet}$ (Atalay & Laaksonen, ٢٠٠٢).

Peroxy radicals can remove hydrogen from lipids, such as polyunsaturated fatty acids, resulting in the formation of lipid hydroperoxides and further propegation of the radical pathway by regeneration of alkyl radicals. Hydroperoxides have direct toxic effects for endothelial cells and can also degrade to form the $OH^{\bullet}$; hydroperoxides may also form stable aldehyde, such as malondialdehyde (MDA), which damage membranes by facilitating the formation of protein cross-links and other end products (Halliwell & Gutteridge, ١٩٩٩).

Reactive oxygen species (ROS) can stimulate vascular smooth muscle cell (VSMC) growth. Active free oxygen production, arterial injury, and VSMC proliferation are strongly related to each other. New evidence suggests that certain enzymatic pathways of arachidonic or linoleic acid metabolism can participate in the formation of free radicals and lipid peroxides in the vascular and renal systems. It has been suggested that certain lipooxygenase (LO) enzymes that reacts with arachidonic or linoleic acid play an important role in atherosclerosis by

inducing the oxidation of low-density lipoprotein (LDL) (Glugliano *et al*., ١٩٩٦).

A series of free radical catalyzed perooxidation products of arachidonic acid, called isoprostanes, can be formed in a cyclo-oxygenase-independent manner and remain associated with membrane phospholipids until they are released by phospholipase. One isoprostan $PGF_{٢}$ is potentially relevant to diabetic vascular disease based on its potent to contraction of vascular smooth muscle. Antioxidant defense mechanisms are critically important for the ultimate effect of oxidative stress and free radicals on cells and tissues (Murray *et al*., ٢٠٠٠).

١.٥. Oxidative Stress Syndrome

Oxidative stress, an imbalance between the generation of free radicals and antioxidant defense capacity of the body. This resulted in the alteration of the cellular components in term of; DNA break down, protein and lipid Peroxidation, cytotoxicity etc… (Atalay & Laaksonen, ٢٠٠٢).

١.٥.١. Diabetes Mellitus and Oxidative Stress Syndrome

The oxidative stress is significantly increased in diabetes because prolonged exposure to hyperglycemia. Many evidence have indicated that some biochemical pathways strictly associated with hyperglycemia (non-enzymatic glycosylation, glucose auto-oxidation, polyol pathways) can increase the production of free radicals (Glugliano *et al*., ١٩٩٦), figure (١-٤).

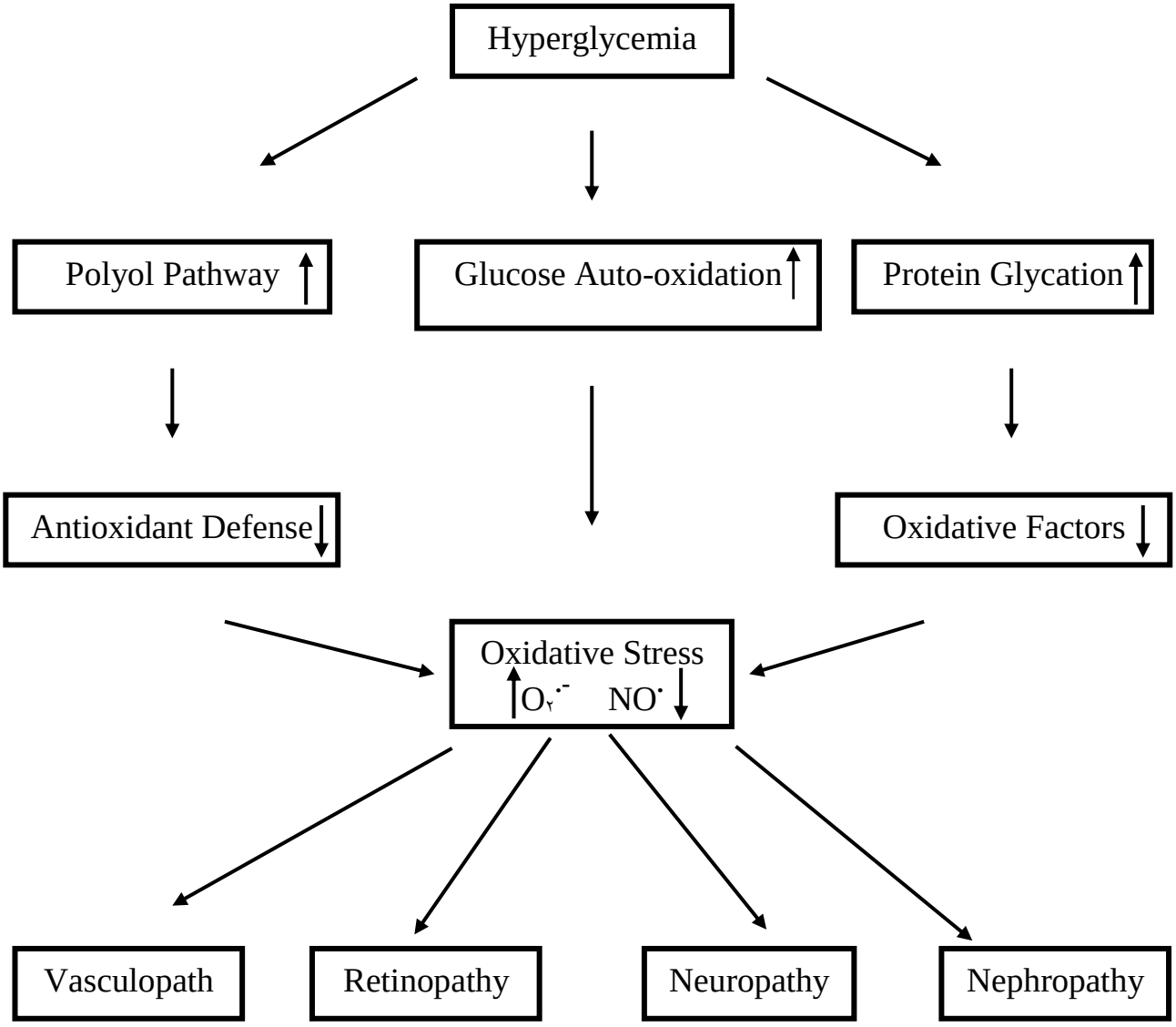
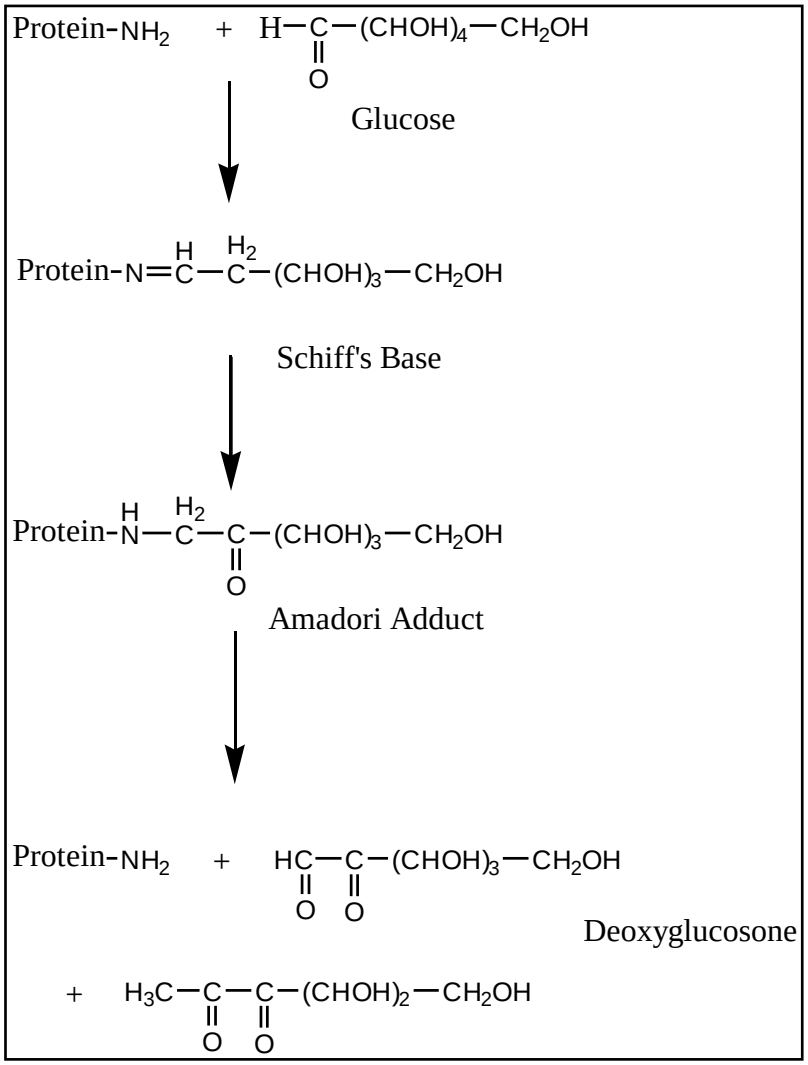


Figure (١-٤): Possible links between hyperglycemia-induced oxidative stress and diabetic complications (Glugliano *et al.*, ١٩٩٦).

١.٥.١.١. Glycation of Protein

H_2O_2 can be formed from glycated of protein. Initially, glucose undergoes a nucleophilic addition reaction with protein to form the schiff base. Formed early glycosylation product, ketomine is chemically reversible and thus is dissociated when blood glucose level returns to normal. However, it subsequently undergoes an Amadori compound. Further reactions, rearrangements, dehydration and cleavage irreversibly results in the formation of brown, insoluble, cross-linking complexes called advanced glycosylation end-products (AGEs) (Glugliano *et al*.,



١٩٩٦), figure (١-٥a).

Figure (١-٥a): Non-enzymatic glycation (Wolff *et al*., ١٩٩١).

Degradation of Amadori product and H_2O_2 formation via both ١,٢- and ٢,٣- enolization pathway and oxidation of enolate anion, figure (١-٥b)

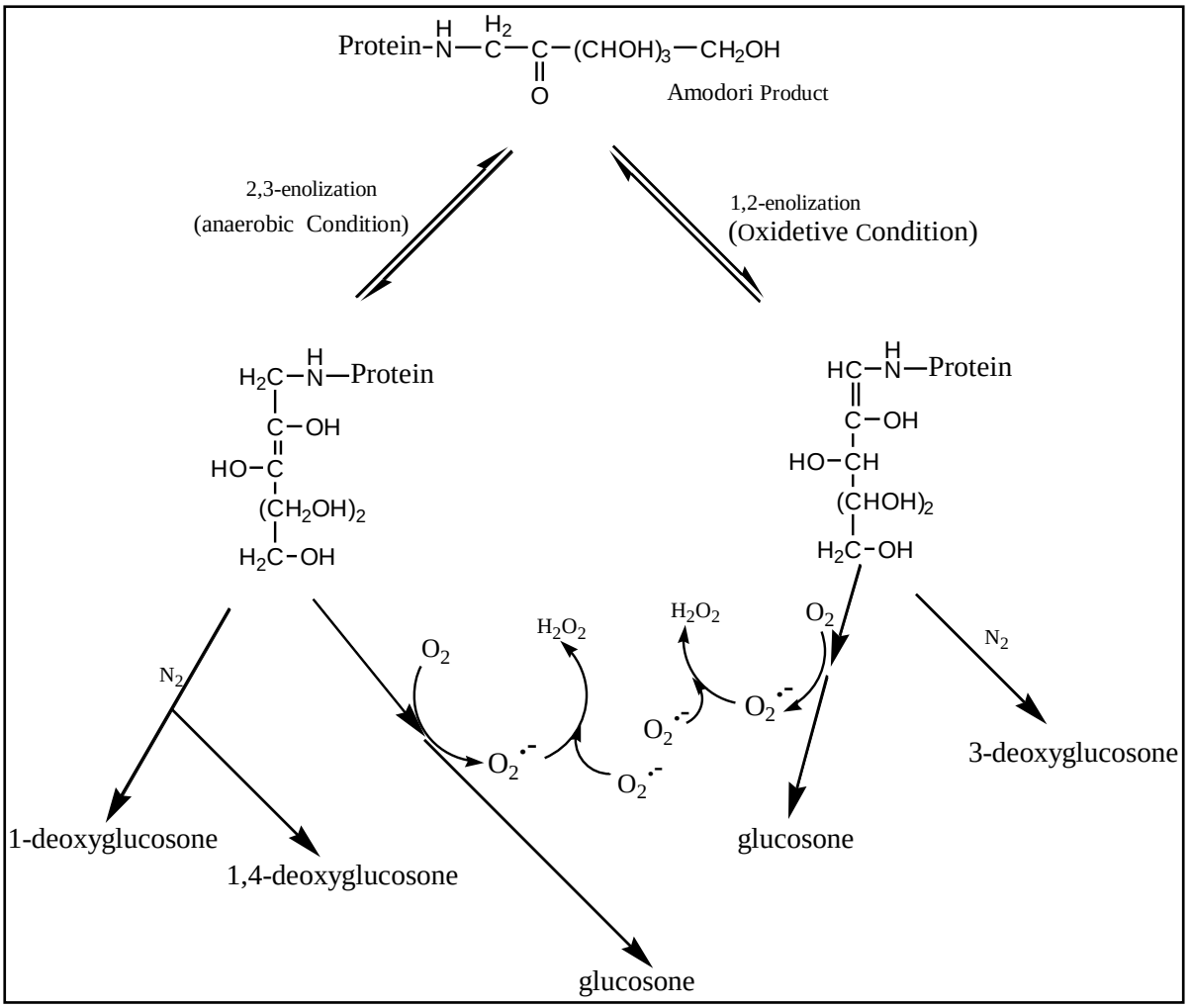


Figure (١-٥b): Degradation of Amadori product and H_2O_2 formation (Elgawish *et al.*, ١٩٩٥)

AGEs tend to accumulate on long-lived macromolecules in tissues. Cross-linking AGE-protein with other macromolecules in tissues results in abnormalities of cell and tissue function. In addition, AGEs contribute to increased vascular permeability in both micro- and macro-vascular structure by binding to a specific macrophage receptor. This process

induced the synthesis and secretion of cytokines such as TNF and IL-١, which causes endothelial dysfunction and induces free radicals generation (Brownlee, ١٩٨٩).

١.٥.١.٢. Glucose Auto-oxidation

Radicals can be formed through glucose auto-oxidation. Through one-enolization and two oxidation reactions the ∝-hydroxyaldehyde part of glucose forms an ∝-ketoaldehyde whereby superoxide is produced in a process requiring oxygen and transition metal ions The oxidizing intermediates formed by autooxidation process is proposed to be the case of some the structures found in diabetes (Wolff & Dean, ١٩٨٧). There is some evidence in vivo that transition metals chelating agents can prevent auto-oxidation in animal diabetes. (Cameron & Cotter, ١٩٩٥), figure(١-٦).

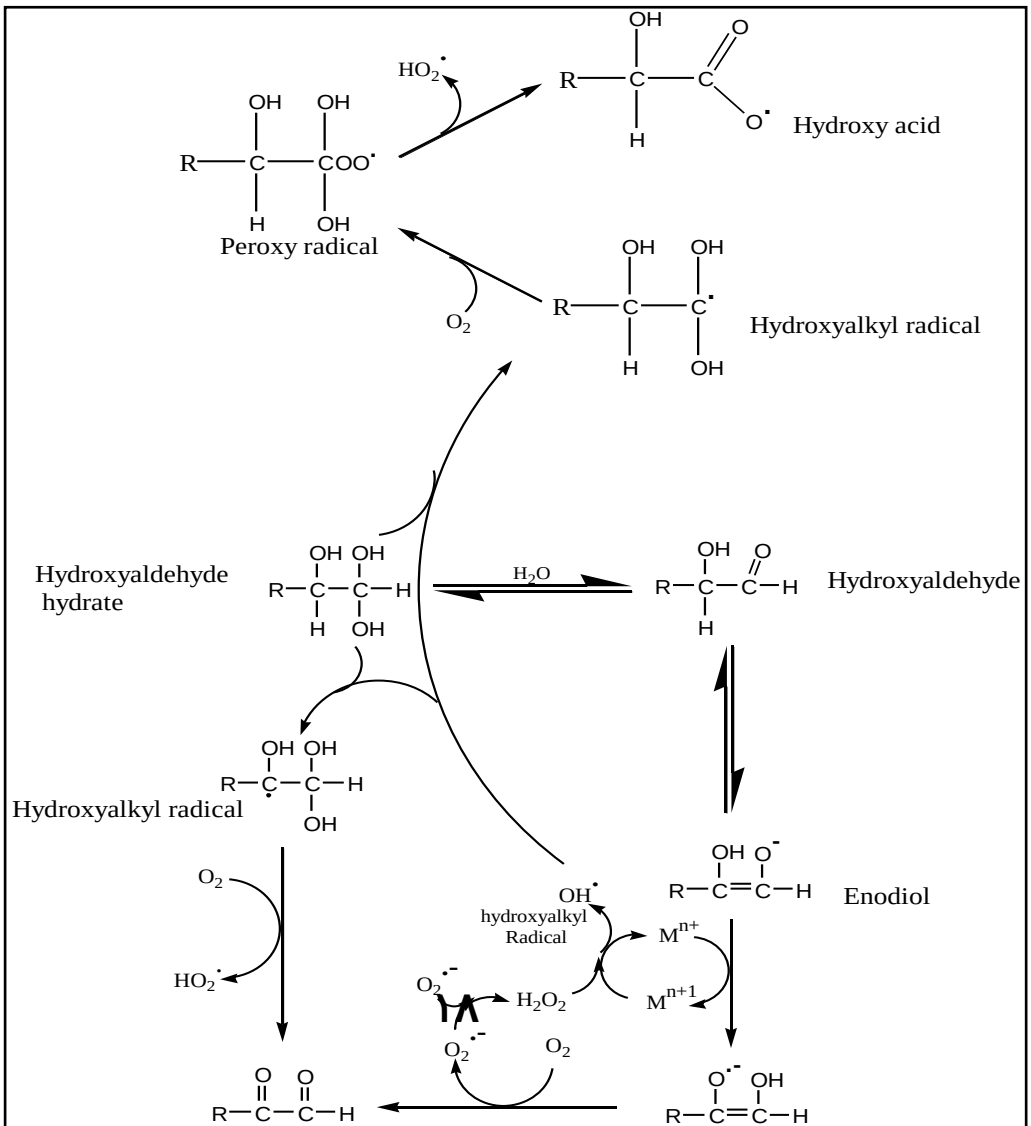


Figure (١-٦): Auto-oxidation of monosaccharide, (Wolff & Dean, ١٩٨٧)

١.٥.١.٣. Polyol Pathways

Polyol pathway can also be generated free radicals, which occurs because elevated glucose levels increases intracellular sorbitol and fructose content due to aldose reductase (AR) and sorbitol dehydrogenase (SDH) activity (Baynes, ١٩٩١), figure (١-٧). AR catalyzes the reduction of glucose by NADPH to sorbitol, which can, in turn, be oxidized to fructose by SDH leading to redox imbalance (NAD^+ / NADH ratio). Oxidation of sorbitol to fructose is coupled with reduction of NAD^+ to NADH. An increase in NAD^+ / NADH ratio is linked to $O_2^{\cdot-}$ formation via the reduction of prostaglandin G_2 (PGG_2) to PGH_2 by prostaglandine hydroperoxidase that uses NADH or NADPH as reducing cosubstrate (Tesfamarian, ١٩٩٣).

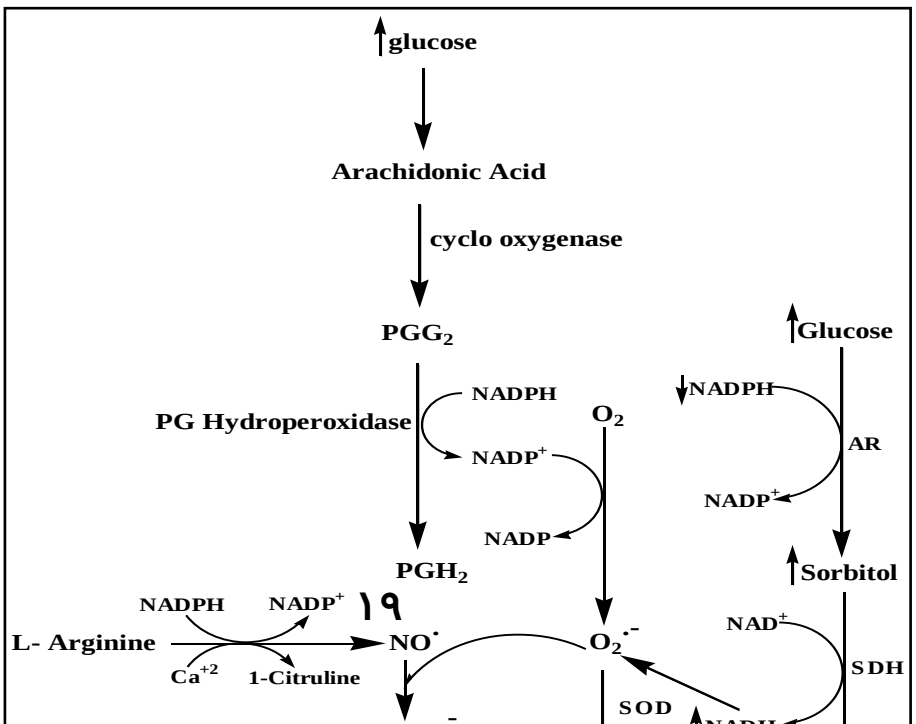
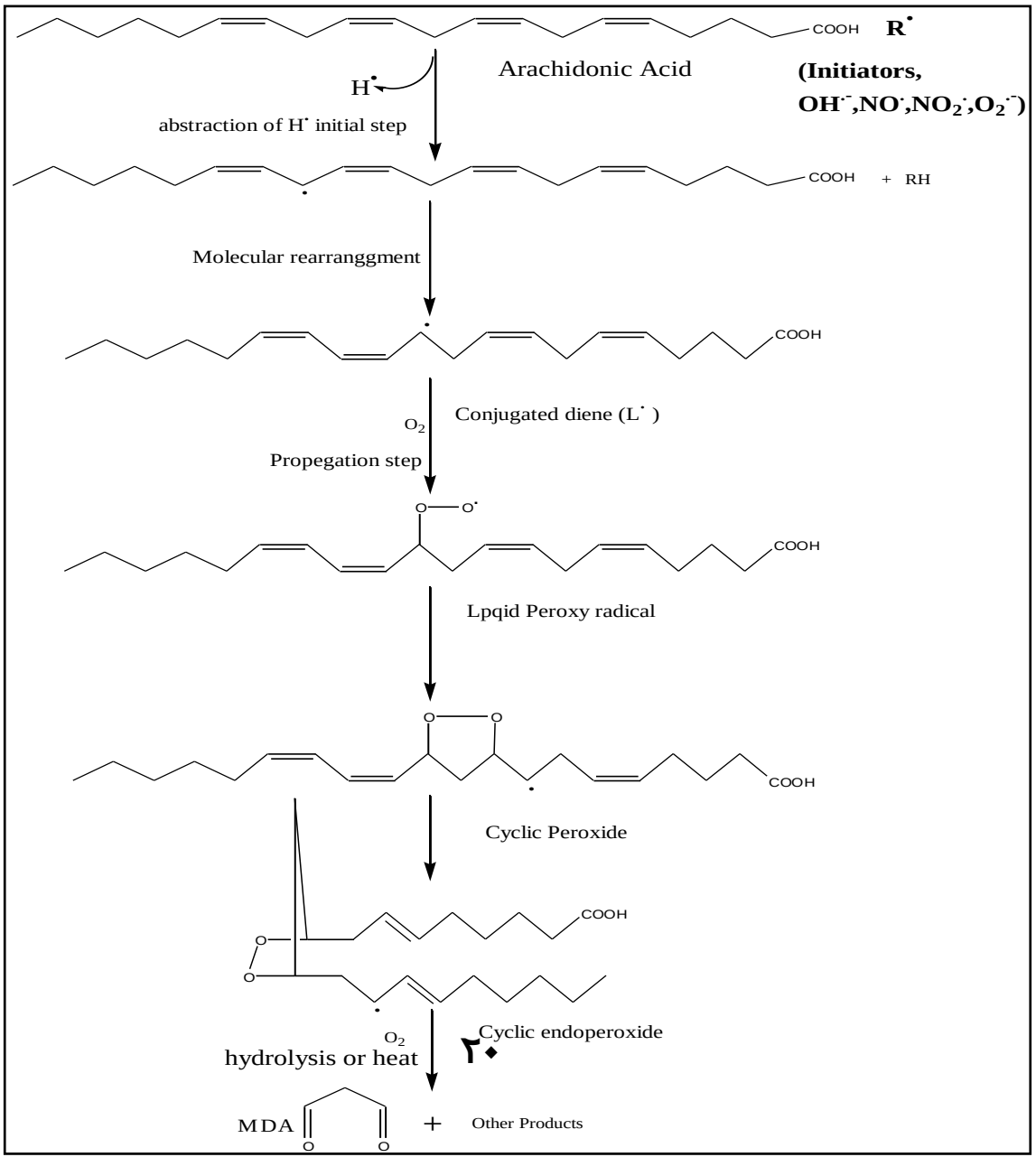


Figure (١-٧): Polyol pathway (Tesfamarian, ١٩٩٣).

١.٥.٢. Specific Biological Alterations in Oxidative Stress Syndrome

١.٥.٢.١. Lipid Peroxidation

Lipid peroxidation is formed by oxidation of membrane



polyunsaturated fatty acids (PUFA) that contain double or triple bonds (interaction of peroxidation aldehyde with phospholipids) leads to release of short-chain aldehydes such as malondialdehyde (MDA) (Lim *et al*., ٢٠٠٠), figure (١-٨).

Figure (١-٨): Mechanism of formation of lipid hydroperoxide (MDA) (Halliwell & Gutteridge, ١٩٩٩).

Lipid peroxidation end products very commonly detected by the measurement of thiobarbituric acid reactive substance (TBARS). This assay has, however, been criticized for the lack of specificity.

Lipid peroxidation as measured by lipid hydroperoxides (Hermes-Lima *et al*., ١٩٩٥) have been shown to correlate closely with TBARS data in tissue and samples. With proper caution TBARS measurement may provide meaningful information (Draper *et al*., ١٩٩٣).

A low density lipoprotein (LDL) particles has ٢٢٠٠ molecules of free fatty acid, half of which is PUFA which is a highly susceptible substrate for free radical reaction. Patient with DM have an increased risk of premature atherosclerosis, which may be due in part to increased oxidizability of LDL (Fuller *et al*., ١٩٩٦).

LDL initially accumulates in the extracellular space of subendothelial space of arteries and through the action of resident vascular cell is mildly oxidized to form minimally oxidized LDL (ox-LDL). Ox-LDL is internalized by the macrophages through scavenger receptor pathway. Native LDL is internalized by the classical receptor pathway with negative feedback so that internalized cholesterol down regulate LDL receptor and prevent further internalization. Such negative feedback does not exist for scavenger pathway. Hence, cholesterol accumulates in the arterial wall (Champe & Harvey, ١٩٩٤).

In addition, ox-LDL inhibits release of Endothelial Derived Relaxation Factor (EDRF) and reduces the action of EDRF on the vessel wall. Ox-LDL stimulates endothelial cells to release a number of biologically active factors like growth factor for vascular cell chemotactic factors (so that resident monocytes are attracted). Ox-LDL causes disturbance of eicosaniod homeostasis and platelets aggregation, activates T lymphocytes in the atherosclerotic lesion stimulating proliferation of smooth muscle cells. Taken all these together, ox-LDL is atherogenic (Akkus *et al*., ١٩٩٦).

Most studies have found increased susceptibility of LDL cholesterol to oxidation in DM patients and elevated levels of plasma, serum, and erythrocyte MDA compared with the control group (Rabini *et al*., ١٩٩٤; Peuchant *et al*., ١٩٩٧; Nourooz Zadeh *et al*., ١٩٩٧; Al-Mashhedani, ٢٠٠٠; Salman, ٢٠٠١).

١.٥.٢.٢. Hyperhomocysteinemia

Homocysteine (Hcy) has become widely accepted as a novel risk marker associated with a therosclerotic cardiovascular disease (CVD) in the coronary, cerebral and peripheral vascular beds (Boers, ٢٠٠٠).

The important role of oxidative-redox stress and hyperhomocysteinemia is biologically plausible because Hcy promotes oxidant in the cells, particularly the endothelium and endothelial nitric oxide synthase (eNOS) reaction through the auto-oxidation of Hcy, formation compounds of disulfide interaction of Hcy thiolactones, and protein homocysteinylation) (Blom, ٢٠٠٠).

Homocysteine is a nonessential nonprotein sulfur-containing amino acid and an intermediary metabolic product derived from demethylated essential amino acid methionine.

The oxidation of two Hcy molecules yields the oxidized disulfide (homocystine), two protons (H^+), and two electrons, which promoting the formation of (ROS).

$$٢\text{Hcy} - \text{SH} + O_٢ \longrightarrow \text{Hcy} - \text{S} - \text{S} - \text{Hcy} + ٢H^+ + ٢e^- \longrightarrow \text{Generating ROS}$$

Auto-oxidation of Hcy ($^{\cdot}OH$, $O_٢^{\cdot -}$, $H_٢O_٢$)

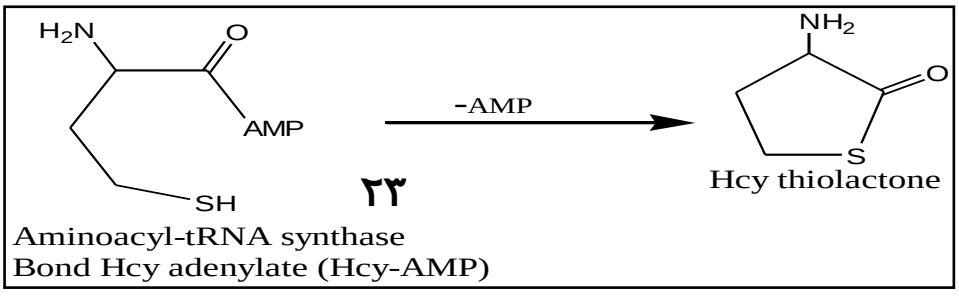
Also, formation of mixed disulfides contributes to the additional formation of ROS.

$$\text{Hcy} - \text{SH} + \text{R} - \text{SH} + O_٢ \longrightarrow \text{Hcy} - \text{S} - \text{S} - \text{R} + \text{ROS}$$

$$\text{Hcy} - \text{SH} + \text{R} - \text{S} - \text{S} - \text{R} \longrightarrow \text{R} - \text{S} - \text{S} - \text{Hcy} + \text{RSH} + \text{ROS}$$

R = any organic compound in the plasma with a thiol group (- SH) accessible to react with Hcy, such as protein, cysteine glutathione, gamma-glutamylcysteine or cystinylglycine (Hayden & Tyagi, ٢٠٠٤).

Additionally, Hcy may undergo complicated rearrangement to form Hcy thiolactone (acyclic thioester), which is chemically reactive and acylates free amino groups such as the side chain lysin groups in proteins. In the process of forming homocysteinylated protein further oxidative stress develops and may lose their biological activity. This results in the modification of protein and in particular the modification of (LDL-cholesterol), which contributes to their retention within the intima and subsequent inflammatory foam cell formation associated with atherogenesis (Hayden & Tyagi, ٢٠٠٤).



The various oxidized forms of Hcy are free and protein-bond Hcy, homocysteine, and Hcy-Cys mixed disulfide, which are defined as total Hcy (tHcy) (Mudd *et al*., ٢٠٠٠).

The results of many recent studies have indicated that elevated plasma levels of Hcy in patients with DM are associated with increased risk of atherosclerosis (Gazzaruso *et al*., ٢٠٠٢; Hayden & Tyagi, ٢٠٠٣; Becker *et al*., ٢٠٠٣; Soinio *et al*., ٢٠٠٤; Hayden & Tyagi, ٢٠٠٤).

Cordoba and coworkers (١٩٩٦) suggested that cysteine and Hcy can induce oxidative modification of LDL. This suggestion is relevant because lipoprotein oxidation is thought to play a key role in the development of atherosclerosis and in the triggering of throbotic events; and Ewadh and Jabir (٢٠٠٢) suggested that extracellular glutathione levels are lowering intracellular glutathione levels of endothelial cells presumably by membrane mediated uptake of Hcy and leading to an accumulation of intracellular Hcy which is induce endothelial cells damage due to glutathione level depletion.

At present, many studies hypothesize that there may exist a folate shuttle, which is operative in the atherosclerosis associated with diabetic as well as, non-diabetic atherosclerosis. This folate shuttle effect may operate as an effective mechanism in the endothelial microenvironment to recouple the oxidatively stressed, uncoupled, and dysfunctional eNOS reaction (Hayden & Tyagi, ٢٠٠٢; Hayden & Tyagi, ٢٠٠٢; Hayden & Tyagi, ٢٠٠٣; Hayden & Tyagi, ٢٠٠٣; Hayden & Tyagi, ٢٠٠٤), figure (١-٩).

There are three critical arms associated with eNOS reaction: the eNOS enzyme, the L-arginine substrate, and the BH_4 cofactor. The eNOS reaction may be uncoupled due to oxidative-redox stress associated with DM atherosclerosis.

When the eNOS reaction is uncoupled, the reaction will become a net producer of $O_2^{\cdot -}$ (instead of $eNO^{\cdot}$ via the eNOS reaction) through the reaction of membranous NADPH, oxygen and the NADPH oxidase enzyme (Hayden & Tyagi, ٢٠٠٤).

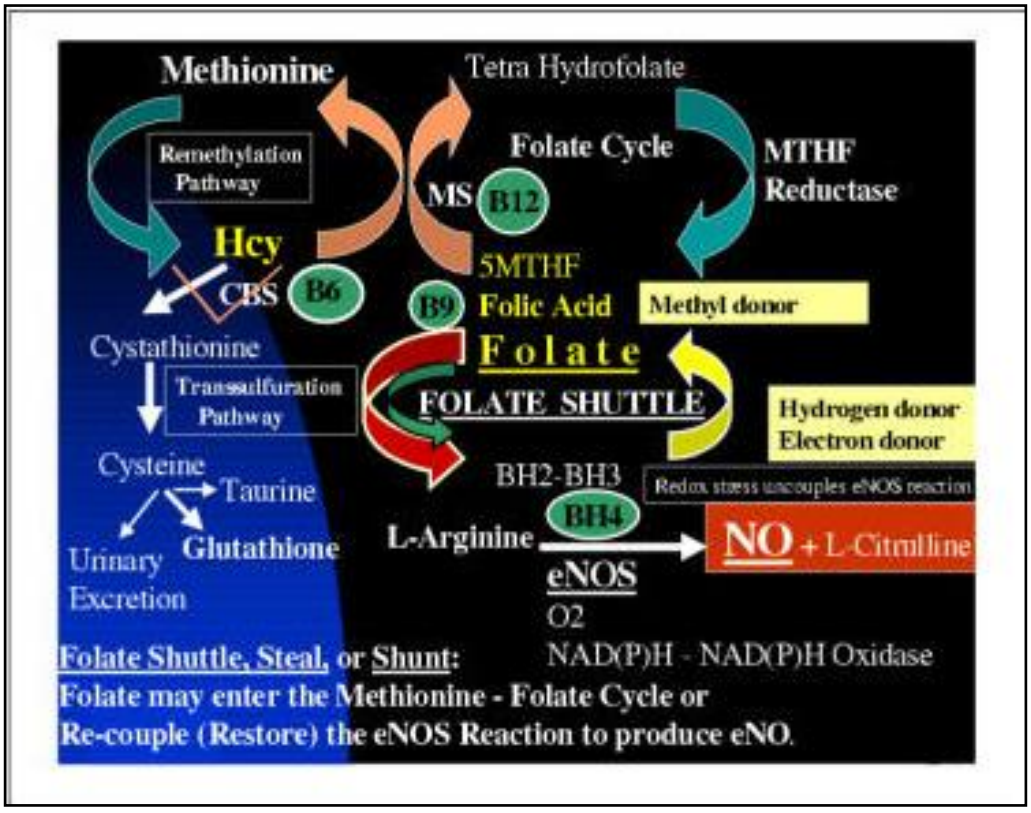


Figure (١-٩): Folate shuttle. MS: methionine synthase, ٥MTHF: ٥,methyltetrahydrofolate, eNOS: endothelial nitric oxide synthase, BH_4: tetrahydrobiopterin, BH_2:

dihydrobiopterin, BH_2: trihydrobiopterin, (Hayden & Tyagi, ٢٠٠٤).

The activated-dysfunctional-uncoupled endothelium is a net producer of $O_2^{\cdot -}$ associated with DM and atherosclerosis. The net production of $O_2^{\cdot -}$ by the uncoupled eNOS reaction in addition to the production of ROS via the auto-oxidation of Hcy results in an excessive oxidative-redox stress (Hayden & Tyagi, ٢٠٠٤).

١.٥.٣. Smoking Effect

The smoke of cigarettes reduces the activity of antioxidant system, this may lead to imbalance between oxidant-antioxidant in favor of the prooxidant leading to potential damage and dominant risk factor of many disease (Michiels *et al*., ١٩٩٤).

In smokers, the mitochonderial respiratory chain function of lymphocyte is disturbed; and it correlates with the degree of oxidative damage of membranes. This mitochondrial dysfunction could contribute to increased endogenous production of ROS (Miro *et al*., ١٩٩٩).

Tobacco smoke as a source of exposure to carbon monoxide (CO), to give high level of hemoglobin carbon monoxide (COHb), which were found to be associated with the prevalence of coronary heart disease (Kannel, ١٩٨١).

Evidence for increased oxidation in smokers compared with non-smokers were also provided by the presence of increased prooxidation products in blood and urine (Morrow *et al*., ١٩٩٥; Mezzetti *et al*., ١٩٩٥).

Effect of smoke nicotine on the function of human polymorphnuclear (PMN) leukocytes was the inhibition of microbeidal function of PMN through inhibition of O_2 production and release of lysosomal enzymes, but nicotine has no effect on migration of PMN to inflammatory sites.

Oxidative damage may also results from ROS generated by the increased and activated phygocytes following cigarette smoking (Chow, ١٩٩٣), and associated with significant increase in circulating nutrophils count and phygocyte derived ROS (Van-Antwerpan *et al*., ١٩٩٥; Sharma *et al*., ١٩٩٧).

Smoking has also been identified as a risk factor for insulin resistance, which can lead to diabetes. Catecholamines, is a type of hormone, are produced in greater quantity in smoker and act as an antagonist to insulin action (Targher, ١٩٩٧).

Cigarette smoke results in the formation of more than a billion oxyradicals in each puff (Borck, ١٩٩٧), therefore, smoking is associated with multiple complications of diabetes-nephropathy (kidney disease) has been shown to be common in type ١ diabetic patients who smoke (Mulhauser, ١٩٩٦), and smoking increases the risk of heart disease in both types of diabetes (Suarez & Barrett-Conner, ١٩٨٤); as well as smoking increases the risk of microalbuminurea in diabetic patients (Chase, ١٩٩١; Ritz *et al*., ١٩٩٦) (microalbuminurea refers to the presence of protein in the urine and can indicate signs of kidney disease).

١.٦. Antioxidant Defense Systems

To refer protection against free radicals and prevent damage to vital biological structures such as cell membranes, proteins, and DNA, nature has provided a defense system in the body. This defense system consists of antioxidants.

Antioxidants can be defined as substances whose presence in relatively low concentrations significantly inhibits the rate of oxidation of lipids, proteins, carbohydrates, and DNA. Antioxidants act as potent electron donors, they donate hydrogen atoms to pair up with unpaired electrons on free radicals. Thus they convert reactive free radicals to inactive substances (Bagchi & Puri, ١٩٩٨).

Antioxidants react with free radicals before more important target molecules like lipids, DNA, etc. are damaged. In doing so the antioxidant is sacrificed (oxidized) and must be regenerated or replaced. The antioxidant radical generated during this process is relatively unreactive and unable to attack vital molecules. Antioxidants prevent damage induced by free radicals and play a very crucial role in preventing or delaying the onset of disease (Langseth, ١٩٩٦), figure (١-١٠).

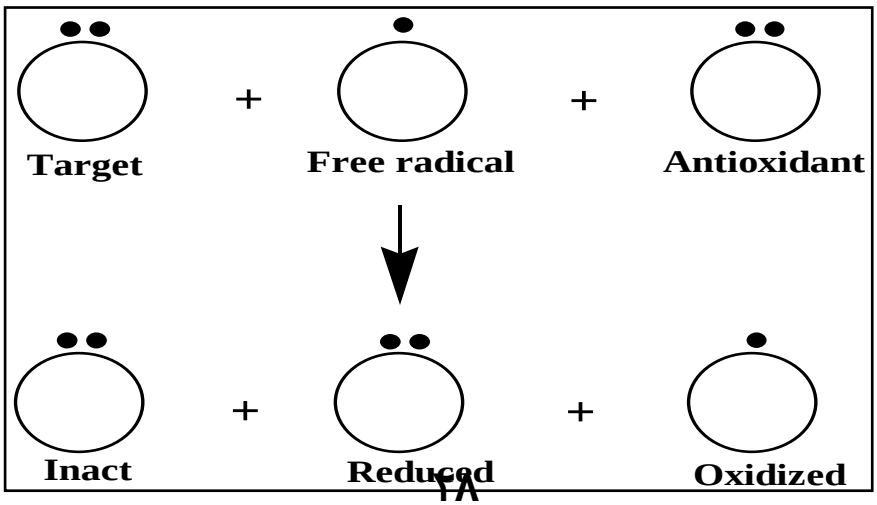


Figure (١-١٠): The interaction of free radicals and antioxidants

The antioxidant defense of the body consists of two main systems (enzymatic antioxidants and non-enzymatic antioxidants).

١.٦.١. Enzymatic Antioxidants

Several antioxidant enzymes are made by various cells in the body. The most three important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) which are made in the body in response to the presence of certain free radicals. Thus, if a body is under higher oxidative stress, and is producing more free radicals, more of the antioxidant enzymes will be made to counterbalance the stress like glutathione S-transferase (GST), and glutathione reductase (GR), figure (١-١١).

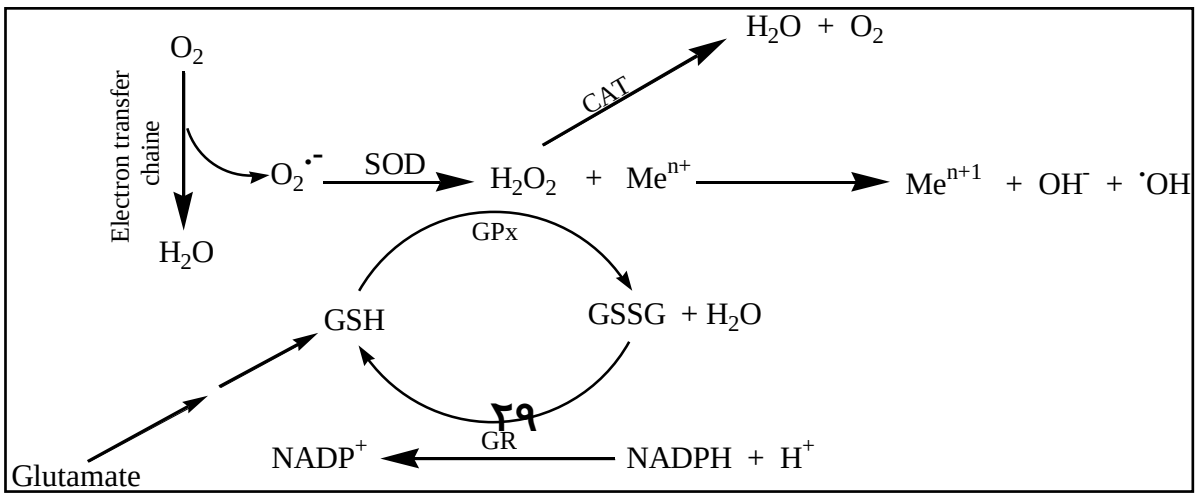


Figure (١-١١): Co-operation of antioxidant enzymes. GSH: glutathione, GSSG: oxidized glutathione, Me^{2+}: transition metal, GR: glutathione reductase, GPx: glutathione peroxidase (Cedeberg, ٢٠٠١).

١.٦.١.١. Superoxide Dismutase (SOD)

SOD (EC.١.١٥.١.١) is a metalloenzyme. There are three major isoforms of SOD; intracellular Cu/Zn-SOD, Extracellular Cu/Zn-SOD (Ec-SOD), and Mn-SOD, with copper and zinc or manganese ions at the active sites, respectively (Cedeberg, ٢٠٠١).

The enzymes are structurally different; Mn-SOD has a molecular weight of ٤٠,٠٠٠ and Cu/Zn-SOD ٣٢,٠٠٠ dalton. Despite different structures, the three isoforms catalyse the same reaction, that is dismutase O_2 to H_2O_2 (Cedeberg, ٢٠٠١).

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Cu/Zn-SOD is mostly in the cytosol of the cell but also to a lesser extent in the lysosomes and the nucleus. Mn-SOD is considered to be a mitochondrial enzyme only. Ec-SOD is found in the plasma and extracellular space (Atalay & Laaksonen, ٢٠٠٢).

$O_2^{\cdot -}$ may react with other ROS such as $NO^{\cdot}$ to form highly toxic species such as peroxynitrite ($ONOO^{\cdot}$), in addition to direct toxic effects (Tasfamarian, ١٩٩٤). Peroxynitrite reacts with the tyrosine residues in proteins resulting with the nitrotyrosine production in plasma proteins, which is considered as an indirect evidence of ($ONOO^{\cdot}$) production and increased oxidative stress. Furthermore, exposure of endothelial cells to high glucose leads to augmented production of $O_2^{\cdot -}$, which may quench

$NO^{\bullet}$. Decreased $NO^{\bullet}$ levels result with impaired endothelial functions, vasodilation and delayed cell replication (Glugliano *et al.*, ١٩٩٦). Alternatively, $O_2^{\bullet-}$ can be dismutated to much more reactive H_2O_2, which through the Fenton reaction can then lead to highly toxic $OH^{\bullet}$ formation (Wolff *et al*, ١٩٩١), figure (١-١٢).

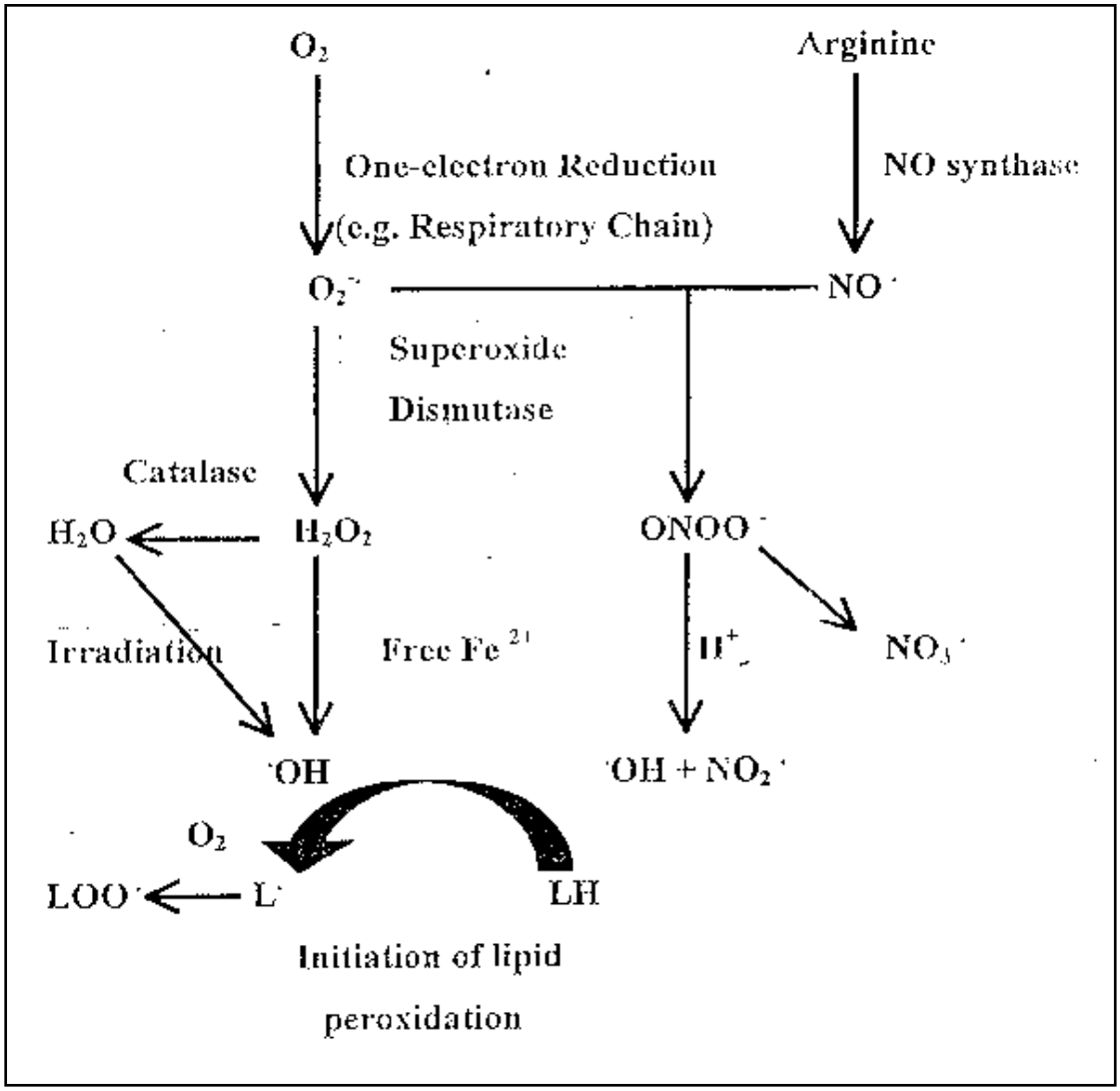


Figure (١-١٢): Suggested pathways for the formation of reactive oxygen species in vivo, (Wolff *et al,* ١٩٩١).

In diabetic, the wide variability among studies, which are shown decreased, increased and unchanged in SOD isoforms activity (Jennings *et al*., ١٩٩١; Walter *et al*., ١٩٩١; MacRury *et al*., ١٩٩٣; Adachi *et al*., ١٩٩٦; Kaneto *et al*., ١٩٩٦) which does not allow conclusions to be drawn as to whether SOD isoform activities are abnormal in diabetic patients, again differences in methodology or study design do not completely explain the conflicting findings among studies.

١.٦.١.٢. Catalase (CAT)

Catalase (EC ١.١١.١.٦) is an oligomer with four ٦٠,٠٠٠ dalton sub-units. It is a hemo protein containing four heme groups. Subcellularly, CAT is mainly localized to the peroxisomes, as much as ٤٠٪ of the total protein content of the peroxisomes has been estimated to be CAT (Murray *et al*, ٢٠٠٠).

The peroxisomes are cytoplasmic organelles in which several oxidative processes occur, e.g. the oxidation and detoxification of several toxic compounds resulting in large amounts of H_2O_2 (Cedeberg, ٢٠٠١).

CAT is found in blood, bone marrow, mucous membranes, kidney, and liver. CAT catalyses the direct composition of H_2O_2 to ground state O_2:

$$2H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

Many studies are found decreased levels of CAT in patients with DM (Kashiwagi *et al*., ١٩٩٤; Vijayalingam *et al*., ١٩٩٧; Peuchant *et al*., ١٩٩٧; Szaleezky *et al*., ١٩٩٩; Cedeberg, ٢٠٠١).

١.٦.١.٣. Glutathione S-Transferase (GST)

The glutathione transferase are recognized as important catalysts in the bio-transformation of xenobiotics, including drugs as well as environmental pollutants. Multiple forms exist, and numerous transferase from mammalian tissues, insects, and plants have been isolated and characterized. Enzymatic properties, reactions with antibodies, and structural characteristics have been used for classification of the glutatione transferase (Mannervik & Danielson, ١٩٨٨).

Mammalian cytosolic GSTs (EC٢.٥.١.١٨) are homodimeric, and the monomers are in the range of ٢٢,٠٠٠ – ٢٩,٠٠٠ dalton. They could be grouped into three distinct classes -α, μ, and π. They are active over a wide variety of substrates with considerable overlap (Goughlin & Hall, ٢٠٠٢).

GST contributes in conjugating drugs, poisons, and other compounds with reduced glutathione and naturalizes the electrophilic side and dissolution in the aqueous cellular and extracellular media, and from there, out of the body (Raza *et al*., ٢٠٠٢).

The transferases increase the nucleophilic properties of reduced glutathione (GSH) and they catalyze the formation of thioether bond by conjugated hydrophobic compounds with GSH to form products consist very stable thioether bond; these products excreted by the bile as GSH conjugates and then cleavage glycine or glutamate and acetylation the

free amino group of cysteinyl residue to give the end product (Mercapturic acid) (Cedeberg, ٢٠٠١).

Some recent studies have found increase in the activity of GST in diabetic patients (Salman, ٢٠٠١; Raza *et al*., ٢٠٠٤).

١.٦.١.٤. Creatine Kinase (CK)

Creatine Kinase (CK, EC ٢, ٧.٣.٢) is a dimer consisting of two sub-units. There are three isoenzymes have been designated as CK-BB (brain type), CK-MB (hybrid type), and CK-MM (muscle type) (Bishop *et al*., ٢٠٠٠).

CK is a major phosphotransfer system in cells with high-energy demand, and it acts in concert with other enzymatic system to facilitate intracellular energetic communication (Neumann *et al*., ٢٠٠٣), thus CK is an enzyme that is associated with ATP regeneration in contractile or transport systems. It predominates physiologic function occurs in muscle cells, where it is involved in the storage of high-energy creatine phosphate. Every contraction cycle of muscle results in creatine phosphate use, with the production of ATP (Dzeja & Terzic, ٢٠٠٣).

$$\text{Creatine phosphate} + \text{MgADP} \overset{\text{CK}}{\rightleftharpoons} \text{Creatine} + \text{MgATP}$$

CK is widely distributed in tissues, with highest activities found in skeletal muscle, heart muscle, and brain tissue. Other tissue sources in which CK is present in much smaller quantities include the bladder,

placenta, gastrointestinal tract, thyriod, uterus, kidney, lung, prostate, spleen, liver, and pancreas (Bishop *et al*., ٢٠٠٠).

During conditions of oxidative stress, the enzyme CK appears to be inactivated via oxidation of the active-site thiol (Reddy *et al*., ٢٠٠٠). Ck is relatively unstable, and its activity is apparently lost as a result of internal disulfide formation (Gunst *et al*., ١٩٩٨).

Gunst and coworkers (١٩٩٨) investigated the effect of endogenous extracellular glutathione concentration on serum CK activity, and they found in patients with multiple organ failure, low serum CK activities were accompanied by extremely low serum glutathione concentration.

Serum CK activity is an indicator commonly used in the diagnosis of heart and skeletal muscle disorders (Bishop *et al*., ٢٠٠٠). Many studies have found that diabetes leads to decrease total CK activity by ٣٥٪ and also decreased CK-BB mRNA levels (Popovich *et al*., ١٩٨٩; Popovich *et al*., ١٩٩١; Kelley *et al*., ٢٠٠٢).

١.٦.٢. Non-enzymatic Antioxidants

١.٦.٢.١. Glutathione (GSH)

GSH is γ-glutamylcysteinylglycine, the most abundant non-protein thiol compound in mammalian cells, is biosynthesized from amino acid precursors (Murray *et al*., ٢٠٠٠)

Tissue glutathione plays a central role in antioxidant defense. The hydrogen atom of SH group abstraction is one of the most important aspects of GSH reactivity considering its function as an antioxidant.

GSH can undergo oxidation reactions with free radicals through two types of process:

١. One-electron Process; in which GSH is oxidized to form a sulfur-centered glutathione radical ($GS^{\bullet}$) (Halliwell & Gutteridge, ١٩٩٩) as shown in reaction (١):

$$GSH + R^{\bullet} \longrightarrow GS^{\bullet} + RH \text{ --------- Reaction (١)}$$

$GS^{\bullet}$ can also be formed through photoionization of glutathione anion (GS^{-}) (reaction ٢) or through electron transfer to a metal ion (reaction ٣) (Buettner & Oberley, ٢٠٠١):

$$GS^{-} \longrightarrow GS^{\bullet} + e^{-} \text{ ---------- Reaction (٢)}$$

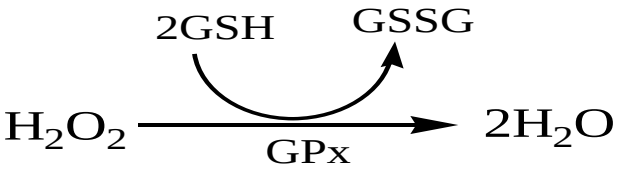
$$GS^{-} + M^{n+} \longrightarrow GS^{\bullet} + M^{(n-١)+} \text{ ---------- Reaction (٣)}$$

٢. Two-electron Processes; in which two molecules of reduced GSH are oxidized to the oxidized form of GSH (reaction ٤) (Buettner & Oberley, ٢٠٠١):

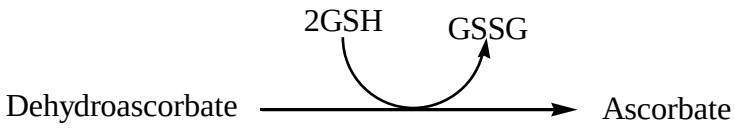
$$٢GSH \longrightarrow GS - SG + ٢H^{+} + ٢e^{-} \text{ ---------- Reaction (٤)}$$

The action of GSH are:

١. Reduced GSH detoxifies ROS such as $H_٢O_٢$ and lipid peroxides directly or in a GPx catalyzed mechanism (Murray *et al.*, ٢٠٠٠)



٢. GSH also regenerates the major aqueous and lipid phase antioxidants, ascorbate and α-tochopherol (Atalay & Laaksonen, ٢٠٠٢):



٣. GSH as a product of glutathione reductase (GR)-catalyzed reactions to maintain the balance between GSH and GSSG. In fact, the ratio of reduced to oxidized GSH within cells is often used scientifically as a measure of cellular toxicity. GSSG is reduced to GSH by GR which uses NADPH as a cofactor:

$$GSSG + NADPH + H^{+} \xrightarrow{GR} 2GSH + NADP^{+}$$

glutathione reductase (GR) can also catalyze reduction of certain "mixed" disulfides, such as that between GSH and coenzyme A (Peuchant *et al*., ١٩٩٧).

٤. GSH as a direct free radical scavenger.

٥. GSH participates non-enzymatically and enzymatically.

Many recent studies have found decrease plasma GSH levels in different disease states especially those associated with oxidative stress like DM (Thornalley *et al*., ١٩٩٦; Peuchant *et al*., ١٩٩٧; Al-Mashhadani, ٢٠٠٠; Salman, ٢٠٠١; Cedeberg, ٢٠٠١;).

In diabetic patient GSH deficiency resulting from reduced concentration of NADPH is used by polyol pathway where glucose is reduced to sorbitol by aldose reductase. An increased activity in this pathway causes a depletion of reduced GSH, which may weaken the antioxidant defense (Cedeberg, ٢٠٠١; Buettner & Oberley, ٢٠٠١), figure (١-٧).

١.٦.٢.٢. Vitamin E (α-Tochopherol)

Vitamin E is a fat-soluble powerful antioxidant and is the primary defense against potentially harmful oxidation that causes disease and aging (Bishop *et al*., ٢٠٠٠).

Vitamin E is the collective name for eight compounds, four tochopherol and tocotrienols, found in nature. It is a fat-soluble substance present in all cellular membranes and is mainly stored in adipose tissue, the liver and muscle (Bagchi & Puri, ١٩٩٨).

α-tochopherol is the predominant isomer in plasma. The actions of vitamin E are:

١. It is essential for the integrity, stability and function of cell membranes, and nerve cells. Vitamin E provides protection to lipid present in cell membranes against free radicals.

٢. It scavengers intracellular as well as extracellular free radicals.

٣. Vitamin E protects polyunsaturated fatty acids in cell membranes from peroxidation. It prevents oxidation of LDL-cholesterol particles, the most crucial step in the development of atherosclerosis.

٤. It increases insulin sensitivity. Thus Vitamin E helps in improving control of diabetes.

٥. It also lowers plasma fibrinogen levels.

٦. Vitamin E improves endothelial function by increasing release of $NO^{\bullet}$, which dilates blood vessels.

٧. It enhances body's immune system.

٨. Vitamin E decreases platelet adhesion and platelet aggregation.

٩. It decreases the risk of cancer, ischemia and reperfusion injury, cataract, arthritis and certain neurological disorders.

١٠. It also inhibits the conversion of nitrites in stomach to nitrosamines.

١١. It is a singlet oxygen quencher; singlat oxygen ($^{1}O_2$) is very active non-radical species, which is an excited form of O_2. $^{1}O_2$ is generated by activation of peroxidase enzyme in biological systems and under aerobic condition, as a result of oxidation of hypoxanthine by xanthine oxidase enzyme (Keith, ١٩٩٩):

$$\text{Enzyme} - H_2 + 2O_2^{\cdot\cdot} \longrightarrow \text{Enzyme} + 2H^+ + 2O_2^{\cdot -}$$

$$2H^+ + 2O_2^{\cdot -} \longrightarrow O_2 + H_2O_2$$

$$H_2O_2 + O_2^{\cdot -} \longrightarrow OH^{\cdot} + OH^- + {}^{1}O_2$$

$^{1}O_2$ can damage DNA and be mutagenic, but vitamin E inhibits this process by neutralizing these highly reactive and unstable $^{1}O_2$ molecules.

١٢. Vitamin E protects double bonds of β-carotene from oxidation and thus exhibits a sparing effect.

The mechanism of action vitamin E is that it exhibits its antioxidant action by providing hydrogen atom to pair up with unpaired electrons on free radicals. Thus it converts free radicals to stable and inactive substances (Gillham *et al.*, ٢٠٠٠), figure (١-١٣).

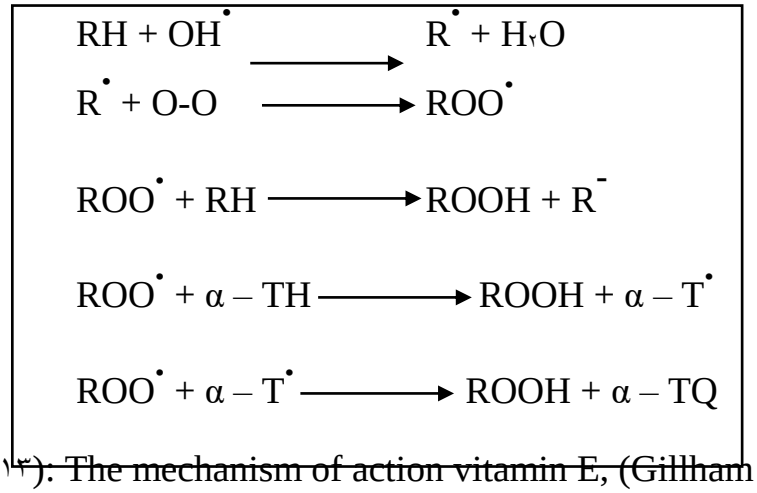


Figure (١-١٣): The mechanism of action vitamin E, (Gillham *et al*., ٢٠٠٠).

During this process, vitamin E is oxidized. Vitamin E does not have antioxidant action. Vitamin C helps in regeneration of oxidized E and converts it to active form. Thus there is a synergistic action between vitamin C and vitamin E (Murray *et al*., ٢٠٠٠).

١.٦.٢.٣. Vitamin C (Ascorbic Acid)

Vitamin C is a water-soluble antioxidant vitamin. It neutralizes free radicals in the plasma, cytoplasm and extracellular fluid. Vitamin C is not synthesized in the body. Vitamin C is taken from the diet and absorbed from small intestines and it is transported through leukocytes and platelets (Murray *et al*., ٢٠٠٠).

The actions of vitamin C are:

١. Vitamin C helps in regeneration of oxidized vitamin E in cell membranes (Cedeberg, ٢٠٠١), figure (١-١٤).

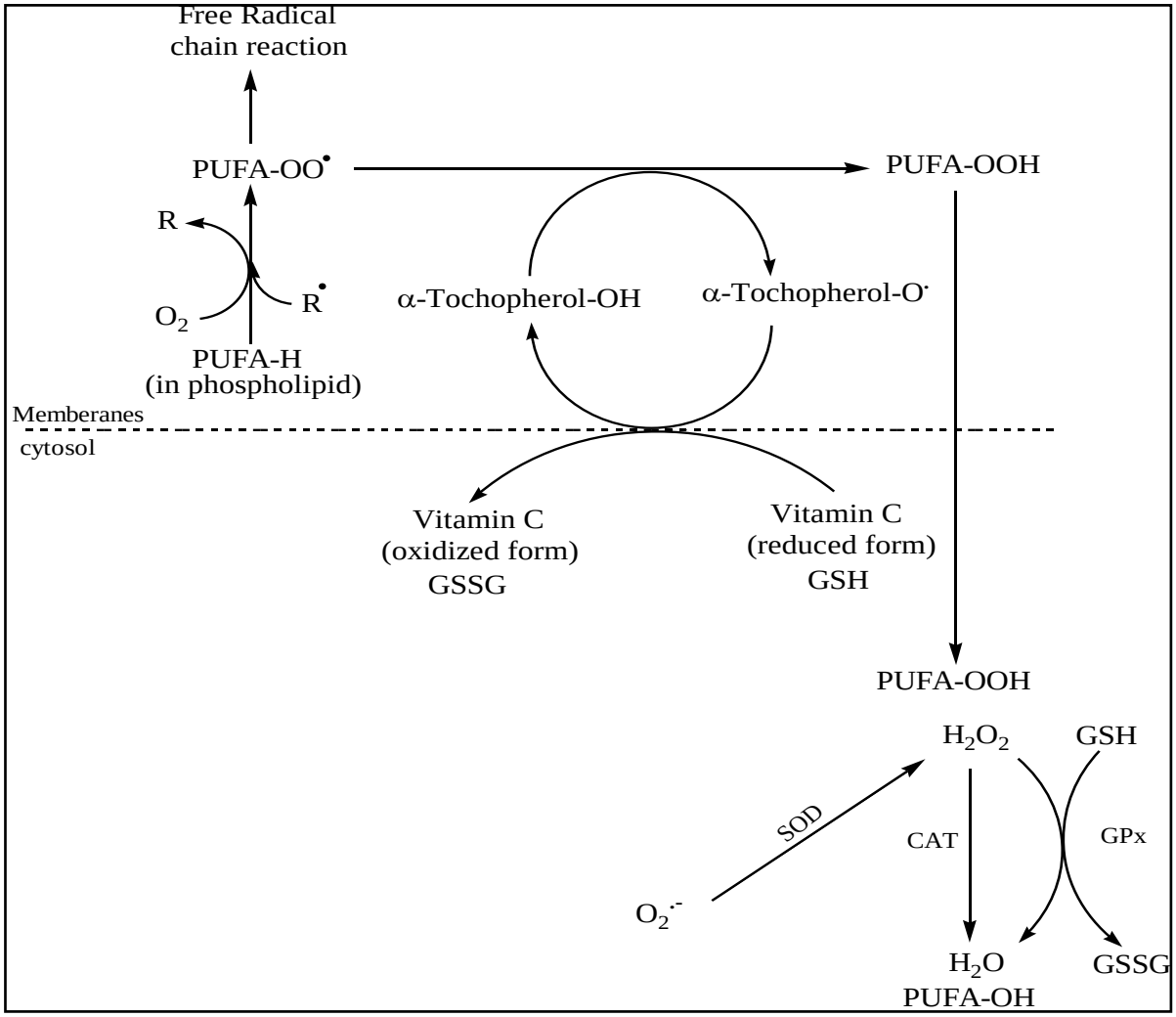


Figure (١-١٤): The interactions between ascorbate and α-tochopherol in trapping free radicals (Murray *et al*., ٢٠٠٠)

٢. Vitamin C effects on plasma lipids by reduction in total cholesterol and LDL-Ch, increase in HDL-Ch levels, and decrease in serum triglyceride levels, thus reducing the risk of cardiovascular disease (Tousoulis *et al*., ٢٠٠٣).

٣. It also lowers blood pressure (Murray *et al*., ٢٠٠٠).

٤. Vitamin C improves endothelial function (Cedeberg, ٢٠٠١).

٥. It reduces the incidence of cataract and certain cancers.

The mechanism of action of vitamin C in diabetic patients is that vitamin C exhibits its antioxidant action by donating hydrogen atom to free radicals (Atalay & Laaksonen, ٢٠٠٢), figure (١-١٥).

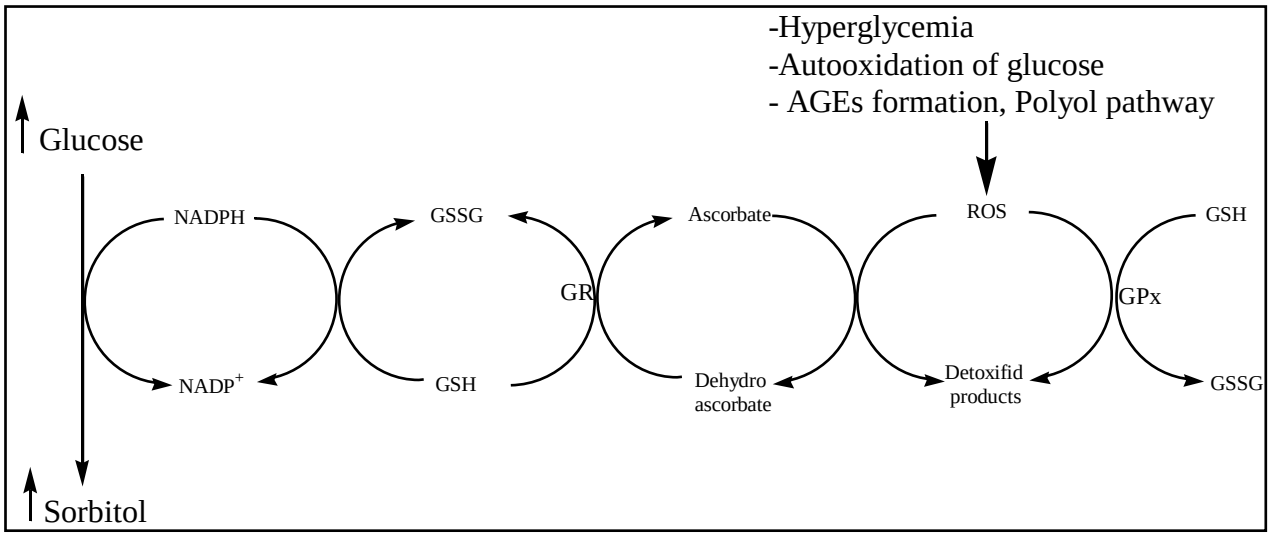


Figure (١-١٥): Mechanism of vitamin C to scavenger of free radicals (Atalay & Laaksonen, ٢٠٠٢).

١.٦.٢.٤. Vitamin A (Retinol)

Vitamin A is often used as a collective term for several related biologically active molecules (retinoids). They are retinol, retinal, retinoic acid (all trans), and ١١-cis-retinal (formed by photoisomerization of all trans-retinal). Retinol is a primary alcohol containing an β-ionone ring with an unsaturated side chain. Retinol is found in animal tissues as a retinyl ester with long-chain fatty acids. Retinal is the aldehyde derived from the oxidation of retinol. Retinal and retinol can readily be interconverted. Retinoic acid is the acid derived from the oxidation of retinal. Retinoic acid cannot be reduced in the body and therefore cannot give rise to either retinol or retinal (Champe & Harvey, ١٩٩٤).

Some carotenoids (provitamin A) like β-carotene act as a precursor of vitamin A; which are synthesized by all plants and by some animals, but not by man (Murray *et al*., ٢٠٠٠).

Scission of β-carotene at the central double bond results in the formation of retinal, which is reduced to retinol (vitamin A) by an NADH-dependent enzymatic reaction. This conversion takes place mainly in the liver (Tietz *et al*., ١٩٧٩).

Retinol and its provitamin, β-carotene, are effective antioxidants and considered the most powerful singlet oxygen quenchers (Handelman, ٢٠٠١). β-carotene can dissipate the energy of $^{١}O_{٢}$, thus preventing this active molecule from generating free radicals. It is efficient at low oxygen pressure (Murray *et al*., ٢٠٠٠).

$$^{١}O_{٢} + \beta\text{-carotene} \longrightarrow {}^{٣}O_{٢} + \beta\text{-carotene}$$

١.٧. Minerals

Minerals may be divided into two groups: (١) macro-minerals, which are required in amount greater than ١٠٠ mg/dL, and (٢) micro-minerals (trace elements), which are required in amount less than ١٠٠ mg/dL (Bishop *et al*., ٢٠٠٠).

Trace elements appear to play a key role in most biological functions. The effect of these elements cannot be replaced by another element. It has been reported that action of every small amount of trace and ultratrace elements are necessary for optimal performance of whole organism, and the lack of a small amount of them can result in clinical abnormalities (Luxton, ١٩٩٩). On the other hand, deficient of these

elements is associated with impairment of function and excess concentrations may be toxic (Bishop *et al*., ٢٠٠٠).

Trace elements are required in very small amounts to maintain health and they often form essential parts of enzymes and hormones, which regulate metabolism (Luxton, ١٩٩٩).

Some of the trace and ultratrace elements have a scavenging property as antioxidant to free radical generation, thus the alteration in their levels may be considered a biochemical marker of free radical generation, such as zinc, selenium, copper, iron, and chromium.

١.٧.١. Iron

Iron is an essential trace element. Most iron in the body is incorporated into heme of hemoglobin and a smaller proportion as myoglobin, heme-containing enzyme like catalase, and iron-sulfur proteins (Bishop *et al*., ٢٠٠٠).

Iron is a first-line prooxidant, it contributes to regulate the clinical manifestations of numerous systemic diseases, including diabetes, atherosclerosis, and deoxyribonucleic acid (DNA) damage (Halliwell & Aruma, ١٩٩١; Meneghini, ١٩٩٧; Fernandez-Real *et al*., ٢٠٠٢).

Iron is intimately linked to oxidative stress. It participates through the Fenton reaction, in the formation of highly toxic free radicals such as $OH^{\bullet}$ and $O_2^{\bullet -}$ and causes oxidative damage (McCord, ١٩٩١). Oxidative stress influences both glucose and iron metabolism (by decreasing internalization of insulin and increased ferritin synthesis) (Reif, ١٩٩٢).

١.٧.٢. Copper (Cu)

Copper is an essential trace element, a small amount of circulating copper is bond to albumin, whereas most is incorporated into ceruloplasmin (Murray *et al*., ٢٠٠٠).

An important role for copper is as a component of enzymes or proteins involved in redox reaction (Noto *et al*., ١٩٨٣) like:

١. Cytochrome c oxidase catalyzes the reduction of oxygen to water in the last step of the electron transport chain (Murray *et al*., ٢٠٠٠).

٢. Cytosolic SOD, containing copper and zinc, catalyzes the conversion of $^{٢}O_{٢}^{\cdot-}$ to $O_{٢}$ and $H_{٢}O_{٢}$.

Copper deficiency associated with low concentration of ceruloplasmin and leads to accumulation iron in the liver and causes increase free radical production (Harris, ١٩٩٥), and lack of the copper-containing antioxidant enzyme related to the shortened life of erythrocytes and neutrophils (Percival, ١٩٩٥), and excess copper can cause free radicals production and damage tissues (Bishop *et al*., ٢٠٠٠).

١.٧.٣. Zinc (Zn)

Zinc is an essential trace element. It is cofactor for more than ٣٠٠ enzymes, and zinc-containing enzymes are found in every enzyme class. Alkaline phosphetase, alcohol dehydrogenase, carbonic anhydrase, DNA polymerase, and SOD are containing zinc (Murray *et al*., ٢٠٠٠).

Zinc enzymes are essential to growth, wound healing, reproductive function, the immune system, and protection from free radicals damage (Cunningham-Rundles, ١٩٩٦).

Oxidative stress syndrome in human with DM results in disturbance of zinc (Kinlaw *et al*., ١٩٨٣). Zinc has an important role in modulating the immune system and its dysfunction in DM may be related in part to the status of zinc (Murray *et al*., ٢٠٠٠). Zinc is found in biological systems only in the (+٢) valance state. This is the result of the extra stability associated with a filled d-orbital electron configuration (Luxton, ١٩٩٩). Diet low in zinc are associated with zinc deficiency include skin lesion, diarrhea, dwarfish, and susceptibility to infection (Burtis & Ashwood, ١٩٩٩; Bishop *et al*., ٢٠٠٠)

١.٧.٤. Chromium (Cr)

Trivalent chromium ($Cr^{٣+}$) is an essential trace element for animals and humans. Its deficiency in organisms causes e.g. disturbance of carbohydrate and lipid metabolism, hypoglycemia, impaired glucose tolerance (Terpilowska & Zaporwska, ٢٠٠٤).

$Cr^{٣+}$ may improve insulin sensitivity, which can modify the risk of diabetes and cardiovascular disease (CVD), therefore, when chromium supplementation is beneficial for preventing CVD among people with diabetes (Rajpathak *et al*., ٢٠٠٤).

The determination of clinical chromium deficiency is difficult, because there is no accurate biochemical indicator of chromium status (Belinda & Connell, ٢٠٠١; Vincent, ٢٠٠٤).

Mechanism of Action

Chromium appears to act by enhancing or potentiating insulin's actions. No chromium-containing enzyme has been discovered, and the biologically active form of chromium is still uncertain. The actions of chromium have been attributed to an increase in the number of insulin receptors (Belinda & Connell, ٢٠٠١; Rajpathak *et al*., ٢٠٠٤).

Numerous researchers have been investigating the effects of chromium supplements on glycemic control in type ١,and ٢ diabetes mellitus disease (Anderson *et al*., ١٩٩٧; Franz & Bantle, ١٩٩٩; Javanovic *et al*., ١٩٩٩; Ravina *et al*., ١٩٩٩; Trow *et al*., ٢٠٠٠).

١.٧.٥. Selenium (Se)

Selenium is an essential trace element. The essentiality of selenium results from its present as a necessary component to form the active center, seleno group (-SeH), of GPx, thioredoxin reductase and of other seleno-enzymes (Tihant *et al*., ١٩٨٩; Murray *et al*., ٢٠٠٠). Selenium is widely distributed in nature and found in combination with sulfide and other minerals (Hayes, ٢٠٠١).

Selenium is a component as a cofactor of the enzyme GPx, which protects polyunsaturated fatty acid in the cell membrane from oxidative damaging effect of peroxide by decomposing these oxidizing compounds. GPx catalyzes the reduction of H_2O_2 and organic hydropeoxides in the presence of GSH (Bedwal *et al*., ١٩٩٣), figure (١-١٦).

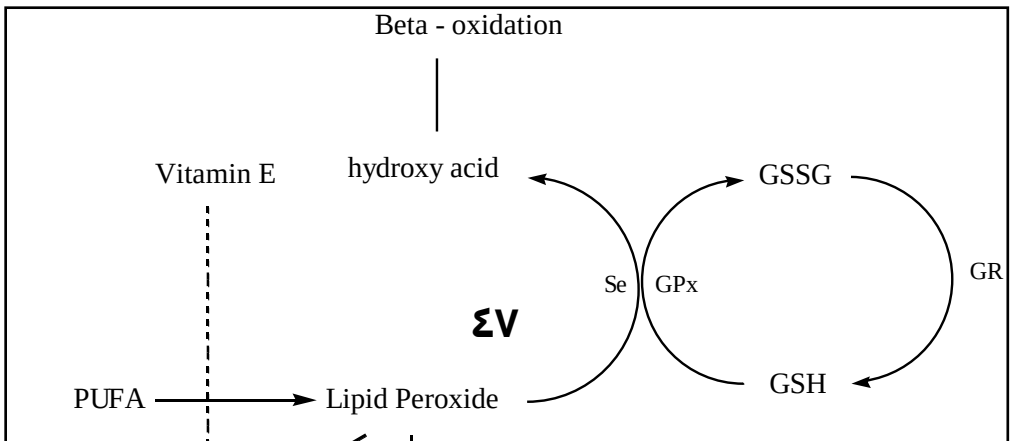


Figure (١-١٦): The relationship between GPx, Se, and Vitamin E (Gillham *et al*., ٢٠٠٠).

Thus selenium deficient cells are less capable of scavenging intracellular peroxides after exposure to exogenous H_2O_2 (Knight, ١٩٩٩). Therefore, selenium was suggested as antioxidant acting as a free radicals scavenger (Palmer & Paulson, ١٩٩٧).

In addition, selenium and vitamin E have been demonstrated to spare one another antioxidant requirements against lipid peroxides (Beckg, ١٩٩٨).

Aim of the Study

The aim of the present work is to investigate different aspects of reactive oxygen species (ROS) involvement in diabetes mellitus disease.

Since the objective of this work to study the oxidative stress of patients with DM by test the following hypotheses:

١. Oxidative stress influences on the activity of antioxidant enzymes and concentration of antioxidant vitamins, glutathione, trace elements and homocysteine levels in serum of diabetic patients.

٢. Diabetes mellitus is generator of oxidative stress, therefore the concentration of diabetes mellitus markers of oxidative stress damage correlates with diabetic complications.

٣. Indicate the predominant free radical in type ١ and ٢ diabetes mellitus indirectly by some free radical markers.

٤. Smoking influences on the enzymatic, and non-enzymatic antioxidants.

٥. Investigate the correlation between different variables of antioxidants and which is the high related changes with diabetes mellitus, and represented as important marker of diabetes mellitus.

٢.١. Place of work

This research was conducted in Al-Qadisiya Governorate, Al-Diwaniya General Hospital; Department of Biochemistry, College of Medicine, Al-Nahrain University, and Department of Chemistry, College of Sciences, Babylon University.

٢.٢. Subjects

The study samples included ٥٠ Patients suffering from type ٢ of diabetes (٢٨ males, and ٢٢ females) aged between ٣٥ and ٦٥ years; and ٥٠ patients with type ١ diabetes (٣٢ males and ١٨ females) aged between ١٣ and ٦٥ years, controlled with ٥٠ healthy individuals (٣٨ males and ١٢ females) aged between ١٥ and ٦٥ years. The practical work was expended ١٥ months period, beginning in October ٢٠٠٣ and ending in December ٢٠٠٤.

٢.٣. Samples

The study samples of patients were collected from Al-Diwaniya General Hospital between October ٢٠٠٣ to December ٢٠٠٤, and the blood was drawn from venous of fasting patients recently diagnosed with diabetes mellitus (type ١ and type ٢) and healthy subjects were used as control.

All tests were performed on serum, some of the blood was allowed to clot on crushed ice, and then centrifuged (٤٥٠ X g for ١٠ minutes) to be used serum samples immediately for detection of variable in this study, and others was stored at deep freezing (-٢٠°C) until using.

٢.٤. Determination of Serum Malondialdehyde (Burtis & Ashwood, ١٩٩٩)

Principle: -

The principle of the following method was based on the spectrophotometric measurement of the color, occurred during the reaction with thiobarbutric acid (T B A) with MDA, figure (٢-١).

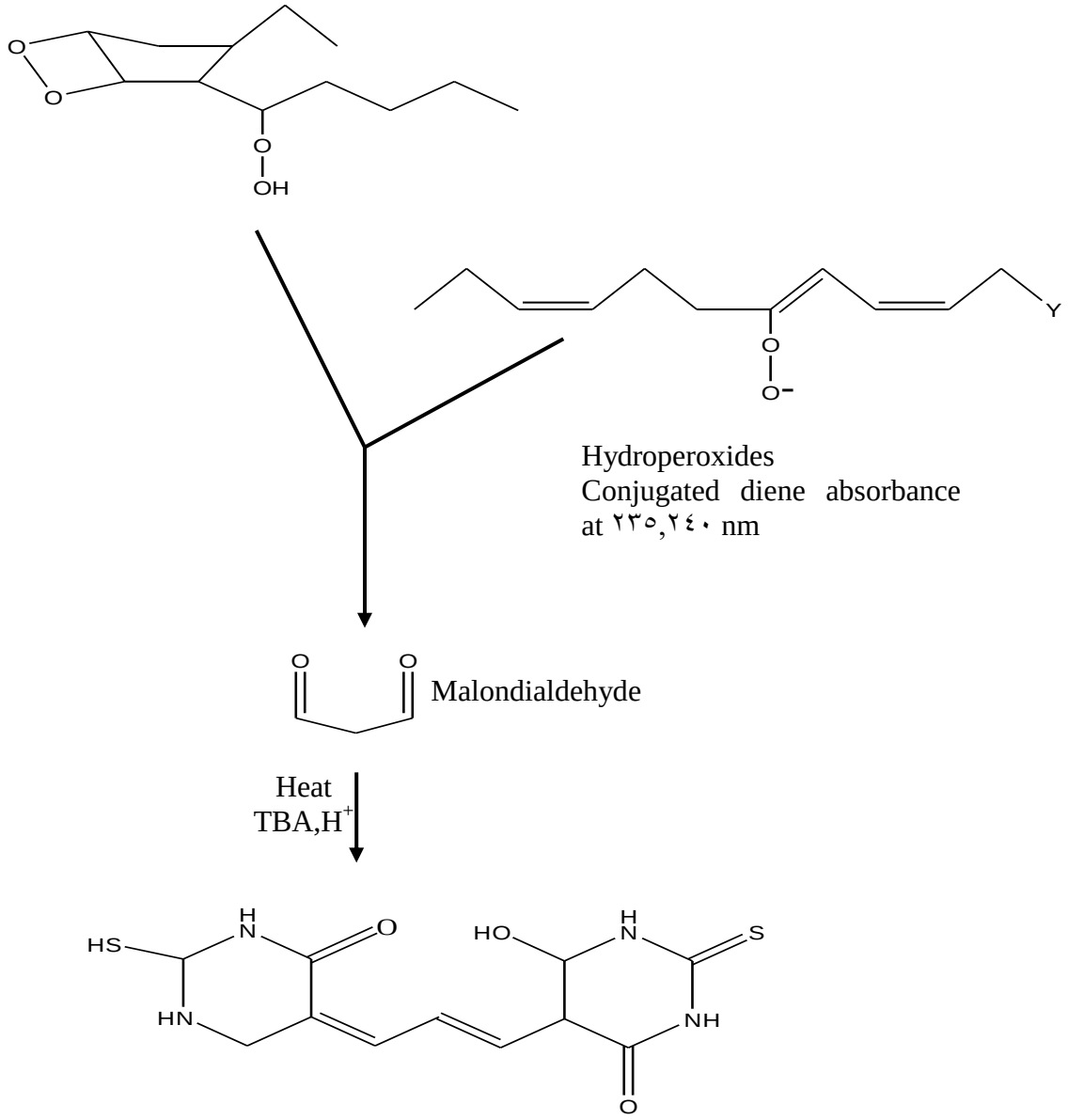


Figure (٢-١): Schematic diagram for assessment lipid peroxidation via determination the byproduct; malodialdehyde (Luenc J., ١٩٩٠)

Regents: -

١- ١٧.٥٪ Trichloroacetic acid (TCA).

٢- ٠.٦ % Thiobarbutric acid (TBA).

٣- ٧٠ % Trichloroacetic acid.

Procedure: -

١. Two set of tubes were prepared as follow:

Reagent	Sample	Blank
Serum	١٥٠ µl	-
TCA (١٧.٥٪)	١ ml	١ ml
TBA (٠.٦٪)	١ ml	١ ml
All tubes were mixed well by vortex, incubated it in boiling water bath for (١٥) minutes, then allowed to cool		
TCA (٧٠٪)	١ ml	١ ml

٢. The mixture was let to stand at room temperature for ٢٠ minutes and centrifuged at ٤٥٠X g for ١٥ minutes, and the supernatant was taken out to read the absorbence of sample at ٥٣٢ nm by using spectrophotometer instrument (type CE١٠١١, CECIL Company, France).

Calculation: -

$$\text{The conc. of MDA} = \frac{A_{\text{of sample at ٥٣٢ mn}}}{L \times \varepsilon} \times D$$

where:

L = light path (١cm)

ε = Extinction coefficient (١.٥٦ X ١٠^٥ $M^{-١}$ $cm^{-١}$)

D = Dilution factor

$$D = \frac{\text{١ ml (volume used in reference)}}{\text{٠.١٥ (volume of the sample)}} = ٦.٧$$

٢.٥. Determination of Serum Total Homocysteine (tHcy) (Pastore *et al.*, ١٩٩٨; Shanshel *et al.*, ٢٠٠٣)

Regents: -

Reagents were prepared for total and free thiol concentrations, with the exception of the ammonium format nitrate buffer. This solvent was prepared by the addition of ٣.٠ ml of formic acid (M.Wt.= ٤٦.٠٣ gm/mole) and ٤.٠ ml of nitric acid (M.Wt.= ٦٣.٠١gm/mole) to ١٩٠٠ ml of HPLC-grade water followed by the addition of ammonium hydroxide to bring the pH to ٣.٠٥ HPLC-grade water was used to adjust the final volume to ٢٠٠٠ ml.

Procedure: -

All test were performed on serum, which was stored at –٢٠°C until HPLC analysis. The separation was carried out on shimadzu LC-٦A system with two pumps and SCI-٦A system controller, ١٠٠ µl of sample was injected on to shimpack CIC-ODS column (٢٥٠ X ٤.٦ mm i.d) protected by ODS guard column (٥٠ X ٤.٦ mm i.d) . A linear gradient from ٠.٠١ M format-ammonium nitrate buffer (pH ٤.٣) and acetonitrile from (٠- ١٠.٥ % in ١١ minutes).

The Column elutes were monitored by Rf X ١٠A Fluorescent detector operating at an excitation wavelength of ٣٦٥ nm and an emission wavelength of ٤٧٥nm. The detector coupled to chromatopack CR-٨A data processor. The peak of homocysteine was separated and the recoveries of total homocysteine added to serum were ٩٩.٢٪.

Calibration curve for tHcy analyze was prepared by dissolving ٨.٧ mg of DL-Hcy in ١٠٠ ml of ٠.١ mol/L of HCl containing ١٠٠ mM/L of DTT. Calibration curve was established by serial diluting the mixed working solution .The linearity of the assays was studied in the ٠-٢٥٠ µmol/L range. The calibration curve shows a linear relationship between peak area and concentration as shown in Figure (٢-٢).

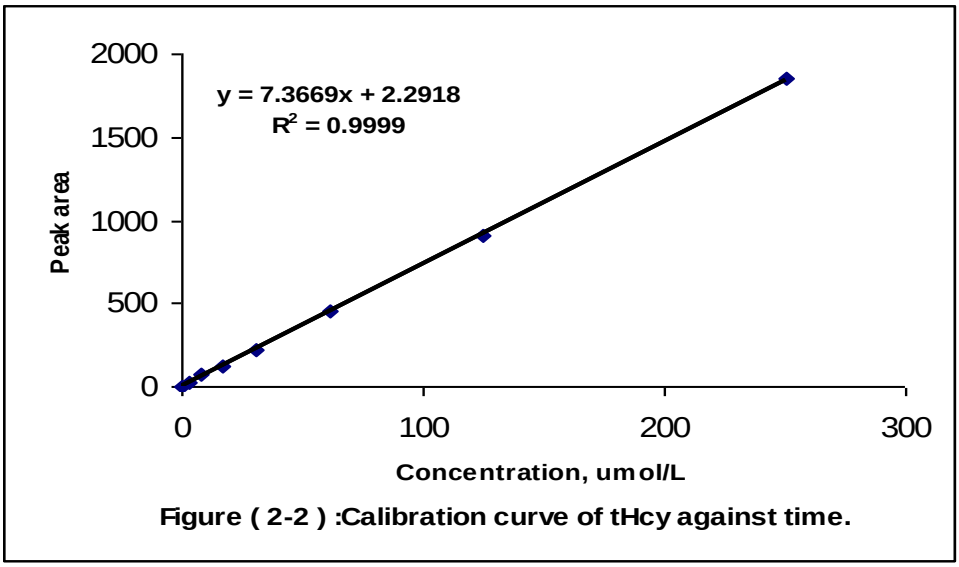


Figure (2-2) :Calibration curve of tHcy against time.

As well as figure (٢-٣) shows the chromatogram of homocysteine for samples and standard.

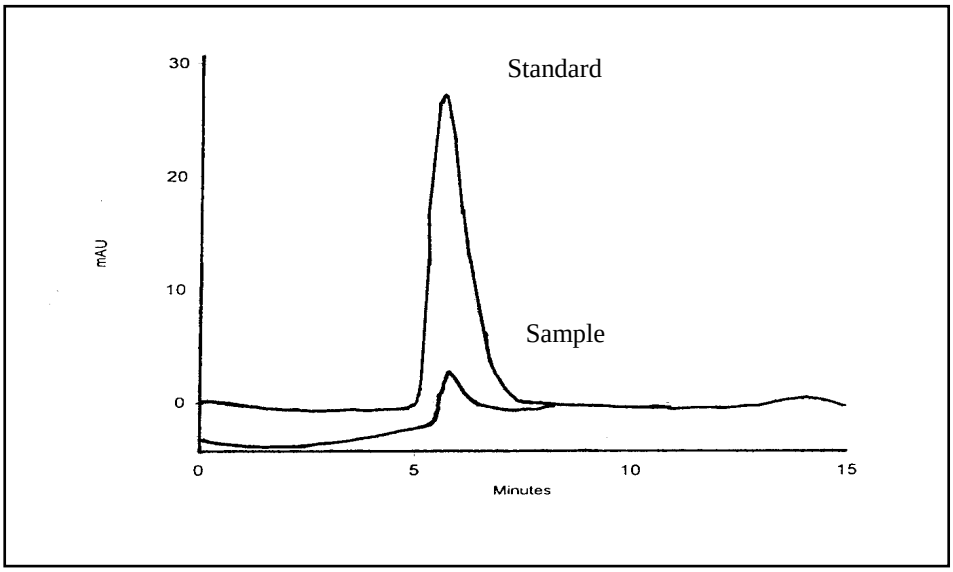


Figure (٢-٣): Chromatogram of homocysteine samples and standard.

Operating condition: -

Column: Shimpack CIC – ODS (٢٥٠ x ٤.٦ mm i.d.)

Protected by $ODSC_{١٨}$ (٥٠ x ٤.٦ mm i.d.)

Mobile Phase: Acetonitrite + Ammonium format nitrate buffer, pH ٤.٣.

٢.٦. Assay the Activity of Serum Superoxide Dismutase (SOD) (Winterboun *et al.*, ١٩٧٥)

Principle: -

Ec-SOD activity in serum was determined using a modified Photochemical nitroblue tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitor, figure (٢-٤).

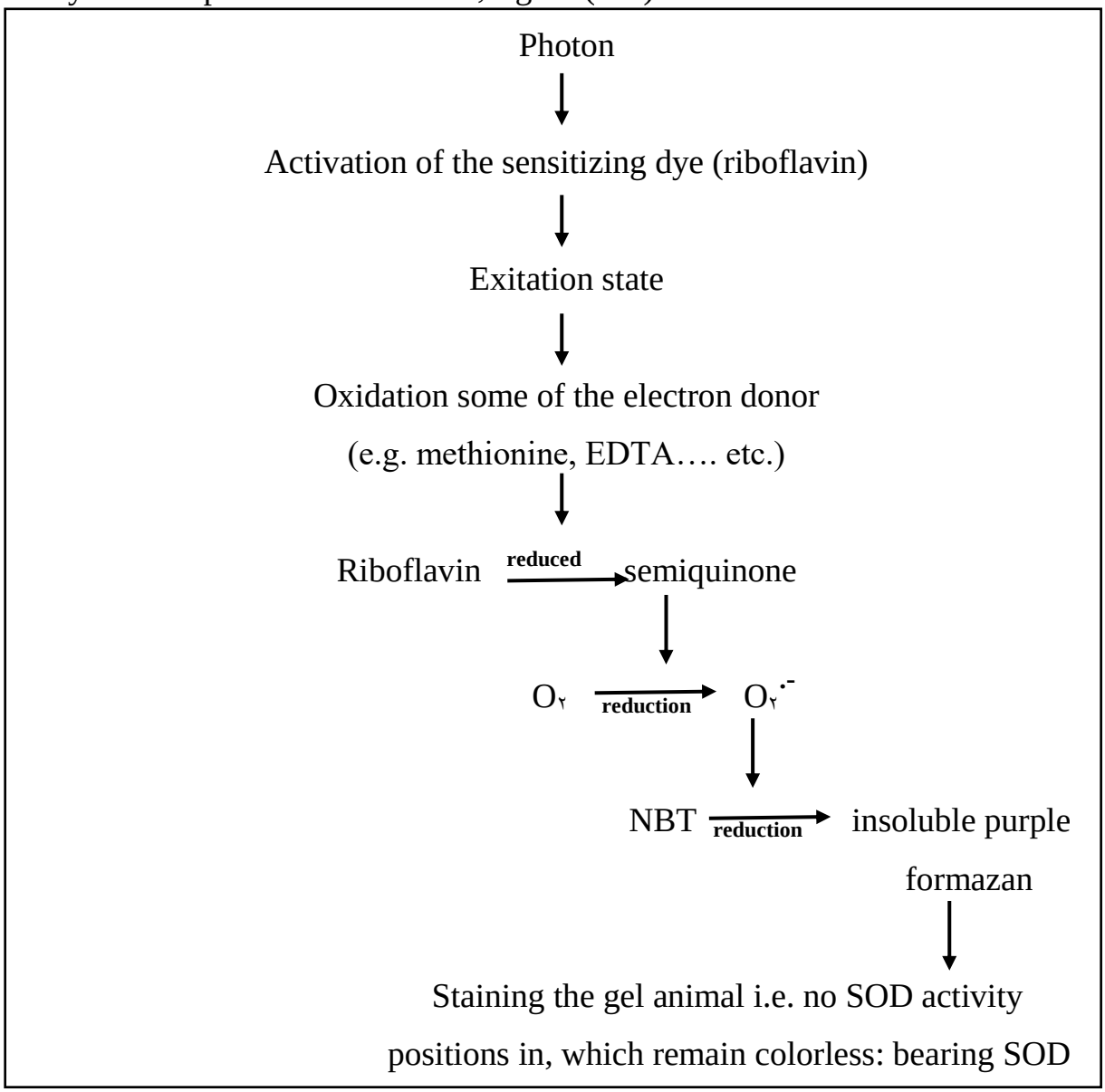


Figure (٢-٤): Schematic diagram showed the of detection SOD activity based upon nitroblue tetrazolium stain (Al-Zamely,٢٠٠١).

Regents: -

١. Working phosphate buffer: ٥٠ mM, pH ٧.٨ containing (٠.١mM) and triton x-١٠٠ (٠.٠٢٥٪). This solution was prepared as follow:

Solution A: Dipotassium hydrogen orthophosphate ($K_٢HPO_٤$) (٥٠ mM). This solution was prepared by dissolving ٨.٧٠٩ gm of $K_٢HPO_٤$ in about ٢٥٠ ml deionized water and then total volume is completed to ١ L.

Solution B: Potassium dihydrogen oryhophosphate ($KH_٢ PO_٤$) (٥٠ mM). This solution was prepared by dissolving ٦.٨٠٥ gm of $KH_٢PO_٤$ in about ٢٥٠ ml of deionized water, then total volume is completed to ١L.

Then ٨٠٠ ml of solution A was mixed with ٢٠٠ml of solution B, the pH was adjusted to ٧.٨. The working buffer solution was prepared by adding ٠.٠٣٧٥ gm EDTA, and ٠.٢٥ ml of triton x-١٠٠ in phosphate buffer (٥٠ mM), pH ٧.٨ and the total volume was completed up to ١L with phosphate buffer.

٢- Triton x-١٠٠ (١٪ w/v) in deionized water.

٣- Nitrobluetetrazolium -٢HCl (NBT-٢HC١ (١.٧٣mM) was prepared by dissolving ٠.٠١٤١gm of NBT -٢HCl in small amount of deionized water, and then total volume is completed to ١٠ ml.

٤- L-Methionine solution (٠.٢M) was prepared by dissolving ٠.٣ gm of L-methionine in small amount of deionized water, and then total volume is completed to ١٠ ml.

٥- Sodium cyanide solution (٢ mM) was prepared by dissolving ٠.٠١١gm of sodium cyanide in small amount of deionized water, and then total volume is completed to ١٠ ml.

٦- Riboflavin solution (١١٧ mM) was prepared by dissolving ٠.٠٠١١gm of riboflavin in small amount of deionized water, and then total volume is completed to ٢٥ ml.

٧- Reacting mixture solution was prepared by mixing ١١٧.٠ ml of reagent ١; ٠.٧٥ ml of reagent ٢; ١ml of reagent ٣, and ١.٢٥ ml of reagent ٤.

Procedure: -

١. Three set of tubs were prepared as follows:

Reagents	Sample	Control	Blank
Reacting mixture	٣ ml	٣ ml	٣ ml
Sodium cyanide	٠.٠٤ ml	٠.٠٤ ml	٠.٠٤ ml
Serum	٠.١٥ ml	-	-
Working buffer solution	٠.٥٢ ml	٠.٦٧ ml	٠.٦٧ ml
Mix by vortex in a glass test tube			
Riboflavine	٠.٠٣٨ ml	٠.٠٣٨ ml	٠.٠٣٨ ml

٢. All tubes were mixed, and the absorbance of sample and control was read immediately at ٥٦٠ nm by using spectrophotometer instrument (model ٢١, Milton Roy Company, France).

٣. All tubes, except blank test tube, were illuminated for ١٠ minutes at ٢٥٠°C by two fluorescent lamps (٢٠ watts each) incubated in an aluminum foil lined box (٧٥ X ٥٠ X ٢٠) cm^3 (Al-Zamely, ٢٠٠١).

٤. At the end of illumination time, the tubs were removed and immediately read absorbance at ٥٦٠ nm.

٥. The standard curve for the inhibition of SSOD enzyme activity was prepared by using different volumes of serum from normal person (٢٠, ٤٠, ٦٠, ٨٠, ١٠٠, ١٢٠, ١٦٠, ٢٠٠, ٢٤٠, ٢٦٠, ٣٠٠ µl), then repeated the above steps, and the absorbance before and after illumination was read at ٥٦٠ nm for each volume.

Calculation: -

١- Calculated the percentage of enzyme inhibition for different volume of serum from normal person by using the following equation

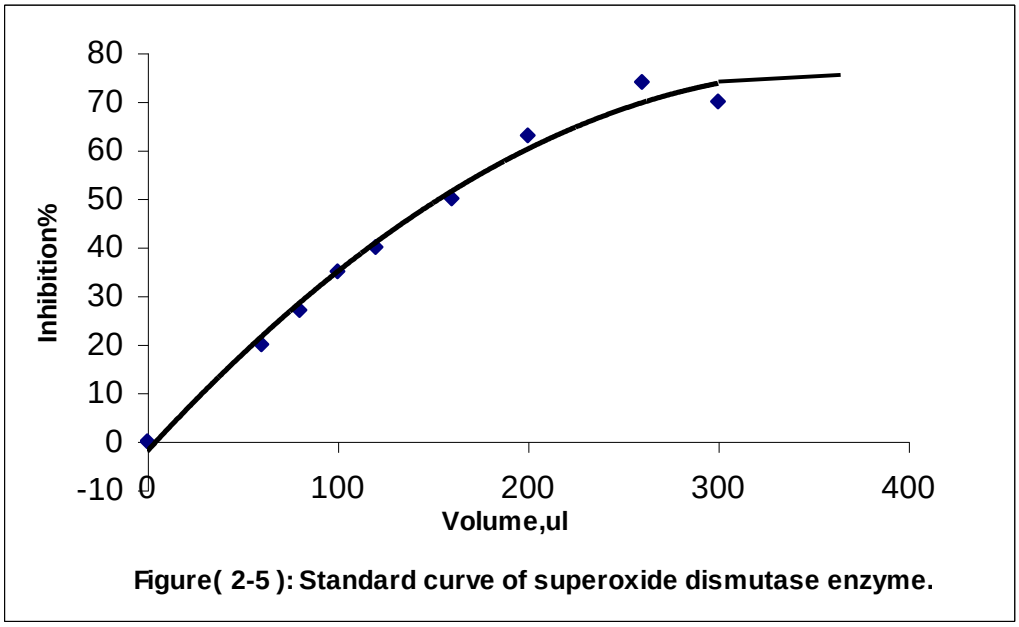
$$\text{Inhibition \%} = \frac{/C/ - /T/}{/C/} \times ١٠٠$$

Where:

/C/ = absorbance of control after illumination - absorbance of control before illumination

/T/ = absorbance of sample after illumination - absorbance of sample before illumination

٢. The standard curve of SOD enzyme was drawn between the percentage of enzyme inhibition and the different volume of serum from normal individual, figure (٢-٥).



Figure(2-5): Standard curve of superoxide dismutase enzyme.

٣. The half of the inhibition was calculated from the standard curve, which it is equal to the unit of SOD enzyme, so that

½ inhibition = ٣٧.٠٪

٤. Then the activity of the SOD enzyme can be calculated from the following equation:

$$\textbf{Activity of SOD enzyme (U/ml)} = \frac{\textbf{Inhibition \% of sample}}{\textbf{½ of the inhibition\% from the standard curve,٣٧٪}}$$

٢.٧. Assay the activity of serum Catalase (CAT) (Aebi, ١٩٧٤)

Principle: -

Catalase (CAT) activity was determined by the decrease in absorbance due to H_2O_2 consumption (ε = ٠.٠٤ mM^{-1} cm^{-1}) (Mueller *et al*., ١٩٩٧)

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Reagents: -

١. Phosphate buffer solution: ٥٠ mM, pH ٧.٠, this solution was prepared as follow:

A- Potassium dihydrogen orthophosphate (KH_2PO_4) (٥٠ mM). This solution was prepared by dissolving ٦.٨١ gm of KH_2PO_4 (١٣٦.٠٩ gm /mole) in small amount of distilled water, and then total volume is completed to ١L.

B- Disodium hydrogen orthophosphate (Na_2HPO_4) (٣٨.٧٧٢ mM). This solution was prepared by dissolving ٦.٩ gm of Na_2HPO_4 (١٤١.٩٦ gm/mole) in small amount of distilled water, and then total volume is completed to ١L.

Then ٣٩٠ ml of solution A was mixed with ٦١٠ ml of solution B, and the pH was adjusted to ٧.٠.

٢. Hydrogen peroxide (H_2O_2) (٣٠ mM).

Fresh solution of hydrogen peroxide was prepared by dilution ٠.٣٤ ml of ٣٠٪ H_2O_2 with phosphate buffer to ١٠٠ ml.

Procedure: -

١. ٥٠ µl of serum was diluted with ٥ ml of phosphate buffer solution (٥٠ mM), pH ٧.٠ immediately prior to assay.

٢. Two sets of tubes were prepared as follow:

Reagents	Sample	Blank
Phosphate buffer solution, pH ٧.٠	-	١ ml
Diluting serum	٢ ml	٢ ml

H_2O_2	١ ml	-

The reaction was started by adding hydrogen peroxide (freshly prepared), then all tubes were mixed immediately and the initial absorbance after ١٥ seconds (t_1) and the final absorbance after (٣٠) seconds (t_2) were read at ٢٤٠ nm by using spectrophotometer (model ٢١, Milton Roy Company, France).

Calculations: -

Enzyme activity in unit (U) was expressed as the rate constant of first reaction (K) is used according to the following equation:

$$K = \frac{V_t}{V_s} X \frac{٢.٣}{\Delta_t} X \log \frac{E_1}{E_2} X ٦٠$$

Where:

K: is rate constant
Δ_t: is ($t_2 - t_1$) and it is equal to ١٥ seconds.
E_1: is the initial absorbance at ١٥ seconds.
E_2: is the final absorbance at ٣٠ seconds.
V_t: is total assay volume.
V_s: is sample volume in the assay mixture.

٢.٨. Assay the activity of serum Glutathione S-Transferase (GST) (Habig *et al.*, ١٩٧٤)

Principle: -

The activity was determined using ١-chloro-٢,٤-dintrobenzene (CDNB) as substrate, figure (٢-٦).

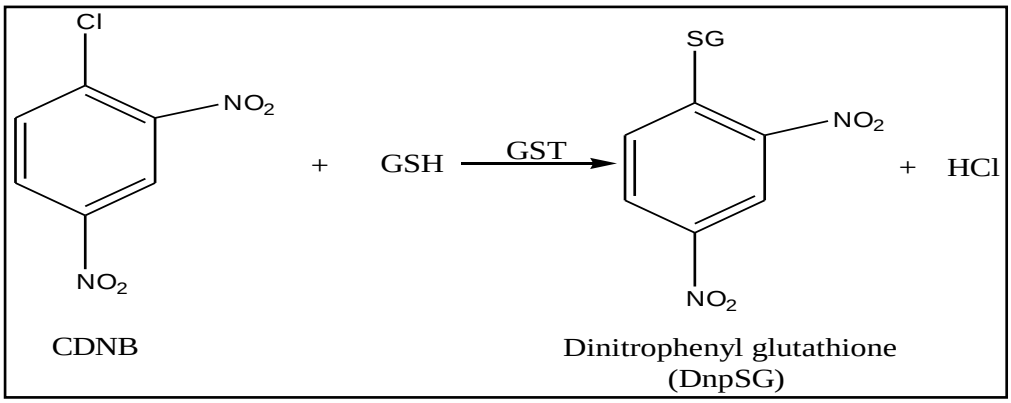


Figure (٢-٦): Reaction between ١-chloro-٢,٤-dinitrobenzene (CDNB) and glutathione in the presence of glutathione S-transferase (GST) enzyme, (Yildiz & Kurman, ٢٠٠٤).

Reagents: -

١. Glutathione solution (٢٩.٩٣ mM).

This solution was prepared by dissolving ٠.٠٩٢ gm of glutathione (٣٠٧.٣٣gm/mole) in small amount of distilled water, and then total volume is completed to ١٠ ml.

٢. ١-chloro-٢,٤-dinitrobenzene (CDNB) (٢٢.٤٦٣mM).

This solution was prepared by dissolving ٠.٤٥٥gm of CDNB (M.Wt=٢٠٢.٥٥gm/mole) in small amount of ethanol (٩٥٪), and then total volume is completed to ١٠٠ ml.

٣. Phosphate buffer solution, pH ٦.٢٥.

This solution was prepared by dissolving ٠.٤٣٥٥gm of dipotassium hydrogen orthophosphate and ٣.٠٦٠gm of potassium dihydrogen orthophosphate in small amount of distilled water, and then total volume is completed to ٢٥٠ ml. Then the pH was adjusted to ٦.٢٥.

Procedure: -

١. Two sets of tubs were prepared as follow:

Reagents	Sample	Blank
Phosphate buffer pH ٦.٢٥	٢.٧ ml	٢.٧ ml
Serum	١٠٠ µl	-
Distilled water	-	١٠٠ µl
CDNB solution	١٠٠ µl	١٠٠ µl
After ٣ minutes add GSH solution	١٠٠ µl	١٠٠ µl

٢. Then, all tubes were mixed and the absorbance after each ١minuts for ١٠ minutes was read at ٣٤٠ nm by using spectrophotometer (model ٢١, Milton Roy Company, France).

Calculation: -

Enzyme activity was expressed as (U/L) is calculated according to the following equation:

$$\text{Activity of GST (U/L)} = \frac{\Delta A/١٠ \times V_t \times ١٠٠٠}{\varepsilon \times V_s \times d}$$

Where:

ΔA: the difference between the first absorbance at ١^{st} minute and tenth absorbance at ١٠^{th} minute.

V_t: the total volume.

V_s: the sample volume

ε: extinction coefficient (٩.٦ $mM^{-١}$ $cm^{-١}$).

d: light path (١ cm).

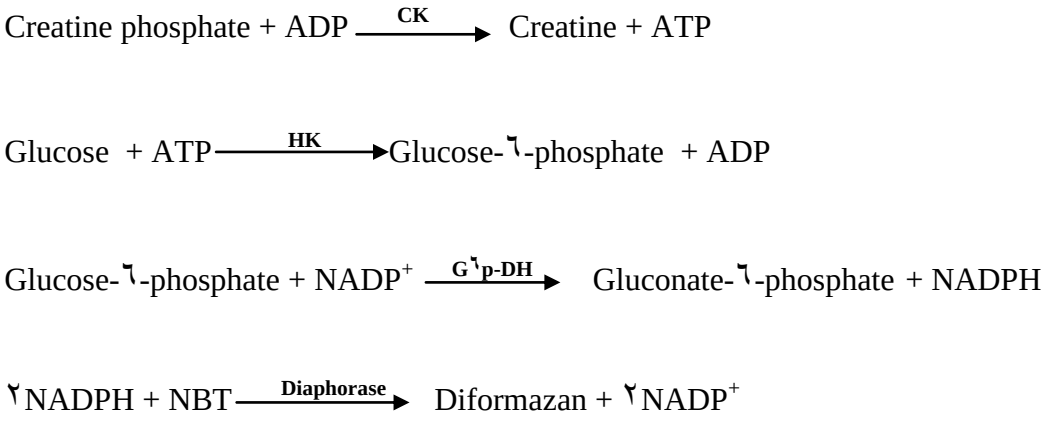
$$\text{Activity of GST (U/L)} = \frac{\Delta A/١٠ \times ٣ \times ١٠٠٠}{٩.٦ \times ٠.١ \times ١}$$

٢.٩. Assay the activity of serum Creatine Kinase (CK) by Using Commercially available Kit (Randox-U.K.)

Principle: -

Creatine Kinase (CK) utilizes creatine phosphate as substrate to act as the initial catalyst for series of reactions resulting in the formation of

NADPH as outlined in the coupled enzyme assay to CK activity and it is used to reduce nitro blue tetrazolium (NBT), in the presence of diaphorase, to give the blue, violet color of diformazan which has an absorption maximum around ٥٦٠ nm. The reaction is stopped by the addition of hydrochloric acid.



Reagents: -

١. Buffer /Glucose solution, pH ٦.٤

This solution was prepared from imidazaol buffer (٠.١mol/L), glucose (٢٠ mm/L), magnesium acetate (١٠ mm/L), EDTA (٢.٠mm/L), and NBT (٠.٧٣mm/L)

٢. Color Reagent

This solution was prepared from ADP (١.١mm/l), AMP (٤.٠mm/L), diadenosine (١٠.٦٣ mM/L), NADP (٠.٨mm/L), creatine phosphate (٢٥ mm/L), glutathione (١٢mm/L), hexokinase (HK)(٢٥٠٠U/L), glucose ٦-phosphate dehydrogenase(G٦P-DH)(١٥٠٠U/L), and diaphorase (٢٥٠٠U/L)

٣. Hydrochloric acid, (٠.١N).

٤. Standard of creatine kinase (١٨٨ U/L)

Procedure: -

١. Four sets of tubes were prepared as follow:

Reagents	Sample	Standard	Reagent blank	Sample blank
Color reagent solution	٠.٥ ml	٠.٥ ml	٠.٥ ml	٠.٥ ml
All tubes were incubated for (٣) minutes at ٣٧°C				
Serum	٠.٠٥ ml	-	-	-
Standard	-	٠.٠٥ ml	-	-
Distilled water	-	-	-	٠.٠٥ ml
All tubes were incubated for (١٠) minutes at ٣٧°C				
HCl ٠.١ N	٥ ml	٥ ml	٥ ml	٥ ml
Serum	-	-	-	٠.٠٥ ml

The absorbance of the sample (A_{sample}), standard ($A_{standard}$), and sample blank ($A_{sample\ blank}$) were measured against the reagent blank at ٥٦٠ nm by using spectrophotometer (type CE١٠١١, CECIL Company, France).

Calculation: -

$$\text{CK activity in sample} = \frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times \text{Conc. of standard}$$

Concentration of standard =١٨٨ U/L

٢.١٠. Determination of Serum Glutathione (Burtis & Ashwood, ١٩٩٩)

Principle: -

٥,٥-Dithiobis (٢-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at ٤١٢ nm and is directly proportional to the GSH concentration, figure (٢-٧).

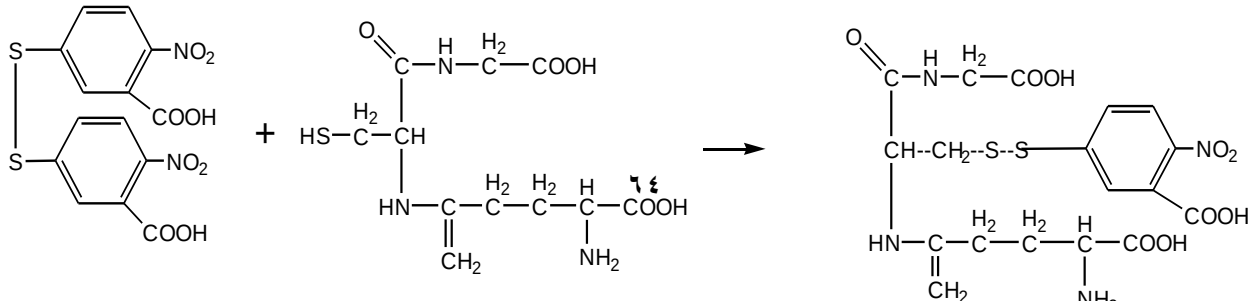


Figure (٢-٧): Reaction between glutathione (GSH) and ٥,٥-Dithiobis (٢-nitrobenzoic acid) (DTNB) (Sedlak & Lindsay, ١٩٦٨).

Reagents: -

١. ٤٪ ٥-sulfosalicylic acid.

٢. ٠.١mM Ellman's reagent was prepared from DTNB (٥-٥-dithiobis (٢-nitrobenzoic acid).; M.Wt = ٣٩٦.٣ gm/mole) in phosphate buffer pH ٨.

٣. Phosphate buffer pH ٨ (this solution was prepared by a mixture of ٠.٦ M KH_2PO_4, and ٠.٨M Na_2HPO_4).

٤. ٥mM Glutathione standard solution was prepared by dissolving ٠.٠١٥٣gm of glutathione (M.Wt.=٣٠٧.٣٣) in small amount of distilled water, then total volume is completed to ١٠ ml.

Procedure: -

١. Two sets of tubes were prepared as follow:

Reagent	Sample	Blank
Serum	١٥٠ µl	-
٤٪ ٥-sulfosalicylic acid	١٥٠ µl	١٥٠ µl
sample test tube was mixed, and centrifuged at ٤٥٠ X g at ٤°C for (٥) minutes, then:		
Supernatant	١٥٠ µl	-
Ellman's Reagent	٤.٥ ml	٤.٥ ml

All tubes were mixed, and the absorbance of sample was read at ٤١٢ nm

by using spectrophotometer instrument (type CE ١٠١١, CECIL Company, France).

٢. The calibration curve of glutathione was prepared from dilution the glutathione standard solution in distilled water to prepare the following concentration: ١, ٥, ١٥, ٢٠, ٣٠, ٤٠, ٥٠, ١٠٠, ٢٠٠, ٣٠٠, ٥٠٠ µM, then the above steps were repeated and the absorbance was read at ٤١٢nm for each concentration.

Calculation: -

The calibration curve of glutathione was drawn between absorbance and different concentration of glutathione, then concentration of serum glutathione of sample was calculated from it, figure (٢-٨).

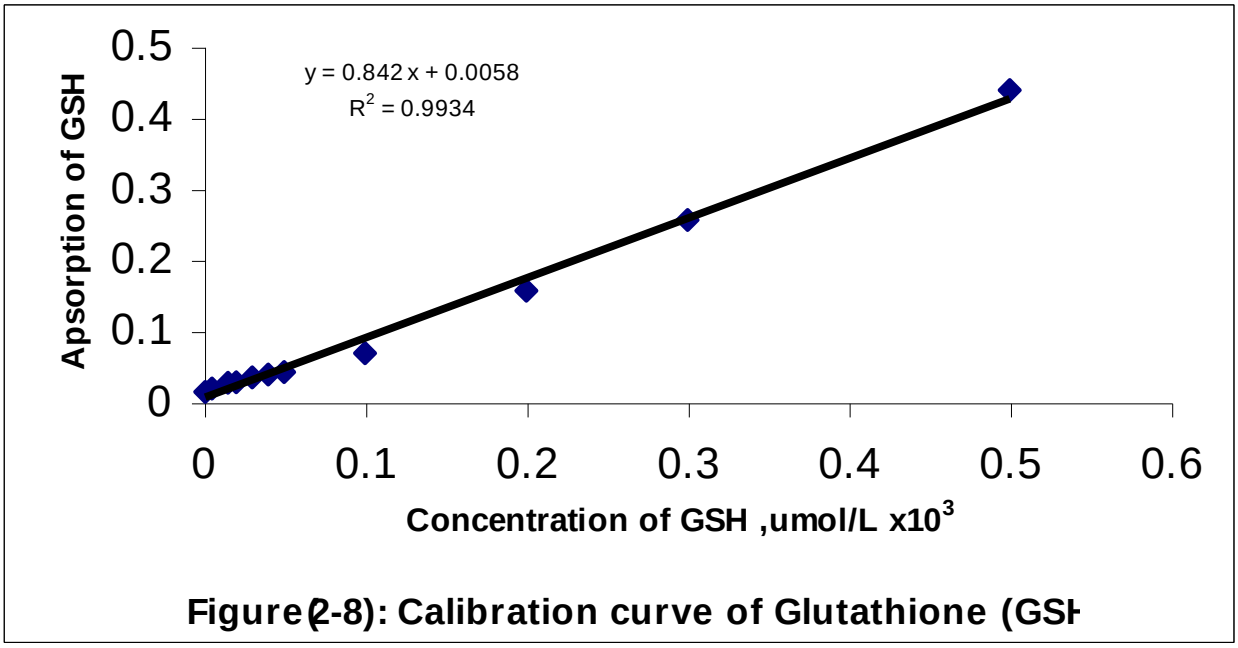


Figure (٢-٨): Calibration curve of Glutathione (GSH

There is another way to calculate the concentration of serum glutathione by using the following equation:

$$\text{The concentration of GSH} = \frac{A_{\text{of sample}}}{\varepsilon \times L}$$

Where:

ε = Extinction coefficient (١٣٦٠٠ M^{-1} cm^{-1})

L = Light path (cm).

٢.١١. Determination of Serum Antioxidant Vitamins

٢.١١.١. Determination of Serum Vitamin (E) by HPLC Technique (Vatassery *et al.*, ١٩٨٣ cited in Salman, ٢٠٠١)

Reagents: -

١. Antioxidant Solution:

This solution was prepared by dissolving ١٠ gm of ascorbic acid and ٠.١ gm of pyrogallol in small amount of ethanol and distilled water in proportional (١:٩), then total volume is completed to١٠٠ ml.

٢. Standard Solution of Vitamin E:

This solution was prepared by dissolving ٠.٠١ gm of tochopherol acetate in small amount of hexane then total volume is completed to ١٠٠ ml.

٣. The mobile phase contains ٩٩٪ of hexane and ١٪ of distilled water.

Procedure: -

١. ٢ ml of antioxidant solution was added to ١٠٠ µl of serum.

٢. The above solution was mixed well and dried by nitrogen gas.

٣. ١٠٠ µl of hexane was added, mixed well and centrifuged, then the supernatant was taken out.

٤. It was dried again by nitrogen gas, then ٠.٥ ml of chloroform and methanol mixture in proportional (١:٢) was added.

٥. The supernatant was injected onto silica column (٢٥٠ x ٤.٦ mm i.d.), flow rate ٢ ml / min.

٦. The column elutes were monitored by UV spectrophotometer at ٢٨٧ nm.

٧. The concentration of serum vitamin E was expressed as µmol / L.

٨. ٢ ml of standard solution of vitamin E (٠.٠١ gm/dl) was injected onto silica column flow rate ٢ml/min., then the column elutes were monitored by UV spectrophotometer at ٢٨٧ nm, figure (٢-٩).

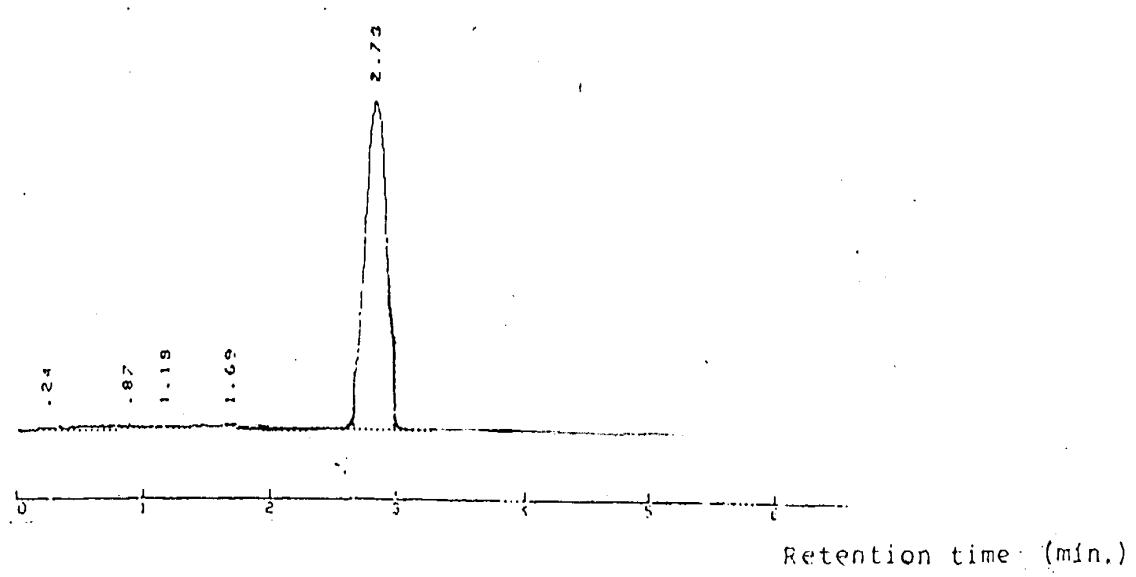


Figure (2-4):Analysis of standard fat-soluble vitamin E.
Operating condition:
Column: Silica (250 x 4.6 mm i.d.)
Mobil phase: (99:1 v/v) hexane: distilled water.
Flow rate: 2 ml / min. UV at 287nm.

٢.١١.٢. Determination of Serum Vitamin A by HPLC Technique (Chow & Omaye, ١٩٨٣ cited in Salman, ٢٠٠١)

Reagents: -

١. Standard Solution of Vitamin A

This solution was prepared by dissolving ٥٠ gm of retinyl-palmitate in small amount of hexane, then total volume is completed to ٥٠ ml.

٢. Methanol ٩٩٪ was represented the mobile phase.

Procedure: -

١. ٢٠٠ µl of serum was pipette into test tube, and a mixture of chloroform and methanol (١:٢) was added for test tube.

٢. The above mixture was mixed well and centrifuged at ١٠٠٠ X g for ١٠ minutes, then the chloroform was taken out.

٣. ١٠٠ µl of chloroform phase was mixed with ١٠٠ µl of standard solution of vitamin A, then injected on to $ODSC_{١٨}$ column, flow rate ١ ml / minutes.

٤. The column elutes were monitored by UV spectrophotometer at ٣٣٠ nm.

٥. The concentration of vitamin A was expressed as µmol/L.

٦. ٢٠٠ ml of standard solution of vitamin A (١٠٠ mg/dl) was injected onto $ODSC_{١٨}$ column, flow rate ١ ml/min., and the elutes were monitored by UV spectrophotometer at ٣٣٠ nm, figure (٢-١٠).

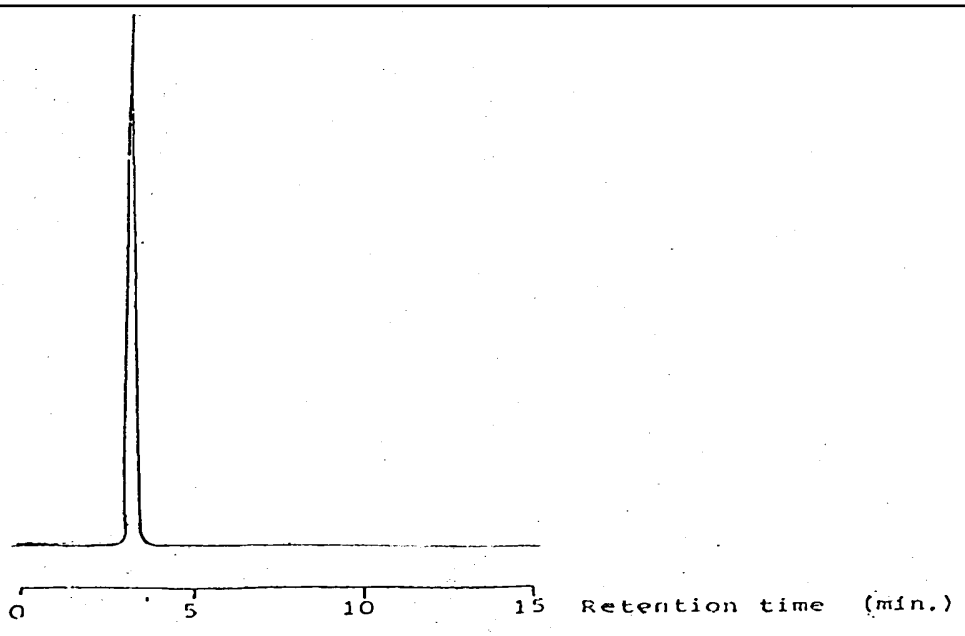


Figure (٢-١٠): Analysis of standard fat-soluble vitamin A
Operating condition:
Column: $ODSC_{18}$ (150 x 6.0 mm i.d.)
Mobil phase: (99% methanol)
Flow rate: 1 ml / min. UV at 330 nm.

٢.١١.٣. Determination of Serum Vitamin C by HPLC Technique (Rose & Nahrwold, ١٩٨١ cited in Salman, ٢٠٠١)

Reagents: -

١. Perchloric acid solution (٠.٣٥ mol/L).

This solution was prepared by taking out ٥ ml of concentrated perchloric acid, and the volume was completed to ١L with distilled water.

٢. Mobile Phase

This solution was prepared by dissolving ١.٠٨٨gm of sodium acetate trihydrate and ٠.٢gm of disodium EDTA in small amount of distilled water, then total volume is completed to ١L. The pH was adjusted to ٦.٠ by glacial acetic acid, then filtered with ٠.٤٥ µm milliporfilter.

٣. Standard Solution of Vitamin C:

This solution was prepared by dissolving ٥٠ mg of ascorbic acid in ٥٠ ml of perchloric acid and EDTA mixture (prepared by dissolving ١٠٠ mg of EDTA in small amount of distilled water, then total volume is completed to ١L) and addition of ٤.٣ ml of concentrated perchloric acid.

Procedure: -

١. ٠.٥ml of perchloric acid solution was added (٠.٣٦ mole/L) to ٠.٥ml of serum.

٢. The above solution was mixed well, then centrifuged at ١٩٠٠ X g for ١ minute.

٣. ٢٠ µl of supernatant was taken out, and injected on to $ODSC_{١٨}$ column, flow rate ١ml/minute.

٤. The column elutes were monitored by UV spectrophotometer at ٢٥٤ nm.

٥. The concentration of vitamin C was expressed as μmol/L.

٦. ٠.٥ ml of standard solution of vitamin C (١٠٠ mg/dl) was injected onto $ODSC_{١٨}$ column, flow rate ١ ml/min., and the column elutes were monitored by UV spectrophotometer at ٢٥٤ nm, figure (٢-١١)

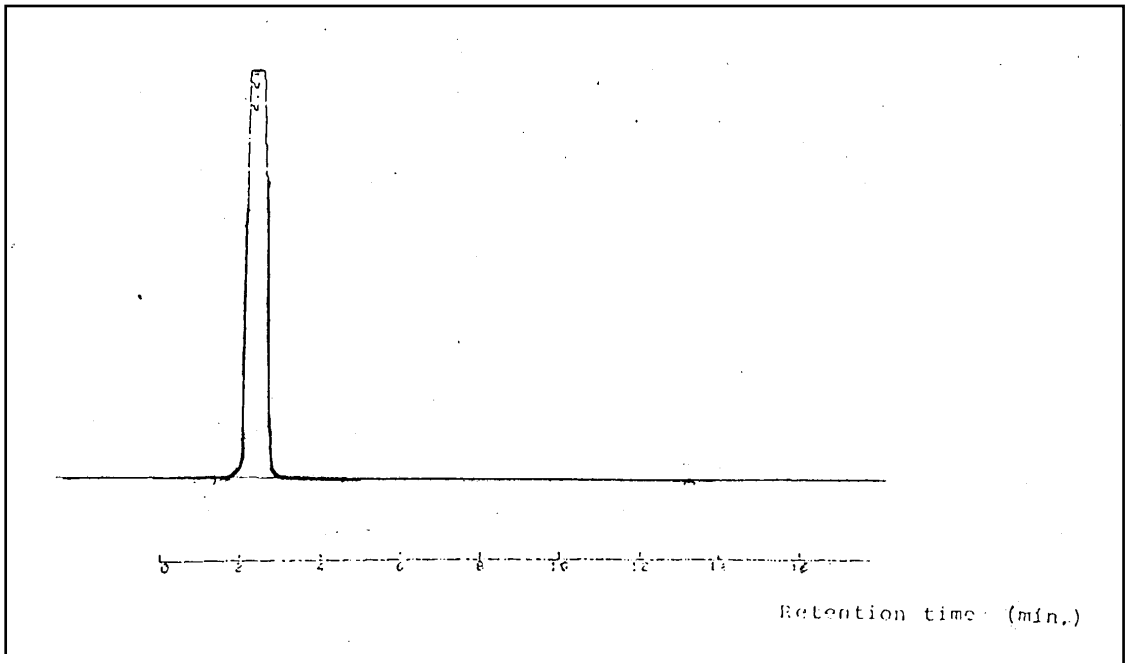


Figure (٢-١١): Analysis of standard water-soluble vitamin C.
Operating condition:
Column: $ODSC_{18}$ (150 x 6.0 mm i.d.)
Mobil phase: (Sodium acetate trihydrate + Sodium EDTA, PH 6.0)
Flow rate: 1 ml / min. UV at 254 nm.

٢.١٢. Determination of Serum Trace Element

٢.١٢.١. Determination of Serum Iron by Using Commercially Available Kit (Randox – UK)

Principle: -

Ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexed with the chromogen, a sensitive iron indicator, to reduce blue chromophore, which absorbs maximally at ٥٩٥nm.

Reagents: -

١. Chromogen, ٢٢.٢ mmol/L.

It was prepared from feren(٣-(٢-pyridyl)–٥,٦-bis(٥–sulfo–٢–furyl)–١,٢,٤–triazine, disodium salt hydrate)

٢. Reductant (ascorbic acid) ١.٣mol/L.

٣. Acetate buffer solution, ٠.٠٨٧mol/L, pH ٤.٦٥.

It was prepared from dimethyl sulphoxide and sodium azide.

٤. Standard was prepared from ferric sulfate ٣٥μmol/L.

Procedure: -

١. Three sets of tubs were prepared as follows:

Reagents	Reagent blank	Sample	Standard
Buffer solution, pH ٤.٦٥	٢.٠ml	٢.٠ml	٢.٠ml
Reductant, ١.٣ mol/L	٠.١ml	٠.١ml	٠.١ml
Double distilled water	٠.٥ml	-	-
Standard, ٣٥.٨ μmol/L	-	-	٠.٥ml
Serum	-	٠.٥ml	-
All tubes were mixed, and the initial absorbance of the sample and of the standard were read at ٥٩٥ nm against the reagent blank by using spectrophotometer (type CE ١٠١١, CECIL Company, France).			
Chromogen, ٢٢.٢ mm/L	٠.١ ml	٠.١ ml	٠.١ ml

٢. Then, all tubes were mixed, and incubated for ٥ minutes at ٢٠ – ٢٥°C. The final absorbance was read against reagent blank. Initial absorbance was subtracted from final absorbance to give ΔA for sample and standard.

Calculation: -

$$\Delta A = A_{٢} - A_{١}$$

$$\text{Concentration of Iron (μmol/L)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ X Conc. Of standard}$$

Where the concentration of standard is ٣٥.٥ μmol/L.

٢.١٢.٢. Determination of Serum Copper by Using Commercially Available Kit (Randox – UK)

Principle: -

Copper is an essential trace element in human nutrition and a component of many metalloenzymes. At pH ٤.٧ copper, which is bond to ceruloplasmin, is released by reducing agent, it then reacts with a specific color reagent, ٤(٣,٥-Dibromo-٢-pyridylazo)-N-Ethyl-N-(٣-Sulphopropyl) aniline, to form a stable, colored chelate.

Reagents:-

١. Acetate buffer solution (٠.٢ mm/L), pH ٤.٧.

٢. Chromogen (٠.٢ mm/L): It was prepared by dissolving ٤-(٣,٥-Dibromo-٢-pyridylazo)-N-Ethyl-N-(٣-sulphopropyl) aniline in acetate buffer, pH ٤.٧.

٣. Ascorbic acid (١.٣ mol/L). It was prepared by dissolving ٤.٨٨٨ gm of ascorbic acid with ٢٠ ml of acetate buffer, pH ٤.٧.

٤. Standard was prepared from copper sulfate (٣١.٥ µm/L)

Procedure: -

١. Three sets of tubes were prepared as follows:

Reagents	Reagent Blank	Standard	Sample
Double distilled water	٦٠ µl	-	-
Serum	-	-	٦٠ µl
Standard (٣١.٥ µm/L)	-	٦٠ µl	-
Ascorbic acid (١.٣ mol/L)	١٠٠٠ µl	١٠٠٠ µl	١٠٠٠ µl
All test tubes were mixed, and allowed to stand for ٦٠ seconds at ٣٧°C. The initial absorbance ($A_١$) of sample and standard were read at ٥٨٠nm against the reagent blank by using spectrophotometer (type CE ١٠١١, CECIL Company, France).			
Chromogen (٠.٢mm/L)	٢٥٠ µl	٢٥٠ µl	٢٥٠ µl

٢. Then, all tubes were mixed, incubated for ٥ minutes at ٣٧°C and the final absorbance ($A_٢$) was read against reagent blank.

Calculation: -

$$\text{Concentration of copper (μmol/L)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ X Conc. Of standard}$$

Where:

ΔA_{sample}: refers to $(A_{٢} - A_{١})$ of sample

$\Delta A_{standard}$: refers to $(A_{٢} - A_{١})$ of standard

Concentration of standard is ٣١.٥ μmol/L.

٢.١٢.٣. Determination of Serum Zinc by Atomic Absorption Technique (Burtis & Ashwood, ١٩٩٩)

Principle: -

Zinc is essential trace element, and deficiencies cause profound clinical consequences. The determination of sample or serum zinc concentration by atomic absorption spectrophotometer (AAS) is the simplest and analytically most reliable test for the routine assessment of zinc. In AAS, the element is merely dissociated from its chemical bonds and place in an unexcited or ground state (neutral atom). Thus, the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow band width corresponding to its own line spectrum.

Procedure: -

١. Different concentrations of working zinc standard solutions were prepared from stock zinc sulfate solution (٥ mg/L) to be used for drawing the standard curve of zinc.

٢. The standards, samples, and quality control specimens were diluted ٥-fold with distilled water. They were mixed well and taken for the

measurement of zinc concentration at ٢١٣.٩ nm by using atomic absorption spectrophotometer (Buck model scientific ٢١٠ UGP).

٢.١٢.٤. Determination of Serum Chromium by Atomic Absorption Technique (Burtis & Ashwood, ١٩٩٩)

Principle: -

Chromium ($Cr^{+٣}$) is an essential trace element for animals and humans. The commonly used instrumental technique for determination chromium in biological specimens is AAS.

Procedure: -

١. The working standards, samples were diluted and taken out ١٠ µl of diluted solutions, which were aspirated directly to the flame and measurement of chromium concentration at ٣٧٥.٩nm by using atomic absorption spectrophotometer (Buck Model Scientific ٢١٠ UGP).

٢. Different concentrations of working chromium standard solutions were prepared from stock chromium chloride solution (١mg/ml) to be used for drawing the standard curve of chromium.

٢.١٢.٥. Determination of Serum Selenium by Flameless Atomic Absorption Spectrophotometer (FAAS) (Burtis & Ashwood, ١٩٩٩)

Principle: -

Atomic absorption measurement suffers from physical, chemical, and spectral interference, which can significantly affect accuracy if they are not adequately overcome. In order to overcome these problems using FAAS technique to determination of selenium, with Zeeman effect background correction. However selenium deficiency can be determined by measuring it by using FAAS, Shimadzu AA-٦٨٠. Oven thermostat is put at

٢٠٠٠°C for ٣ seconds (atomization temperature) and ٢٢٠٠°C for ٣ seconds (cleaning temperature); the instrument is equipped with a heated graphite furnace model HGA ٢٢٠٠. Argon gas (٩٩.٩٩٩٪ purity) was used as purge gas at a flow rate of ٥٠ ml/min argon gas flow was maintained continuously through drying and charring steps and the gas stop mode was used during atomization stage. Lamp special for selenium with wavelength ١٩٦ nm.

Procedure: -

The standards and samples specimens were diluted ٢-fold with deionized water, and taken out ١٠ µl of diluted solutions, which were dispensed into graphite furnace. Standard solutions of selenium (٠.٤٠, ٠.٣٥, ٠.٣٠, ٠.٢٥, ٠.٢٠, ٠.١٥, and ٠.١٠) µmol/L were prepared from H_2SeO_3 and analyzed sequentially.

٢.١٣. Statistical Analysis

The data were analyzed by using student's T-test taking $P \leq$ ٠.٠٥ as the lowest limit of significant of difference and simple linear correlation between two quantitative parameters, and correlation considered significant at $P \leq$ ٠.٠٥.

٣.١. Sampling characteristics

One hundred patients with DM (٥٠ with type ١ DM, and ٥٠ with type ٢ DM), and ٥٠ healthy individuals served as control were enrolled in the present study. Table (٣-١) shows the demographic sampling characteristics of the study.

Table (٣-١): The demographic sampling characteristics of the study.

The characteristics	Type ١ No.=٥٠	Control No.=٢٥	Type ٢ No.=٥٠	Control No.=٢٥
Age (mean ± SE)	٣٢.٠٨± ٢.٣٢٧	٢٧.٠٤± ١.١٩٦	٥١.٢٤±١.٢٢٨	٤٧.٠٤± ١.٧٥٠
Duration of DM (mean ± SE)	١٠.٣٦٤± ١.٣٩٤	-	٥.٦١٦± ٠.٨٤٦	-
Family history (No.)	١٧	-	٣٨	-
Smokers (No.)	١١	١١	١٧	١٣
Non-smokers (No.)	٣٩	١٤	٣٣	١٢
Males (No.)	٣٢	٢١	٢٨	١٧
Females (No.)	١٨	٠٤	٢٢	٠٨

٣.٢. Age

The mean age ± SE of patients with type ١ DM was (٣٢.٠٨ ± ٢.٣٢٧ years *vs* ٢٧.٠٤ ± ١.١٩٦ years of control), while in type ٢ DM was (٥١.٢٤ ± ١.٢٢٨ years *vs* ٤٧.٠٤ ± ١.٧٥٠ years of control). There was no significant difference in age of control and diabetic patients with type ١ DM and type ٢ DM as shown in table (٣-١).

٣.٣. Duration of DM disease

The mean ± SE of duration of DM was (١٠.٣٦٤ ± ١.٣٩٤ years) in patients with type ١ DM, while in type ٢ DM was (٥.٦١٦ ± ٠.٨٤٦ years).

٣.٤. Assessment of reactive oxygen species

The assessment of generation of reactive oxygen species (ROD) in DM disease was achieved via determining the level of total homocysteine (tHcy), and MDA; the end product of lipid peroxidation as shown in table (٣-٢).

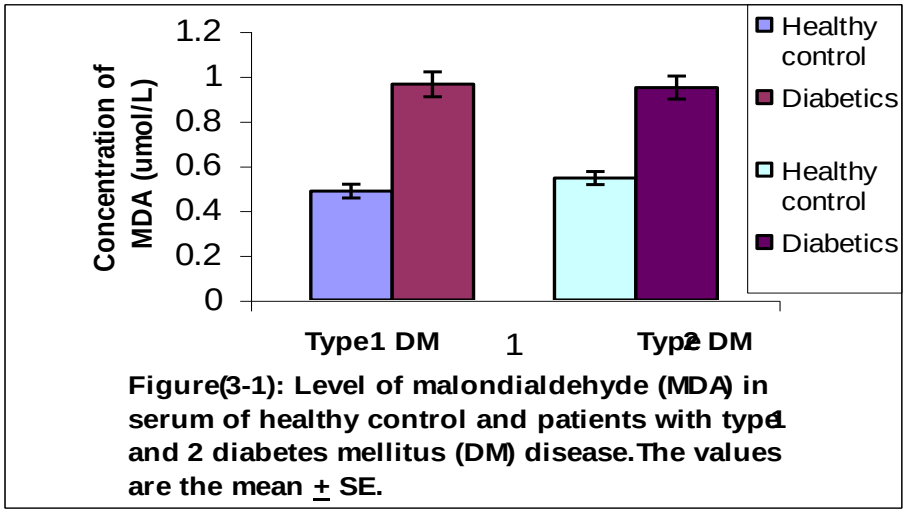
Table (٣-٢): Results of specific biochemical tests in serum of patients with type ١ or type ٢ DM and control .

			MDA (µmol/L)	tHcy (µmol/L)
Type ١ DM No.=٥٠	Mean±SE		٠.٣٩٧± ٠.٠٥٦	٢.٧٦٨± ٠.٣٩١
	SD		٠.٣٩٧	٢.٧٦٨
	Range		١.٦٠٥ – ٠.٣٨٦	٢٦.٢٥ – ١٤.٦٦
	٩٥% C.I.	Lower	٠.٨٦٩	١٩.٦٢٤
		Upper	١.٠٥٧	٢٠.٩٣٦
Control no.=٢٥	mean±SE		٠.٤٨٦ ± ٠.٠٣١	١٠.٧٤١± ٠.١٩٧
P-Value			S	S
Type ٢ DM No.= ٥٠	mean±SE		٠.٩٤٨±٠.٣٦٣	٢١.٩٧± ٠.٣٩٥
	SD		٠.٠٥١	٢.٧٩٩
	Range		١.٨١ – ٠.٣٨٣	٣٠.١١ – ١٨.١
	٩٥%C.I.	Lower	٠.٨٦٣	٢١.٣٠٧
		Upper	١.٠٣٣	٢٢.٦٣٣
Control No.=٢٥	mean±SE		٠.٥٤٥±٠.٠٢٩	١٠.٦٣٣±٠.١٨١
P-Value			S	S

٣.٤.١. Lipid Peroxidation

Dien Conjugation (DC) and thiobarturic acid reactive species (TBARS) are widely used as indicators of lipid peroxidation. DC is a measure of early events of lipid peroxidation reactions whereas TBARS measure end products of lipid peroxidation; MDA, (Vasankari *et al*., ١٩٩٥).

In this work, there was significant difference in malondialdehyde (MDA) levels between controls and diabetic patients (with type ١, and type ٢ DM) P $\leq$ ٠.٠٥, but there was no significant difference between type ١ DM and type ٢ DM. Therefore serum MDA levels in patients with type ١, and type ٢ DM are higher than control by ١.٩٨١ and ١.٧٣٩ times of control levels respectively, figure (٣-١), table (٣-٢).



Figure(3-1): Level of malondialdehyde (MDA) in serum of healthy control and patients with type1 and 2 diabetes mellitus (DM) disease. The values are the mean ± SE.

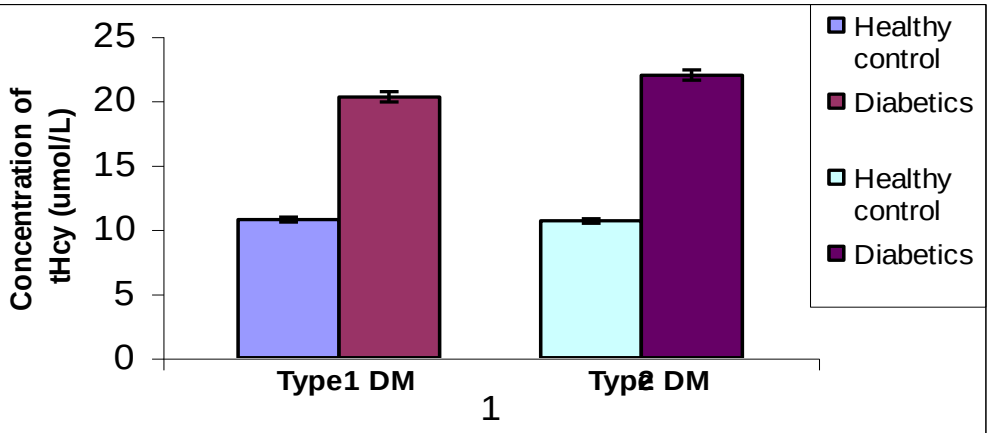
Elevated levels of lipid peroxidation products in serum of diabetic subjects and rats have been shown in several studies (Dominguez *et al*., ١٩٩٨; Al-Mashhadani, ٢٠٠٠; Salman, ٢٠٠١; Marra *et al*., ٢٠٠٢; Hadwan, ٢٠٠٥). The results of this study indicated that serum MDA levels are elevated in diabetic patients. Higher levels of MDA is associated with reduced of antioxidant activity and increased oxidative stress (Marangon *et al*., ١٩٩٨; Al-Zamely, ٢٠٠٠; Al-Mashhadani, ٢٠٠٠; Sahin *et al*., ٢٠٠١). Low density lipoprotein particles has ٢٢٠٠ molecules of free fatty acids, half of which is PUFA which is a highly susceptible substrate for free radical reaction to form ashort-chain aldehyde such as malondialdehyde, therefore elevated level of MDA might increase susceptibility of diabetic patients to premature atherosclerosis, which may be due in part to increased oxidizability of LDL.

٣.٤.٢. Total Homocysteine (tHcy) Level

In plasma, about ٨٠٪ of homocysteine is bound to proteins (especially albumin), the remainder being present in a free form, either as a disulphide with itself or cysteine, or as reduced homocysteine itself. Usually the sum of these fractions is measured in the laboratory and called serum tHcy concentration.

Hyperhomocysteinemia (elevated level of tHcy) plays an extremely important role for additional oxidative-redox stress regarding the accelerated cardiovascular complications in diabetic patients (Fodinger *et al*., ١٩٩٧). There is no obvious direct biochemical link between methionine-homocysteine metabolism and glucose/carbohydrate metabolism other than insulin induced protein synthesis, which may decrease serum methionine level (Zinneman *et al*, ١٩٦٦). Therefore, elevated level of tHcy may be associated with insulin deficiency that leads to enhanced homocysteine catabolism in the liver, a phenomenon that was reversible upon insulin administration (Jacobs *et al*, ١٩٩٨). As well as elevated level of tHcy in diabetic patients may be associated with low folate levels or cobalamin concentration or renal dysfunction as one of diabetic complications probably also contributes to the higher homocysteine levels (VanGuldener *et al*., ٢٠٠١). Presence of hyperhomocysteinemia (HHcy) may be a predictor for the development of DM (Hayden & Tyagi, ٢٠٠٤). Therefore, there is an interrelationship between Hhcy and DM and/or it its complications (Smulders *et al*., ١٩٩٩).

As shown in table (٣-٢), the mean ± SE of serum tHcy levels in patients with type ١ DM was (٢٠.٢٨ ± ٠.٣٩١ µmol/L *vs* ١٠.٧٤١ ± ٠.١٩٧ µmol/L of control), while in patients with type ٢ DM was (٢١.٩٧٠ ± ٠.٣٩٥ µmol/L *vs* ١٠.٦٣٣ ± ٠.٩٠٥µmol/L of control). There was significant elevation in serum tHcy levels of diabetic patients (with type ١ and type ٢ DM) by about ٥٠٪ from control, $P \leq ٠.٠٥$, figure (٣-٢).



Figure(3-2): Level of total homocystiene (tHcy) in serum of healthy control and patients with type 1 and 2 diabetes mellitus (DM) disease.The values are the mean ± SE.

Several studies pointed out elevated levels of serum tHcy in diabetic patients and suggested that homocysteine (Hcy) shows stronger relationship with cardiovascular complications and death in patients with diabetes than in non-diabetes.

١. The mortality risk associated with HHcy is about ٢ times stronger in diabetics than in non-diabetics (Hoogeveen *et al*., ٢٠٠٠).

٢. The association between retinopathy and HHcy is much stronger in patients with diabetes compared to subjects without diabetes (Hoogeveen *et al*., ٢٠٠٠).

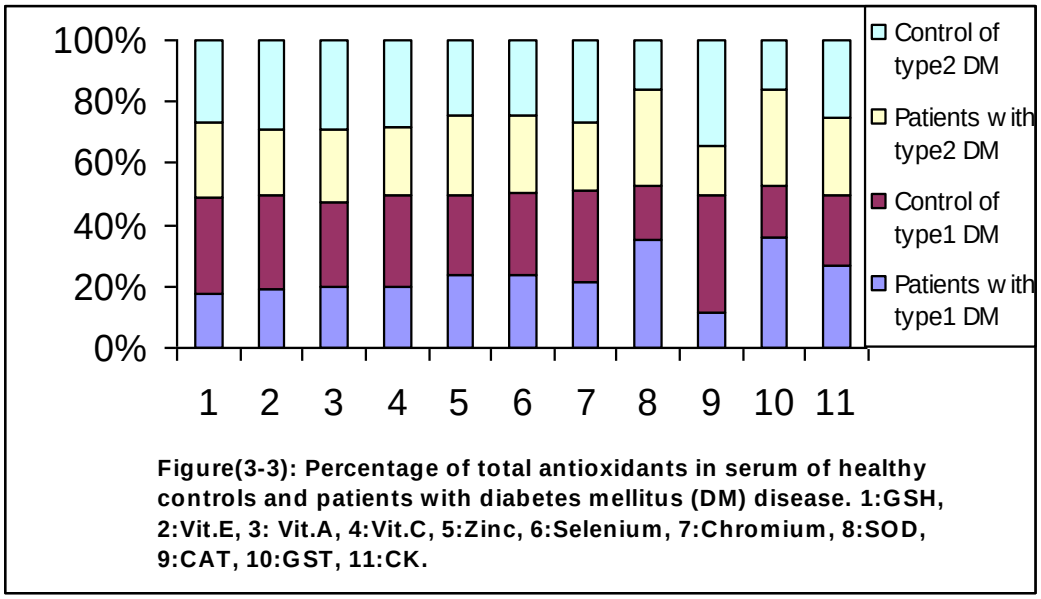
٣. Despite similar plasma Hcy levels, a strong correlation between the extent of coronary abnormalities and plasma Hcy level is found in diabetics, while this relationship is not significant in non-diabetics. (Okada *et al*., ١٩٩٩).

٣.٥. Assessment of Scavenger System

The results of this study indicate the percentage (%) of antioxidant that assessed in this study table(٣-٣),(figure ٣-٣).

Table (٣-٣): Percentage of antioxidants that assessment in this study for diabetic patients (with type ١ or ٢ DM), and control.

Antioxidants	%			
	Type ١ DM	Control	Type ٢ DM	Control
SOD	٣١.٥٤	٢٠.٥٥	٢٦.٩٥	٢٠.٩٦
CAT	٩.٢٥	٣٩.٢٨	١٢.٢٢	٣٩.٢٥
CK	٢٢.٦٠	٢٥.٧٤	٢١.٢٥	٣٠.٤١
GST	٣٢.٠٠	٢٠.٢١	٢٧.١٥	٢٠.٦٤
GSH	١٤.٩٣	٣٣.٣٠	١٩.٩٤	٣١.٨٣
Vitamin E	١٥.٩١	٣٢.٩٩	١٦.٦٨	٣٤.٤٢
Vitamin A	١٦.٧٧	٢٩.٩٤	١٩.١١	٣٤.١٨
Vitamin C	١٦.٧٥	٣١.٧٠	١٧.٦٧	٣٣.٨٨
Zinc	١٩.٧٩	٢٩.٢٥	٢٠.٩٨	٢٩.٩٨
Chromium	١٧.٧٦	٣٢.٣٧	١٨.٠٠	٣١.٨٧
Selenium	٢٠.١٦	٢٩.٦٧	٢٠.٥٦	٢٩.٦١



Figure(3-3): Percentage of total antioxidants in serum of healthy controls and patients with diabetes mellitus (DM) disease. 1:GSH, 2:Vit.E, 3: Vit.A, 4:Vit.C, 5:Zinc, 6:Selenium, 7:Chromium, 8:SOD, 9:CAT, 10:GST, 11:CK.

The results obtained in the present study confirmed tha diabetic patients were presented with depletion of scavengers in a sequence compatible with those reported in any disease related to the oxidative stress syndrome in humans. Figure (٣-٤) demonstrates that antioxidants are depleted from serum at different rates upon exposure to oxidative stress in both type ١ and ٢ DM, with catalase (CAT) activity being depleted first, followed by glutathione (GSH), then α-tocopherol, ascorbate, chromium, vitamin A, zinc, selenium, and lastly CK activity.

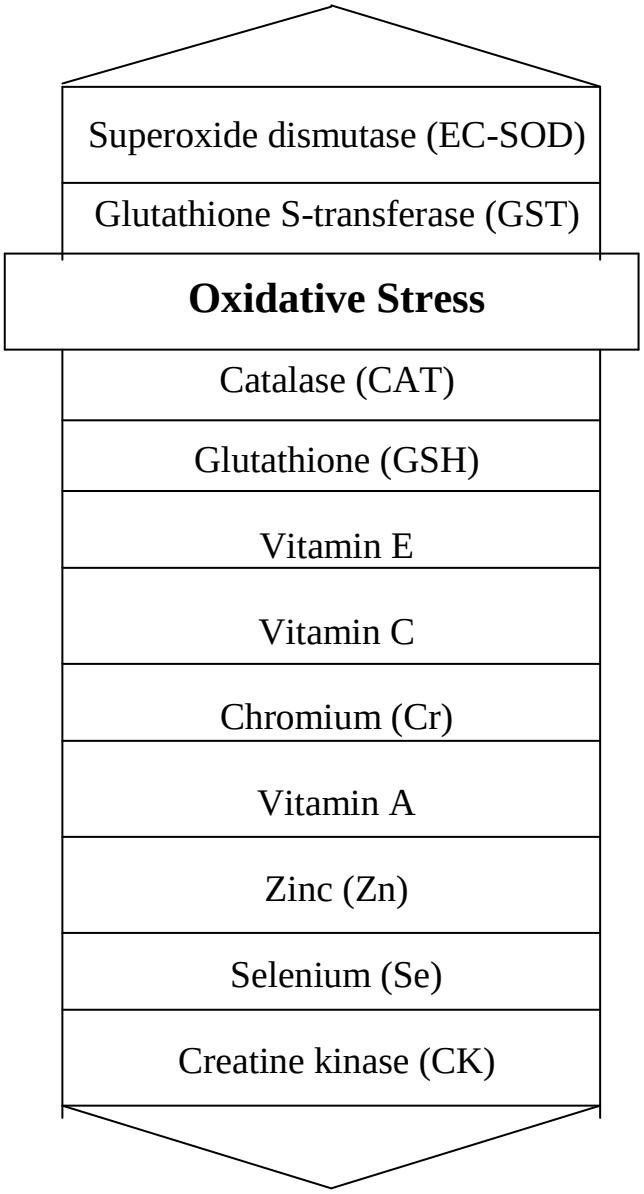


Figure (٣-٤): Depleted of serum antioxidants at different rat upon exposure to oxidative stress in patients with type ١ and ٢ DM.

٣.٥.١. Enzymatic Antioxidants

Table (٣-٤) shows the levels of some antioxidant enzyme.

Table (٣-٤): Levels of several enzymatic antioxidants in serum of patients with type ١ or type ٢ DM and control.

			SOD (U/ml)	CAT (K/ml)	CK (U/L)	GST (U/L)
Type ١ DM No.=٥٠	Mean±SE		١.٥٥٣ ± ٠.٠٣٩	٠.٣٨٣± ٠.٠١٠	١٦٨.٥٦±٣.٨٩٧	٤.٧٣١±٠.١٩٩
	SD		٠.٢٧٦	٠.٠٧٤	٢٧.٥٦٢	١.٤١٣
	Range		٢.٨٧٥– ٠.٥٧٩	٠.٥٩٥ – ٠.٠٨٤	٢٥٩ - ٧٥	٧.٥٩ – ٢.٢
	%٩٥C.I.	Lower	١.٤٨٧	٠.٣٦٦	١٦٢.٠٢٩	٣.٠٥٦
		Upper	١.٦١٨	٠.٤٠٠	١٧٥.٠٩٠	٥.٠٦٤
Control No.=٢	Mean±SE		١.٠١٢±٠.٠٥٤	١.٦٢٦±٠.٠٦٢	١٩٢±٥.٩٥٥	٢.٩٨٨± ٠.٠٥١
P-Value			S	S	S	S
Type ٢ DM No.= ٥٠	Mean±SE		١.٣٢٧±٠.٠٢٧	٠.٥٠٦±٠.٠١٨	١٥٨.٥٢±٣.٠٢٦	٤.٠١٤±٠.١٩٧
	SD		٠.١٩٧	٠.١٣٣	٢١.٤٠٢	١.٣٩٨
	Range		٢.٥٢٦ – ٠.١٣٧	٠.٧٧٦ – ٠.٣٣٢	١٨٤ - ٨٦	٧.٦٩١ – ٢.٣٢
	%٩٥C.I.	Lower	١.٢٨١	٠.٤٧٥	١٥٣.٤٤٨	٣.٦٨٣
		Upper	١.٣٧٣	٠.٥٣٧	١٦٣.٥٩٢	٤.٣٤٥
Control No.=٢٥	Mean±SE		١.٠٣٢±٠.٠٣٠	١.٦٢٥±٠.٠٥٩	٢٢٦.٨±٥.٦٦٢	٣.٠٥٣±٠.٠٣٣
P-Value			S	S	S	S

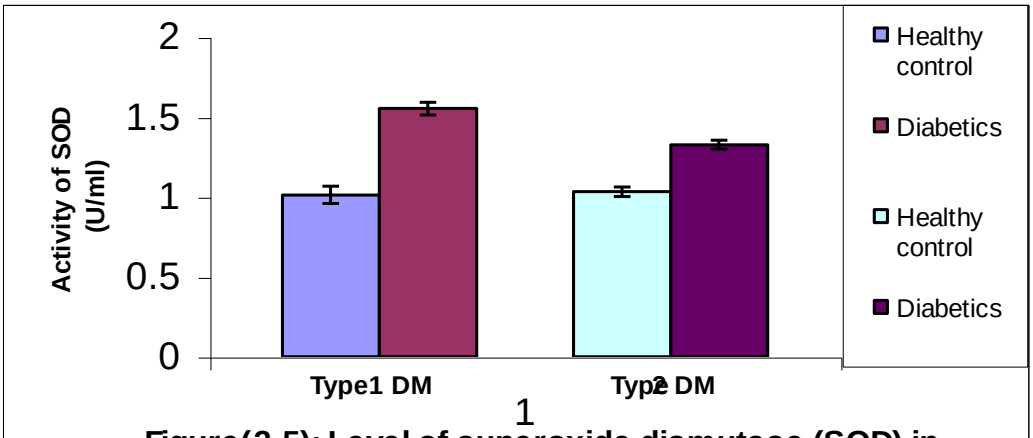
٣.٥.١.١. Extracellular Souperoxide Dismutase (Ec-SOD) Activity.

The primary natural defense against free radicals is to prevent their formation by various enzymes, especially SOD, which is regulating superoxide anion ($O_٢^{\bullet -}$) levels in catalyzing the dismutation of $O_٢^{\bullet -}$ to $H_٢O_٢$ (Frank *et al.*, ٢٠٠٠). The activity of Cu/Zn-SOD increases in many disease

causing oxidative stress (Knight, ١٩٩٩). Therefore, several studies pointed out high levels of Ec-Cu/Zn-SOD activity in diabetic patients (with type ١

and type ٢ DM) (MacRury *et al.*, ١٩٩٣; Adochi *et al.*, ١٩٩٦; Dominguez *et al.*, ١٩٩٨; Hristosozova *et al.*, ٢٠٠٠; Salman, ٢٠٠١).

The activity of Ec-Cu/Zn-SOD is measured by the degree of inhibition of reaction between nitrobluetetrazolume and superoxide anion ($O_2^{\bullet -}$) that is generating during illumination of riboflavin in the presence of electron donor substance (Winterboun *et al.*, ١٩٧٥). However, as seen in the present study, there is a significant increase in serum Cu/Zn-SOD activity levels in patients with type ١ and type ٢ DM, $P \leq$ ٠.٠٥, figure (٣-٥). Table (٣-٤) shows the mean ± SE of serum Cu/Zn-SOD activity in patients with type ١ DM was (١.٥٥٣ ± ٠.٠٣٩ U/ml *vs* ١.٠١٢ ± ٠.٠٥٤ U/ml of control), while type ٢ DM was (١.٣٢٧ ± ٠.٠٢٧ U/ml *vs* ١.٠٣٢ ± ٠.٠٣٠ U/ml of control).



Figure(3-5): Level of superoxide dismutase (SOD) in serum of healthy controls and patients with type1 and 2 diabetes mellitus (DM) disease.The values are the mean ± SE.

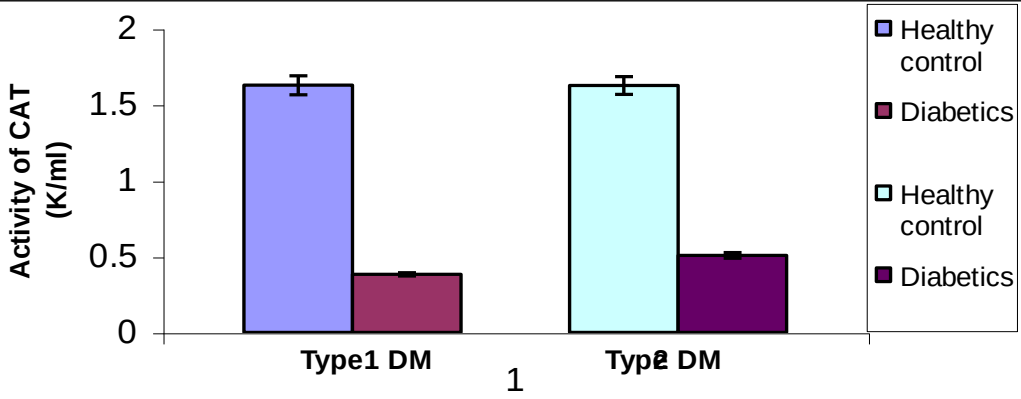
The results of this study reports that high levels of serum Cu/Zn-SOD activity in diabetic patients are indicating to the presence of high level of superoxide anion ($O_2^{\bullet -}$), which represents an indicator to high degree oxidative stress in those patients, therefore increased amounts of substrate of $O_2^{\bullet -}$ this results in a stimulant to increase synthesis of serum Cu/Zn-SOD

in diabetic patients and may protect against free radicals damage (Hristosozova *et al*., ٢٠٠٠).

٣.٥.١.٢. Catalase (CAT) Activity

CAT is a hydrogen peroxide decomposion catalyzing enzyme mainly localized to peroxisomes or microperoxisomes (Atalay & Laaksonen, ٢٠٠٢). Therefore, measurement of CAT could be a valuable biomarker for the generation of H_2O_2 and the degree of oxidative stress (Mueller *et al*., ١٩٩٧; Banerjee *et al*., ٢٠٠٢).

In this study, the results reported that measurement of serum CAT activity in diabetic patients represents the powerful indicator to evaluation of the oxidative stress. However, there was a significant reduction in serum CAT activity levels of diabetic patients (with type ١ and type ٢ DM) than controls, $P \leq$ ٠.٠٥, figure (٣-٦), therefore the mean ± SE of serum CAT activity levels in patients with type ١ DM was (٠.٣٨٣ ± ٠.٠١٠ K/ml *vs* ١.٦٢٦ ± ٠.٠٦٢ K/ml of control), while in patients with type ٢ DM was (٠.٥٠٦ ± ٠.٠١٨ K/ml *vs* ١.٦٢٥ ± ٠.٠٥٩ K/ml of control), table (٣-٤), these found results were in agreement with a great number of previous publications (Ou & Wolff, ١٩٩٤; Szaleczky *et al*., ١٩٩٩; Hristosozova *et al*., ٢٠٠٠; Atalay & Laaksonen, ٢٠٠٠).



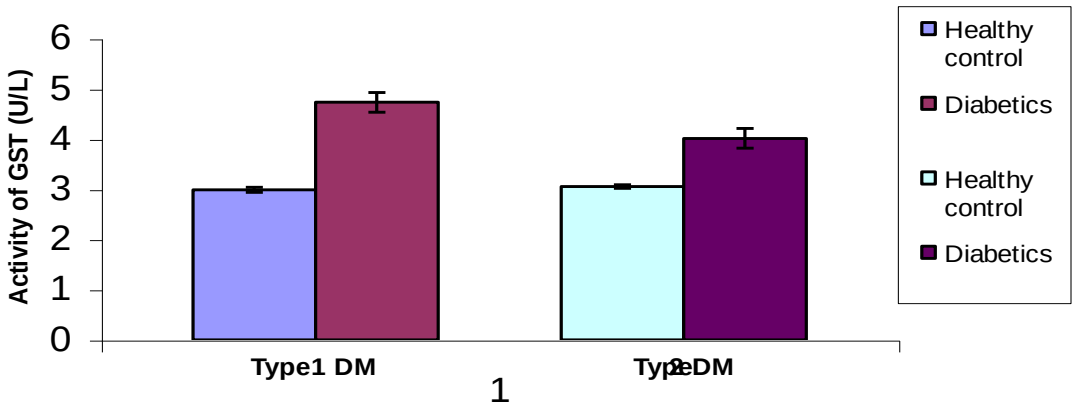
Figure(3-6): Level of catalase (CAT) activity in serum of healthy controls and patients with type1 and 2 diabetes mellitus (DM) disease. The values are the mean± SE

The present results indicate that decrease levels of serum CAT activity in diabetic patients may be due to hyperglycemia that causes degradation of peroxisomes (Hristtosozova *et al*., ٢٠٠٠) or may be due to inactivation of the enzyme CAT that may occur through glycation governed by prevailing glucose concentration. Thus increased glycation in diabetics and subsequent reactions of proteins may effect amino acids close to the active site of the enzyme or disturb the stereochemical configuration and causes structural and functional changes in the molecules (Rahbani *et al*., ١٩٩٩). Furthermore decline levels of serum CAT activity is a good indicator to high concentration of hydrogen peroxide ($H_٢O_٢$) in diabetics that increases oxidative stress and diabetic complications.

٣.٥.١.٣. Glutathione S-Transferase (GST)Activity

GST is an antioxidant enzyme that catalyzes the reaction between reduced glutathione (GSH) and drugs, xenobiotics and other toxic compounds, rendering them more water soluble and finally exerted from the body (Coughlin & Hall, ٢٠٠٢). Previous studies have shown that some chemical compounds, which augments oxygen products, have generated toxic effect such as many drugs and xenobiotics, which contain quinone groups cause free radical. The metabolism of quinone compounds includes formation of semiquinone radical. This radical reduces the molecule oxygen immediately, the superoxide anion ($O_٢^{\cdot -}$) comes into existence and, following that other ROS are generated (Mayesca & Holden, ١٩٩٥; Kanbak *et al*., ١٩٩٦; Inal & Kanbak, ١٩٩٧). However, most studies have reported increased levels of serum GST activity contributed to the increased oxidative stress found in type ١ and type ٢ DM subjects (Ioannides *et al*., ١٩٩٥; Salman, ٢٠٠٠; Mari & Cederbaum, ٢٠٠١; Raza *et al*., ٢٠٠٤).

The results of this study indicate a significant difference was observed in serum GST activity levels between control and diabetic patients (with type ١ and type ٢ DM), P ≤ ٠.٠٥, figure (٣-٧).



Figure(3-7): Level of glutathione S-transferase (GST) activity in serum of healthy control and patients with type1 and 2 diabetes mellitus (DM) disease. The values are the mean ± SE.

Thus the mean ± SE of serum GST activity levels of patients with type ١ DM was (٤.٧٣١ ± ٠.١٩٩ U/L *vs* ٢.٩٨٨ ± ٠.٠٥١ U/L of control), while in patients with type ٢ DM was (٤.٠١٤ ± ٠.١٩٧ U/L *vs* ٣.٠٥٣ ± ٠.٠٣٣ U/L of control), table (٣-٤), this higher levels of serum GST activity in diabetic patients may be due to increased synthesis of this enzyme under oxidative stress to protect the body from toxic compounds (Delmas *et al*., ١٩٩٦).

٣ . ٥ . ١ . ٤. Creatine Kinase (CK) Activity

Serum CK activity is an indicator commonly used in the diagnosis of heart and skeletal muscle disorder (Gunst *et al*., ١٩٩٨). Several studies pointed out that levels of serum CK is decreased in diabetic patients and suggested that decreased levels of serum CK may contribute to diabetic cardiomyopathy complications (Popovich eal., ١٩٨٩; Popovich *et al*., ١٩٩١; Hadwan, ٢٠٠٥).

In this work, serum CK activity in diabetic patients (with type ١ and type ٢ DM) was significantly lower than controls activity, P ≤ ٠.٠٥, figure (٣-٨). The mean ± SE of serum CK activity levels of patients with type ١ DM was (١٦٨.٥٦ ± ٣.٨٩٧ U/L *v*s ١٩٢ ± ٥.٩٥٥ U/L of control), while in patients with type ٢ DM was (١٥٨.٥٢ ± ٣.٠٢٦U/L *v*s ٢٢٦.٨ ± ٥.٦٦٢ U/L of control), table (٣-٤).

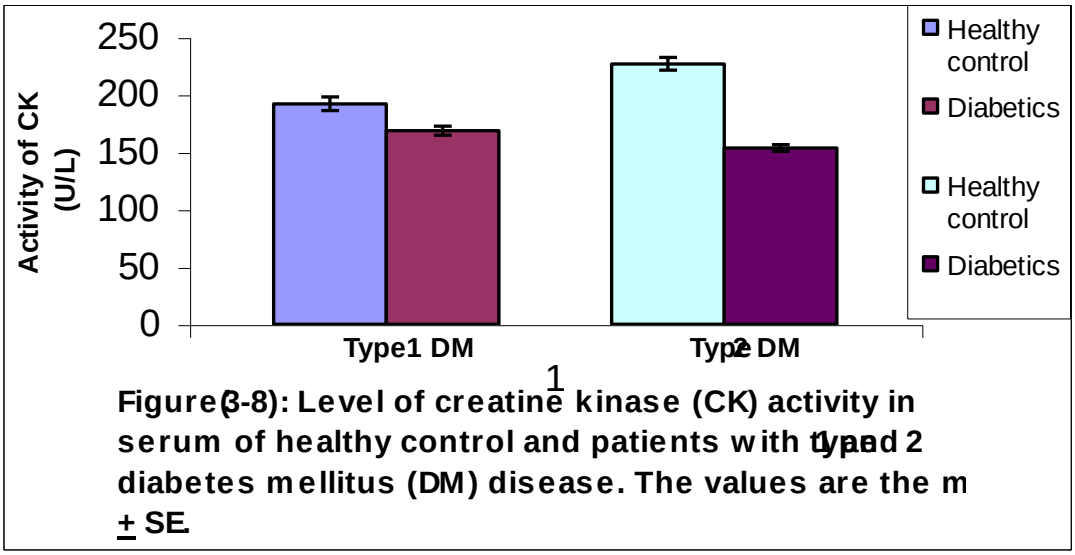


Figure (٣-8): Level of creatine kinase (CK) activity in serum of healthy control and patients with type 1 and 2 diabetes mellitus (DM) disease. The values are the m ± SE.

Serum CK is a protein contain thiol groups that is particularly susceptible to oxidation (Reddy *et al*., ٢٠٠٠), therefore decreased the activity of serum CK results from the oxidation of the essential –SH group of CK by ROS that generated in diabetic patients because prolonged exposure to hyperglycemia and inducing an inactivation of the enzyme (Mekhfi *et al*., ١٩٩٦).

٣.٥.٢. Non-Enzymatic Antioxidants

Table (٣-٥) shows the levels of some non-enzymatic antioxidants.

Table (٣-٥): Levels of several non-enzymatic antioxidants in serum of patients with type ١ or type ٢ DM and control .

			GSH (µmol/L)	Vitamin E (µmol/L)	Vitamin A (µmol/L)	Vitamin C (µmol/L)
Type ١ DM No.=٥٠	Mean±SE		٠.٦٥٣±٠.٠٢٧	٣.١٦±٠.٨٢	١.٢١٢±٠.٠٨٧	٣.٤٥٢±٠.١٦٥
	SD		٠.١٩٣	٠.٥٨	١.٢١٢	١.١٧٠
	Range		١.٢٤ – ٠.١٧	٤.٢٢٥ – ١.٩٨	٢.٣٥ – ٠.٢٣	٥.٤٥ – ١.٢
	%٩٥ C.I.	Lower	٠.٦٠٧	٣.٠٢٣	١.٠٦٦	٣.١٧٥
		Upper	٠.٦٩٨	٣.٢٩٧	١.٣٥٨	٣.٧٢٩
Control No.=٢٥	Mean±SE		١.٤٥٦±٠.٠٧٠	٦.٥٥١±٠.٢٢٢	٢.١٦٣±٠.١٤٠	٦.٥٣٤ ±٠.٢٤٢
P-Value			S	S	S	S
Type ٢ DM No.= ٥٠	Mean±SE		٠.٨٧٢±٠.٠٤١	٣.٣١٢±٠.١٠٦	١.٣٨١±٠.٠٨٠	٣.٦٤٢±٠.١٤٨
	SD		٠.٢٩٦	٠.٧٥٢	٠.٥٧١	١.٠٥٣
	Range		١.٩ – ٠.١٧	٢.٦٧ – ١.٥٦٧	٢.٦٧ – ٠.٢٤٥	٥.٧١٩ – ١.٧
	% ٩٥C.I.	Lower	٠.٨٠٣	٣.١٤٣	١.٢٤٦	٣.٣٩٣
		Upper	٠.٩٤٠	٣.٤٩	١.٥١٦	٣.٨٩١
Control No.=٢٥	Mean±SE		١.٣٩٢±٠.٠٦٠	٦.٨٣٤±٠.٢٣٩	٢.٤٧٠±٠.١٢٤	٦.٩٨٤±٠.٢٤٨
P-Value			S	S	S	S

٣.٥.٢.١. Glutathione (GSH) Level

Serum GSH plays a central role in antioxidant defense (Sen & Hanniuen, ١٩٩٤; Meister, ١٩٩٥). GSH detoxifies ROS such as H_2O_2 and lipid peroxides directly or in a glutathione peroxidase (GPX) catalyzed mechanism. GSH also regenerates the major aqueous and lipid phase antioxidant, ascorbate and α-tocopherol. Glutathione reductase (GRD) catalyzes the NADPH dependent reduction of oxidized glutathione (GSSG)

serving to maintain intracellular GSH stores and a favorable redox status GSH catalyzes the reaction between the –SH group and potential alkylating agents (Murray *et al*., ٢٠٠٠).

Thornalley *et al*. (١٩٩٦) found an inverse correlation between GSH level and the presence of DM complications in type ١ and type ٢ DM patients. However, most studies have also found decreased level of serum GSH in diabetic patients (Jain & McVie, ١٩٩٤; Akkus *et al*., ١٩٩٦; Delmas *et al*., ١٩٩٨; Paolisso *et al*., ٢٠٠٠; Salman, ٢٠٠١; Atalay & Laaksonen, ٢٠٠٢; Hadwan, ٢٠٠٥).

In this work, serum GSH level was significantly decreased in patients with type ١ and type ٢ DM when it is compared with control, $P \leq ٠.٠٥$. Hence, serum GSH level is approximately ٤٥٪ than that of control in patients with type ١ DM, while serum GSH level in patients with type ٢ DM was approximately ٦٢٪ than that of control, figure (٣-٩), table (٣-٥).

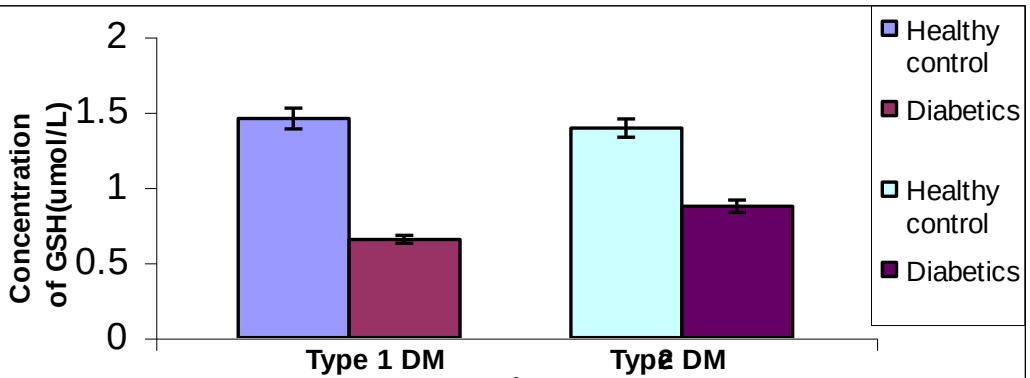


Figure (3-9): Level of glutathione (GSH) in serum of healthy control and patients with type 1 and type 2 diabetes mellitus (DM) disease. The values are the mean± SE

Decreased level of serum GSH in diabetic patients could represent an adaptive response to increased oxidative stress and free radicals generation that oxidized thiol group of GSH and decline reduced glutathione level, therefore it is a good indicator to diabetic complications.

The results of this study show different negative correlation, but not significant between serum GSH level and serum MDA, and tHcy levels, whereas a positive correlation, but not significant, with level of serum CK activity in diabetic patients (with type ١ and type ٢ DM). Furthermore, there was a significant negative correlation ($r = -٠.٣٠٩$, $P \leq ٠.٠٥$) between levels of GSH and levels of GST activity in serum of patients with type ٢ DM, but this correlation was not significant in patients with type ١ DM, table (٣-٦), figure (٣-١٠).

Table(٣-٦): The correlation between the level of glutathione and several biochemical markers related to oxidative stress syndrome.

The correlation of glutathione (µmol/L) *vs*. other variables	**Type ١ DM, No.= ٥٠**		**Type ٢ DM, No.= ٥٠**	
	Correlation coefficient (r)	**P-Value**	**Correlation coefficient (r)**	**P-Value**
vs. MDA (µmol/L)	-٠.٠٠٠٢	**NS**	-٠.٠٠٤٦	**NS**
vs. tHcy (µmol/L)	-٠.١٢٧٥	**NS**	-٠.١٨٦٤	**NS**
vs. GST (U/L)	-٠.٢٠٤٥	**NS**	-٠.٣٠٩٩	**S**
vs. CK (U/L)	٠.٠٥٨١	**NS**	٠.١٢٢٣	**NS**

These results probably were due to the following:

١. Depletion serum GSH levels lead to slightly accumulation of intracellular total homocysteine, which induces endothelial cells damage and slightly increases risk of cardiovascular disease in diabetic patients. These results go with the results of the study of (Ewadh and Jabir, ٢٠٠٢).

٢. Serum GSH has an important role in the protection of cells from the perooxidation injury, therefore decreases the degree of lipid perooxidation via end products; MDA in diabetic patients, these results are in agreement with the results of (Delmas *et al*., ١٩٩٦; Dominguez *et al*., ١٩٩٨).

٣. Presence of high level of toxic compounds in diabetic patients, especially with type ٢ DM (treatment with of hypoglycemic oral such as tablets of daonil, glucophage…etc) induced accumulation of GST enzyme and altered GSH metabolism, therefore toxic compounds increase ROS production that decreases levels of GSH, while increases synthesis of GST enzyme to play a protective role (Inal & Kanbak, ١٩٩٧; Raza *et al*., ٢٠٠٤).

٤. Increased levels of GSH could be slightly reversible loss in activity of CK enzyme through S-glutathionylation mechanism for regeneration of active CK from S-glutathionylated CK and increased level of CK activity (Reddy *et al*., ٢٠٠٠).

$$CK - SG + GSH \rightleftharpoons CK + GSSG$$

A B

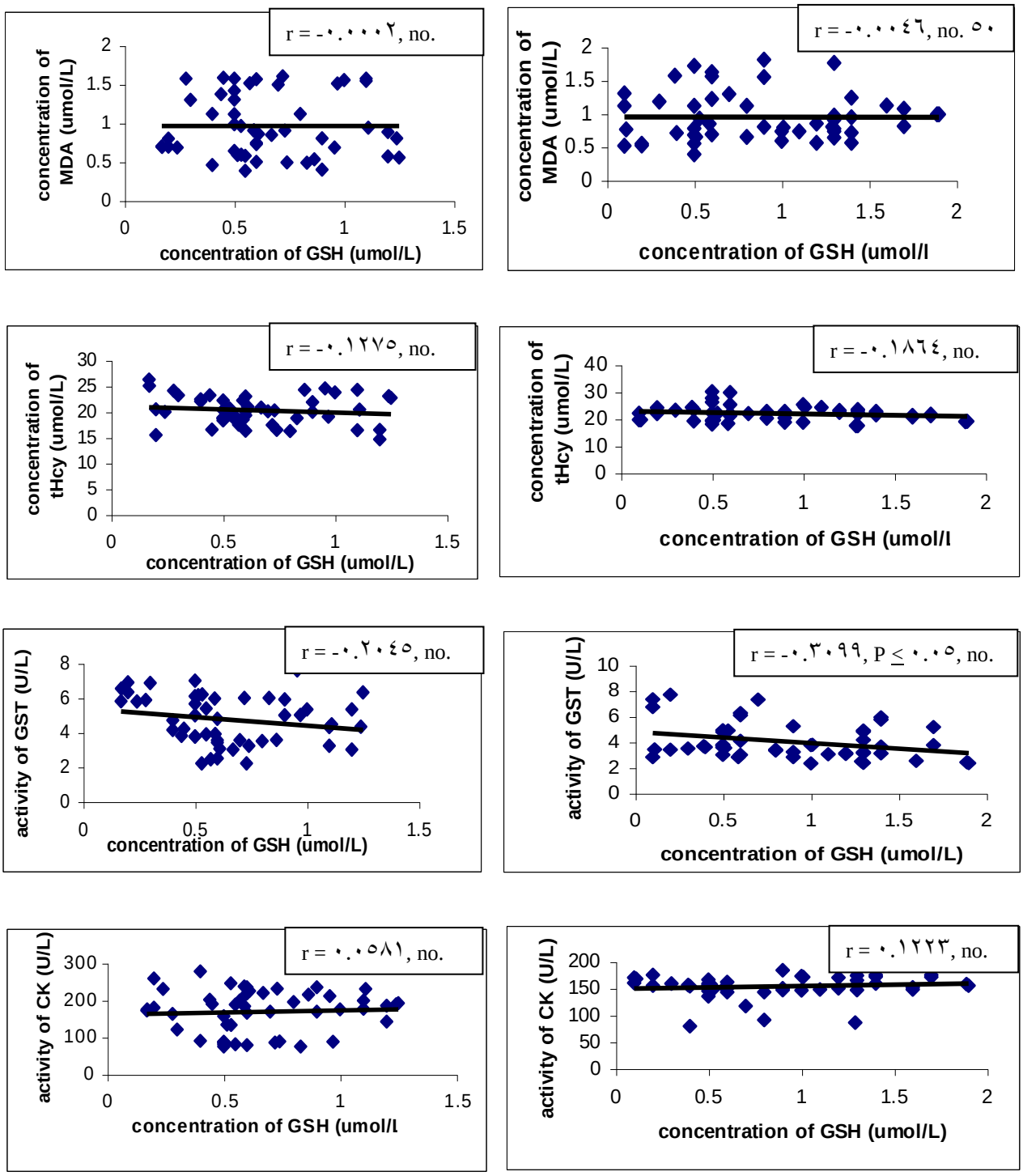


Figure (٣-١٠): Correlation of glutathione (GSH) with level of malondialdehyde (MDA), total homocystiene (tHcy), glutathione S-transferase (GST) activity, and creatine kinase (CK) activity in serum of patients (A) with type ١ diabetes mellitus, and (B) with type ٢ diabetes mellitus disease.

٣.٥.٢.٢. Vitamin E (α-tocopherol) Level

Serum vitamin E is a principle modulator of free radicals activity, it is a potent antioxidant acting as scavenger of reactive oxygen species (ROS) and reduces oxidative stress in DM disease (Paolisso *et al.*, ٢٠٠٠). Numerous studies have shown decrease in vitamin E level in diabetic tissue and blood (Salman, ٢٠٠١; Paolisso *et al.*, ٢٠٠٠; Anderson *et al.*, ١٩٩٩; Bursell *et al.*, ١٩٩٩; Dominguez *et al.*, ١٩٩٨; Tutuncu *et al.*, ١٩٩٨; Fuller *et al.*, ١٩٩٦).

This study indicated that serum vitamin E levels was significantly reduced in diabetic patients compared with control $P \leq$ ٠.٠٥, figure (٣-١١), therefore the mean ±SE of serum vitamin E levels in patients with type ١ DM was (٣.١٦ ± ٠.٨٢μmol/L *vs* ٦.٥٥١ ± ٠.٢٢٢μmol/L of control), while in patients with type ٢ DM was (٣.٣١٢ ± ٠.١٠٦μmol/L *vs* ٦.٨٣٤ ± ٠.٢٣٩μmol/L of control), table (٣-٥).

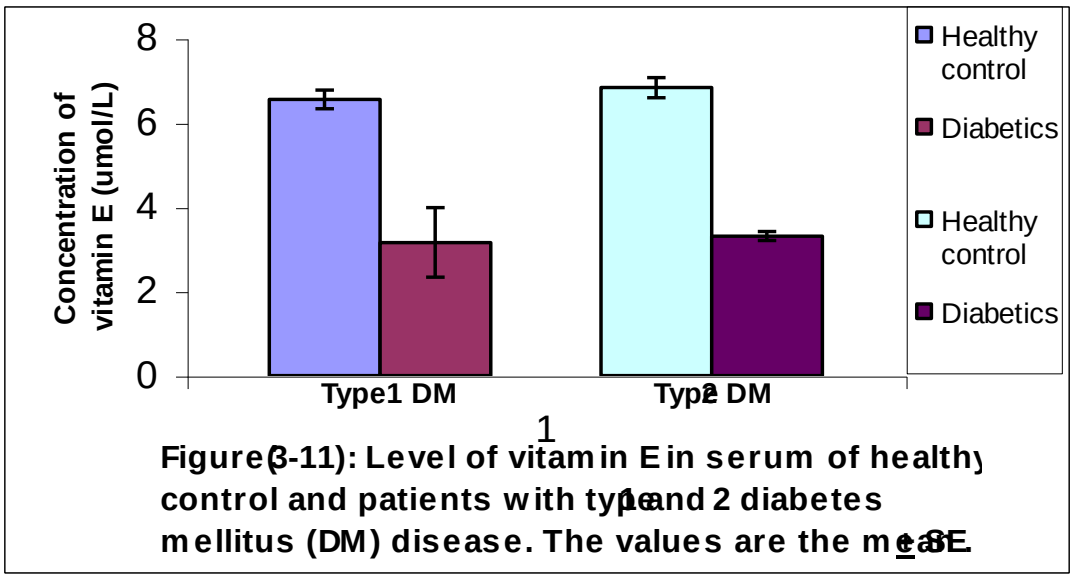


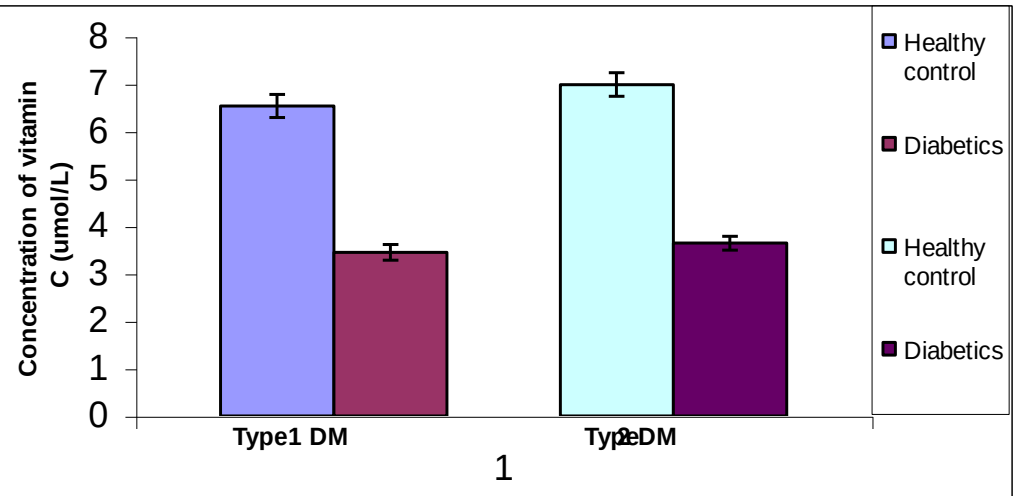
Figure (٣-11): Level of vitamin E in serum of healthy control and patients with type 1 and 2 diabetes mellitus (DM) disease. The values are the mean ± SE.

Reduced level of serum vitamin E possibly is due to increased utilization of this antioxidant in neutralizing free radicals in diabetic patients, thus supplementation of vitamin E to diabetic patients could reduce level of oxidative stress and diabetic complications.

٣.٥.٢.٣. Vitamin C (Ascorbic Acid) Level

Serum vitamin C is a free radical scavenger and interacts with free radicals in the water compartment of cells as well as in the fluid between cells. It is considered to be one of the most important antioxidants in extracellular fluids. Several studies have been shown that patients with diabetes have lower serum levels of vitamin C than non-diabetic subjects, and this is believed to be an important factor contributing to the increase of oxidative stress status and endothelial dysfunction in diabetes (Tousoulis *et al*., ٢٠٠٣; Ceriello, ١٩٩٩; Will & Byers, ١٩٩٦; Paolisso *et al*., ١٩٩٥; Wang *et al*., ١٩٩٤).

The results of this study show the mean ±SE of serum vitamin C in patients with type ١ DM, which was (٣.٤٥٢ ± ٠.١٦٥µmol/L *vs* ٦.٥٣٤ ± ٠.٢٤٢µmol/L of control), while in patients with type ٢ DM, it was (٣.٦٤٢ ± ٠.١٤٨µmol/L *vs* ٦.٩٨٤ ± ٠.٢٤٨µmol/L of control), table (٣-٥). There was a significantly decreased vitamin C levels in serum of diabetic patients than that of control, P ≤٠.٠٥, figure (٣-١٢).



Figure(3-12): Level of vitamin C in serum of healthy control and patients with type1 and 2 diabetes mellitus (DM) disease. The values are the mean± SE.

This decline in levels of serum vitamin C in diabetic patients may be due to (١) reduce renal re-absorption of vitamin C induced by hyperglycemia, (٢) the competition between glucose and vitamin C for the uptake into certain cells and tissues, (٣) the activity of polyol pathway that inhibited affectivity by utilizing high level of vitamin C to avoid hyperglycemia, (٤) and possible secondary depletion due to increased oxidative stress have been proposed (Wang *et al*., ١٩٩٤; Will & Byers, ١٩٩٦).

٣.٥.٢.٤. Vitamin A (Retinol) Level

Serum vitamin A is an important antioxidant. It is a biomarker of oxidative stress in DM disease (Dominguez *et al*., ١٩٩٨).

The present study indicates a significant decrease in serum vitamin A levels in diabetic patients than that of control, $P \leq ٠.٠٥$, figure (٣-١٣). The mean ±SE of serum vitamin A in patients with type ١ DM was (١.٢١٢ ± ٠.٠٨٧µmol/L *vs* ٢.١٦٣ ± ٠.١٤٠µmol/L of control), while in patients with type ٢ DM was (١.٣٨١ ± ٠.٠٨٠µmol/L *vs* ٢.٤٧ ± ٠.١٢٤µmol/L of control), table (٣-٥).

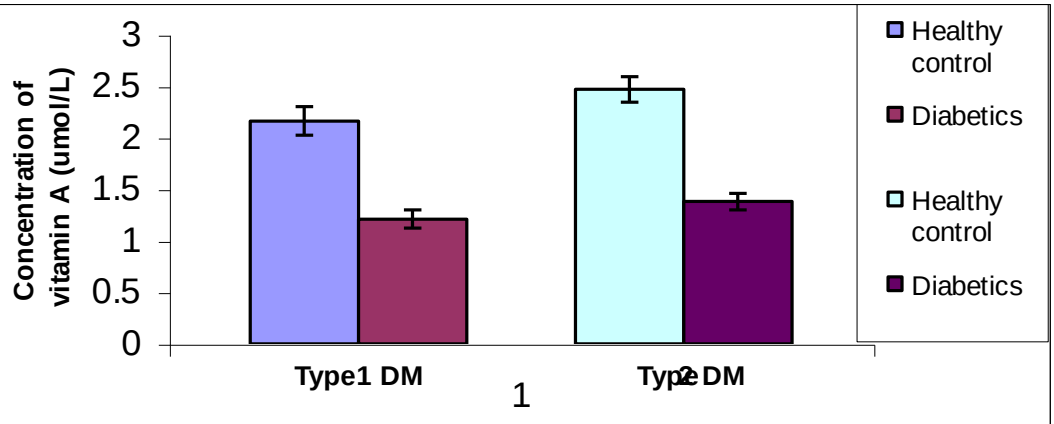


Figure (٣-13): Level of vitamin A in serum of healthy control and patients with type 1 and 2 diabetes mellitus (DM) disease. The values are the mean ± SE.

These results may be due to the increased utilization of this vitamin in the neutralization of free radicals induced by hyperglycemia, thus depletion of serum vitamin A in diabetic patients is due to the increased oxidative stress and diabetic complications. These results are in agreement with the singings of many studies (Delmas *et al*., ١٩٩٦; Domingues *et al*., ١٩٩٨; Bursell *et al*., ١٩٩٩). Therefore, supplementation of vitamin A or its precursor B-carotene to diabetic patients help in the reduction of oxidative stress and diabetic complications.

٣.٥.٢.٥. Trace Elements (Iron, Zinc, Copper, Chromium, and Selenium) Levels.

Some serum trace elements act as antioxidants and prevent membrane peroxidation. Others act directly on glucose metabolism. Ceruloplasmin, the major plasma copper transporting protein, possesses a potent oxidant property. Chromium trivalent ($Cr^{٣+}$) plays an important role in insulin-receptor activation (Terpilowska & Zaporowska, ٢٠٠٤). The function of zinc (Zn) in the body metabolism is based on its enzymatic affinity, way of a Zn-enzyme complex or Zn metalloenzyme. Iron ($Fe^{٣+}$) is stored in the ferittin and iron is released from it by the action of reducing agents that convert $Fe^{٣+}$ to $Fe^{٢+}$, thus ferrittin can act as a source of iron, which induces oxidative stress, and as a mechanism that protects against iron toxicity (Fern *et al*., ٢٠٠٢). Selenium (Se) plays important role in antioxidant defense system (Ulusu & Turan, ٢٠٠٥).

The several studies pointed out that diabetes results in disturbance of these trace elements. Present study indicates a significant decrease in levels of serum iron, Zinc, chromium, and selenium in diabetic patients with type ١ DM by about ٥١٪, ٣٣.٧٪, ٥٠٪, and ٣٣٪ respectively from that of control, $P \leq ٠.٠١$, figure (٣-١٤); while in patients with type ٢ DM there was a significant decrease in about ٦٦٪, ٣٠٪, ٥٠٪, and ٣١% respectively from that of control, $P \leq ٠.٠٥$, figure (٣-١٤), table (٣-٧).

Table (٣-٧): Levels of several trace elements in serum of patients with type ١ or type ٢ DM and control.

			Fe (µmol/L)	Cu (µmol/L)	Zn (µmol/L)	Cr (µmol/L)	Se (µmol/L)
Type ١ DM, No.=٥٠	mean±SE		٨.٦٤٢±٠.٣٢٠	١٧.٠٣±٠.٥٢٢	١٠.٤٩٤±٠.١٢٢	٠.٥٦٢±٠.٠٠٩	٠.٨٥±٠.٠١٣
	SD		٢.٢٦٤	٣.٦٩٤	٠.٩١٨	٠.٠٦٣	٠.٠٨٨
	Range		١٧.٢٥٢ _ ٥.٠٠٣	٢٦.٦٨٧ _ ٢.٣٨١	١٢.٠٦٩ _ ٨.٢٦	٠.٦٩٣ _ ٠.٤٦٦	٠.٩٧٧_ ٠.٦٩٢
	٩٥٪ C.I.	Lower	٨.١٠٥	١٦.١٥٥	١٠.٢٨	٠.٥٤٥	٠.٨٢٩
		Upper	٩.١٧٨	١٧.٩٠٤	١٠.٧٠٨	٠.٥٧٧	٠.٨٧١
Control No.=٢٥	mean±SE		١٧.٦٠٣± ٠.٦١٣	١٢.٩٩١± ٠.٥٨٢	١٥.٥١١± ٠.١٩٩	١.٠٢٣±٠.٠١٨	١.٢٥١±٠.٠١١
P-Value			S	S	S	S	S
Type ٢ DM, No.=٥٠	mean±SE		٦.٨٥٦±٠.٣٠٦	١٥.٨٦±٠.٤١٠	١١.١٢١± ٠.١٢٢	٠.٥٦٩±٠.٠٠٩	٠.٨٦٦±٠.٠١٠
	SD		٢.١٦٩	٢.٩٠٥	٠.٨٨٧	٠.٠٦٢	٠.٠٧٢
	Range		١٣.٨٦١ _ ٢.١٩٦	٣٣.٤٠٨ _ ٧.٦٩٧	٨.٨٧٢_ ٩.٣٣١	٠.٦٨٩_ ٠.٤٥	١.٠٠٥_ ٠.٧٠٢
	٩٥٪ C.I.	Lower	٦.٣٤٣	١٥.١٧٢	١٠.٩٢٢	٠.٥٥٥	٠.٨٤٩
		Upper	٧.٣٦٨	١٦.٥٤٧	١١.٣٢	٠.٥٨٤	٠.٨٨٤
Control No.=٢٥	mean±SE		٢٠.١٠٧± ٠.٧٩٩	١٢.٩٢٦± ٠.٥٩٤	١٥.٨٩٤± ٠.٢٢٩	١.٠٠٨±٠.٠١٦	١.٢٤٨±٠.٠٠٩
P-Value			S	S	S	S	S

The results also indicate a significant increase in levels of serum copper (Cu) in patients with type ١ and type ٢ DM from control by about ٢٤٪ and ٣٨٪ respectively, P ≤ ٠.٠١, figure (٣-١٤).

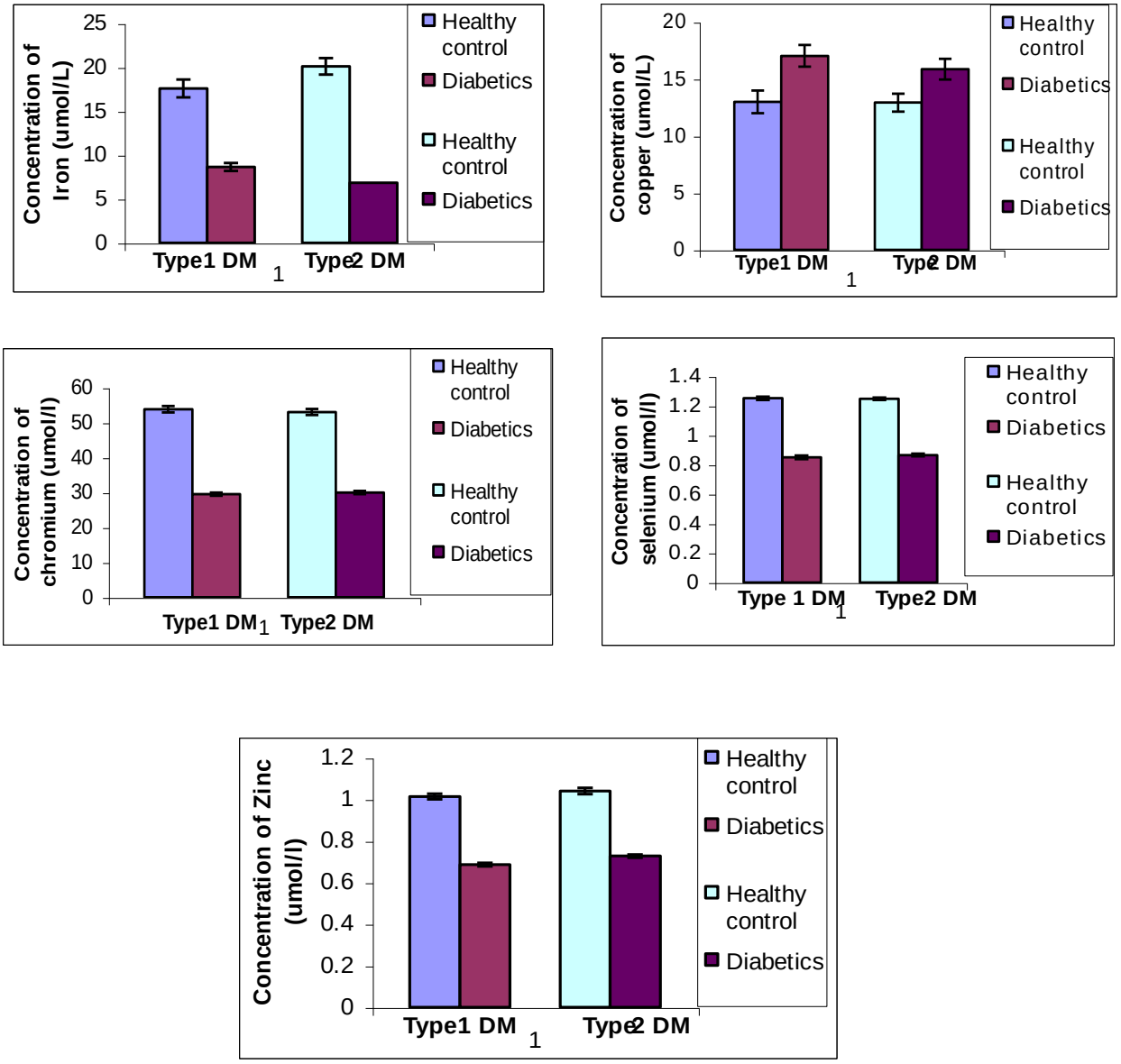


Figure (٣-١٤): Level of some trace elements in serum of healthy control and patients with type ١ and ٢ diabetes mellitus (DM) disease. The values are the mean ± SE.

However, these results may be due to:

١. All studies found increased level of serum iron in diabetic patients but in this work pointed out decreased levels of serum iron in diabetic patients than control may be due to increased drinking tea in Iraq that inhibits iron absorption (Kim & Miller, ٢٠٠٥), thus tea and its phenolic constituent are reported to have antioxidant activity in vitro (Luo *et al.*, ١٩٩٧), the antioxidant action of tea was dependant on the ability of their constituent phenolic compounds to scavenger free radicals and to chelate iron (reduce the utilization of dietary iron), (Samman *et al.*, ٢٠٠١). Or may be due to their low iron diet, treatment of diabetic patients by either insulin or oral hypoglycemia agents could stimulate the increase synthesis of ferritin or act as iron chelator, or may be due to the presence of high level of oxidative stress induced by hyperglycemia, which leads to glycation of transferrin and decreases its ability to bind ferrous iron ($Fe^{٢+}$) (Fujimoto *et al.*, ١٩٩٥), and by increasing the pool of free iron stimulating ferritin synthesis (Juckett *et al.*, ١٩٩٥; Fernandez *et al.*, ٢٠٠٥).

٢. Decreased levels of serum zinc in diabetic patients may be due to reduced level antioxidant enzyme alkaline phosphetase or due to excessive urinary output especially in patients with diabetic nephropathy, glastrointestinal malabsorption or genetic factors or signs of infection during which Zn will act as defense mechanism (Walter *et al.*, ١٩٩١). The antioxidant properties of zinc have been demonstrated in vitro. Zinc may exert its antioxidant effect by decreasing the susceptibility of essential sulfhydryl groups of proteins to oxidation and by competing with prooxidant metals such as iron and copper for biological binding sites (Lokitch *et al.*, ١٩٨٣), these results agree with the results of (Zargar *et al.*, ١٩٩٨).

٣. Decreased levels of serum chromium in diabetic patients may be due to increase in glucose and insulin concentration, which increases urinary Cr output (Vincent, ٢٠٠٤). These results go with the results of (Terpilowska & Zaporowska, ٢٠٠٤).

٤. Decreased levels of serum selenium in diabetes mellitus disease may be due to increased oxidative stress that requires increased utilization of selenium as antioxidant by binding with vitamin E and as a cofactor of glutathione perooxidase (GPx) to scavenge free radical, which acting to detoxify tissue peroxidation (Hayes, ٢٠٠١; Beckg, ١٩٩٨). These results agree with the results of (Ulusu & Turan, ٢٠٠٥)

٥. Increased levels of serum copper in diabetic patients may be due to the presence of oxidative stress by hyperglycemia, which leads to the glycation of ceruloplasmin enzyme, whereas most of copper is incorporated into it, and releases high level of serum copper that is associated with low concentration of ceruloplasmin and iron in the liver and causes increased free radical production in diabetic patients. These results go with the results of the study of (Walter *et al*., ١٩٩١; Zargar *et al*., ١٩٩٨; Salman, ٢٠٠١).

٣.٦. Lipid Peroxidation and Antioxidant Vitamins

The new trend in management of diabetes and decrease lipid peroxidation degree is to use therapeutic antioxidant and scavengers like vitamin A (Fulle *et al*., ١٩٩٦; Tuluncu *et al*., ١٩٩٨;; Bagchi & Puri, ١٩٩٨; Belinda & Connell, ٢٠٠١; Franz *et al*., ٢٠٠٢).

The results of this study reported different negative correlation between serum MDA and serum vitamin E, vitamin C, and vitamin A in patients with type ١ and type ٢ DM. The reason for studies these correlations between MDA and antioxidant vitamins because MDA represented the important indicator for lipid peroxidation, table (٣-٨).

Table (٣-٨): The correlation between the level of lipid peroxidation via MDA and some antioxidant vitamins in diabetics.

The correlation of malondialdehyde(MDA) (µmol/L) *vs*. other variables	**Type ١ DM, No.= ٥٠**		**Type ٢ DM, No.= ٥٠**	
	Correlation coefficient (r)	**P-Value**	**Correlation coefficient (r)**	**P-Value**
***vs*. vitamin E (µmol/L)**	**-٠.٣٤٥**	**S**	**-٠.١٩٧**	**NS**
***vs*. vitamin A (µmol/L)**	**-٠.١٨٦**	**NS**	**-٠.٢٠٦**	**NS**
***vs*. vitamin C (µmol/L)**	**-٠.٠٩٧**	**NS**	**-٠.٣٠٥**	**S**

However, there was a significant negative correlation between serum MDA level and serum vitamin E ($r = -٠.٣٤٥$, $P \leq ٠.٠٥$), whereas there was negative correlation but not significant with vitamin C and vitamin A in serum of patients with type ١ DM, figure (٣-١٠)A, while in patients with type ٢ DM, there was a significant negative correlation between levels of serum MDA and levels of serum vitamin C ($r = -٠.٣٠٥$, $P \leq ٠.٠٥$), and negative correlation but not significant with serum vitamin E and serum vitamin A, figure (٣-١٠)B.

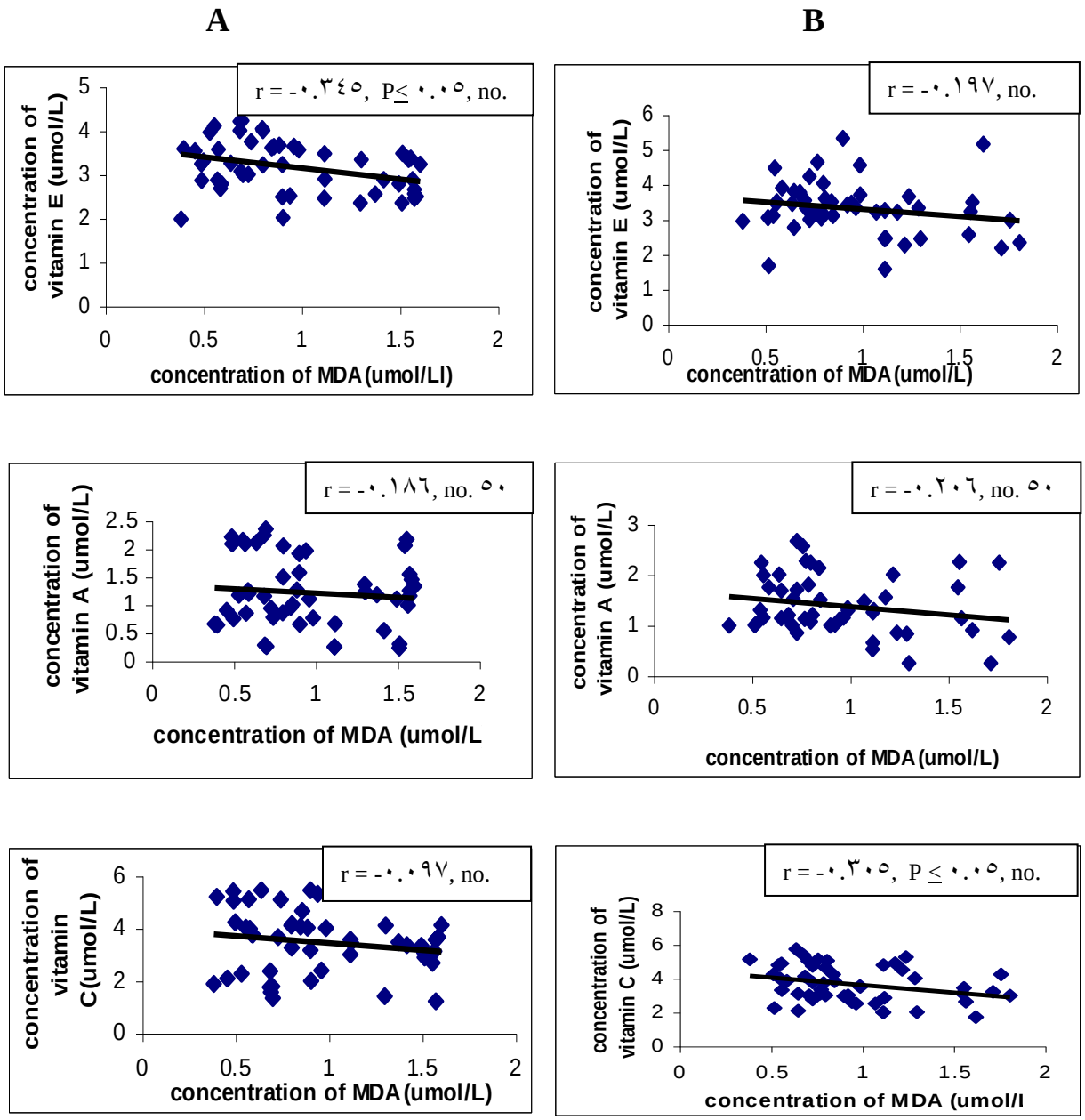


Figure (٣-١٥): Correlation between level of malondialdehyde (MDA) and level of antioxidant vitamins in serum of patients (A) with type ١ diabetes mellitus and (B) with type ٢ diabetes mellitus.

Antioxidant vitamins act as quenchers for free radicals (Paolisso *et al*., ١٩٩٥). Thus, increased levels of serum antioxidant vitamins could reduce the level of MDA, the end product of lipid peroxidation in diabetic patients. However, our results reported that supplementation of vitamin E to patients with type ١ DM and vitamin C to patients with type ٢ DM could reduce lipid peroxidation degree and then reduce the cardiovascular disease complications.

٣.٧. Correlation of Ec-Cu/Zn SOD Activity with Levels of Zinc, Copper, and CAT Activity

Ec-Cu/Zn SOD and CAT are antioxidant enzymes that are cooperative in their action, while copper (Cu), and zinc (Zn) act as cofactors for Ec-Cu/Zn SOD enzyme in humans. In the present study, table(٣-٩) shows the correlation of Ec-Cu/Zn SOD activity with levels of zinc, copper, and CAT activity.

Table(٣-٩): The correlation between the levels of Ec-Cu/Zn SOD activity and levels of CAT activity and some trace elements (copper, and zinc) in diabetics.

The correlation of Ec-Cu/Zn SOD(U/ml) *vs*. other variables	**Type ١ DM, No.= ٥٠**		**Type ٢ DM, No.= ٥٠**	
	Correlation coefficient (r)	**P-Value**	**Correlation coefficient (r)**	**P-Value**
***vs*. Zinc (µmol/L)**	-٠.٢٨	**S**	-٠.٣٠٠	**S**
***vs*. Copper (µmol/L)**	٠.١٦	**NS**	٠.١٧٩	**NS**
***vs*. Catalase (CAT), (K/ml)**	-٠.١٢٦	**NS**	-٠.١٣٠	**NS**

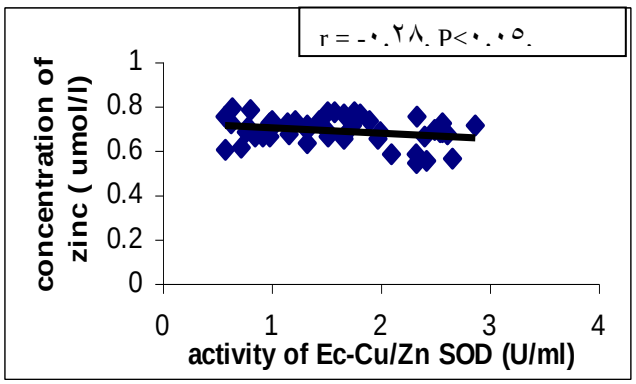
However their was a significant negative correlation between levels of Ec-Cu/Zn SOD activity and levels of zinc in serum of patients with type ١ DM ($r = -٠.٢٨٠$, $P \leq ٠.٠٥$), figure (٣-١٦) A, and in serum of patients with type ٢ DM ($r = -٠.٣٠٠$, $P \leq ٠.٠٥$), figure (٣-١٦) B, table (٣-٩). These

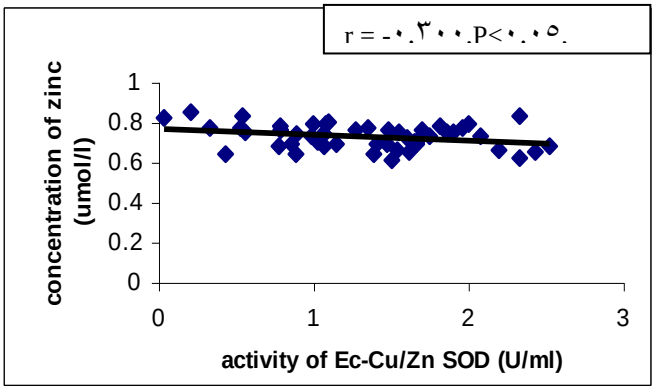
results may be due to that zinc acts as a cofactor for Ec-Cu/Zn SOD enzyme or could be linked to the protective effect of zinc on the protein itself, therefore serum zinc level decreases with the increased synthesis of Ec-Cu/Zn SOD (Faure *et al*., ١٩٩٥).

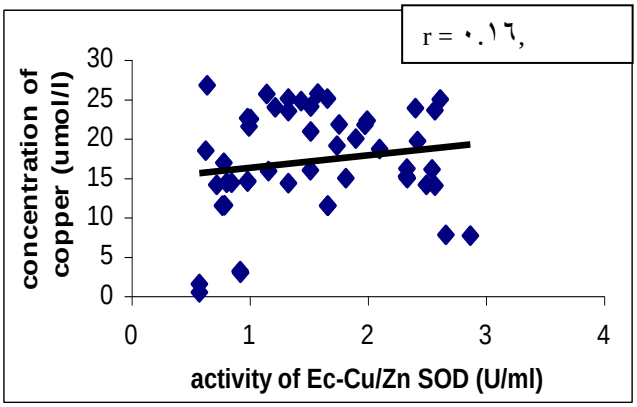
As well as, there was a positive correlation, but not significant, between levels of Ec-Cu/Zn SOD activity and levels of copper in serum of patients with type ١ DM, figure (٣-١٦)A, and in serum of patients with type ٢ DM, figure (٣-١٦)B, table (٣-٩). These results may be due to increase levels of copper in diabetic and healthy individuals lead to increase in free radicals generation, thus increased levels of copper lead to slightly increase in levels of Ec-Cu/Zn SOD activity to defense against ROS damage.

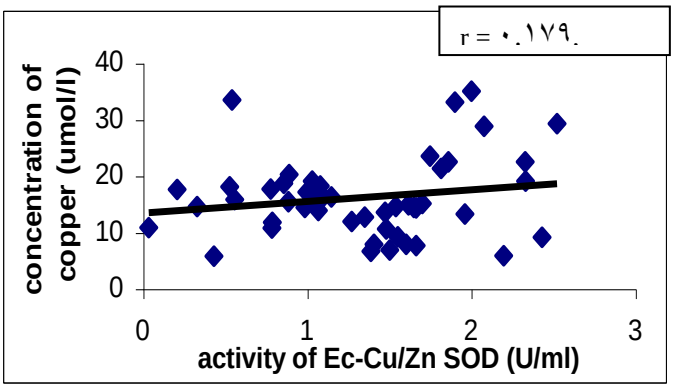
Furthermore, there was a negative correlation, but not significant, between levels of Ec-Cu/Zn SOD activity and levels of CAT activity in serum of patients with type ١ DM, figure (٣-١٦)A, and in serum of patients with type ٢ DM, figure (٣-١٦)B, table (٣-٩). In contrast with controls, there was a positive correlation; this may be due to the presence of oxidative stress syndrome in diabetic patients. This finding is in accordance with that of (Singh & Pathak, ١٩٩٠). Thus, increased levels of Ec-Cu/Zn SOD activity was slightly decreased levels of serum CAT activity in diabetic patients probably due to the presence of high levels of superoxide anion ($O_2^{\bullet -}$) that required high levels of Ec-Cu/Zn SOD activity to stimulate it to high level of hydrogen peroxide (H_2O_2) that consume high levels of CAT activity that is responsible for the decomposition to oxygen molecule and water.

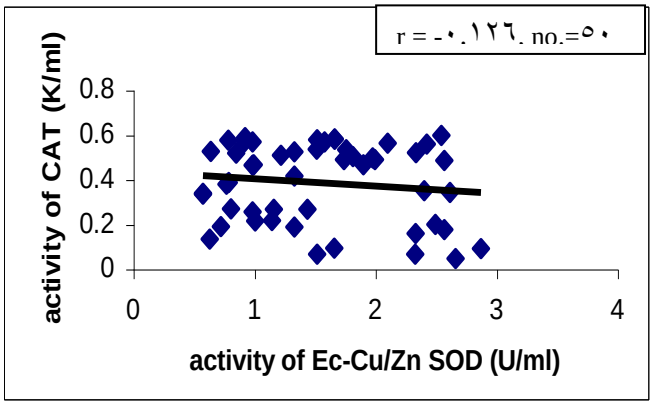
A **B**











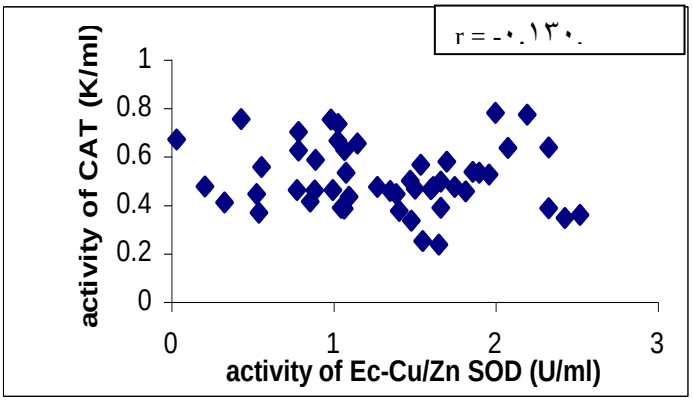


Figure (٣-١٦): Correlation of Ec-Cu/Zn SOD activity with level of catalase (CAT) activity and some trace elements

(copper, and zinc) in serum of patients (A) with type ١ diabetes mellitus and (B) with type ٢ diabetes mellitus disease.

٣.٨. Effect of Smoking on Oxidative Stress Status in Diabetics

Evidence for increased oxidation in smokers compared ith non-smokers were proved by the presence of increased prooxidant products in blood and urine (Morrow *et al*., ١٩٩٥; Mezzetti *et al*., ١٩٩٥).

In this work, the results reported no significant difference in levels of serum MDA, tHcy, Cu, Fe, Zn, Se, Cr, GSH, vitamin C, and GST, Ec-Cu/Zn SOD, CK, CAT activities in serum of smokers with type ١, and ٢ DM than diabetic non-smokers as well as in levels of serum vitamin E of smokers with type ١ DM, and levels of serum vitamin A of smokers with type ٢ DM than that of diabetic non-smokers, table (٣-١٠).

Table (٣-١٠): Effect of smoking on the levels of some biochemical markers related to oxidative stress syndrome in diabetics.

Variables	Type ١ DM, No.=٥٠			Type ٢ DM, No.=٥٠		
	Smokers No.= ١١ Mean±SE	Non-smokers No.= ٣٩ Mean±SE	P-Value	Smokers No.= ١٧ Mean±SE	Non-smokers No.= ٣٣ Mean±SE	P-Value
Age(Years)	٣٣±٣.٧٣٤	٣١.٨٢±٢.٧٧٢	NS	٤٨.١٧٦±٢.٠٥٤	٥٣.٢٧٢±١.٤٩	NS
Duration of DM (Years)	٩.٣٢٥±٢.٢١٧	١٠.٦٥٧±١.٦٨٥	NS	٥.٢٨٤±١.٥٥٦	٠	NS
MDA (µmol/L)			NS	٠.٩٩٩±٠.٠٦٨	٥.٧٨٧±١.٠١٧	NS
tHcy (µmol/L)	٠.٩٦٦±٠.٠٩٩	٠.٩٦٢±٠.٠٣٤	NS	٢٢.٤٥١±٠.٦٦٣	٠.٨٤٨±٠.٠٣٧	NS
Ec-SOD (U/ml)	٢٠.٥٠٧±٠.٨١٠	١٩.٤٧٥±٠.٤٨٤	NS	١.٢٨٨±٠.٠٩٦	٢١.٠٣٧±٠.٤٨١	NS
CAT (K/ml)	١.٥٤٣±٠.١٠٨	١.٥٨٥±٠.٠٥٧	NS	٠.٤٨٨±٠.٠٣١	١.٤٠٢±٠.٠٨١	NS
GST (U/l)	٠.٣٨٢±٠.٠٥٤	٠.٣٨٧±٠.٠١٦	NS	٤.١٧٢±٠.٢٧٧	٠.٥٤٢±٠.٠٢٤	NS
CK (U/l)	٤.٨٧٩±٠.٥٠٤	٤.٦٨٩±٠.٢١٦	NS	١٤٧.١٧٦±٦.٢٤٥	٣.٧٠٩±٠.٢١٠	S
GSH (µmol/L)	١٥٨.٠٩±١٠.١٥	١٧١.٥١٢±٥.٨٠٧	S	٠.٨١٦±٠.٠٦٠	١٥٦.٧٨٧±٣.١٩٧	NS
Vitamin E (µmol/L)	٠.٦٤١±٠.٠٨٤	٠.٦٩٤±٠.٠٢٤	NS	٢.٩٣٥±٠.١٧١	٠.٩٠١±٠.٠٤٧	NS
Vitamin A (µmol/L)	٣.٠٨٤±٠.١٧٣	٣.٤٢٩±٠.٠٨٦	NS	١.٣٢٤±٠.٠٨٩	٣.٥٠٦±٠.١٢٣	NS
Vitamin C (µmol/L)	٠.٧٤٢±٠.١٢٣	١.٣٤٥±٠.٠٩٦	NS	٣.٦١٨±٠.٢١٧	١.٤١±٠.١١٤	NS
Iron (µmol/L)	٣.٤٣٩±٠.٢٣٣	٣.٤٥٥±٠.٢٠٢	NS	٦.٩٦±٠.٥٦٥	٣.٦٨٩±٠.٢٣٢	NS
Copper (µmol/L)	١٠.٢±١.١٣٠	٨.٢٠٢±٠.٣٢٣	NS	١٥.٨٨٣±١.٠٨٣	٦.٦٥٣±٠.٣٣٢	NS
Zinc (µmol/L)		١٦.٩٦±٠.٦٩٣	NS	١١.١٠٦±٠.٢١٤	١٥.٨١٤±٠.٦٧٤	NS
Chromium (µmol/L)		١٠.٥٢٤±٠.١٣٧	NS	٠.٥٦٦±٠.٠١٦	١١.١٣٦±٠.١٣٧	NS
Selenium (µmol/L)		٠.٥٨٣±٠.٠١١	NS	٠.٨٦٢±٠.٠١٩	٠.٥٩٥±٠.٠٠٨	NS
		٠.٨٥٧±٠.٠١٧			٠.٨٧٥±٠.٠٠٩	

	١٧.٢٨١±١.١٨٢ ١٠.٣٨٧±٠.٢٦٠ ٠.٥٦٤±٠.٠١٨ ٠.٨٤٧±٠.٠٢٥					

But there was a significant decrease in level of vitamin A in serum of smokers with type ١ DM, P ≤ ٠.٠٥, figure (٣-٢٣), and vitamin C in serum of smokers with type ٢ DM, P ≤ ٠.٠٥, figure (٣-٢٤) than diabetic non-smokers.

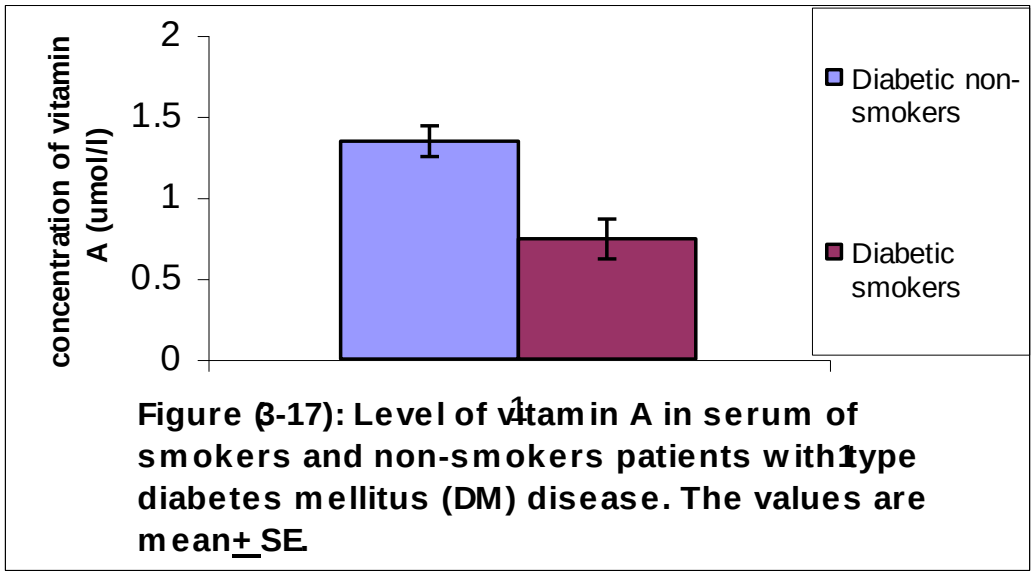


Figure (٣-17): Level of vitamin A in serum of smokers and non-smokers patients with type ١ diabetes mellitus (DM) disease. The values are mean± SE.

These results may probably be due to the following:

١. Smoking may be enhance oxidative stress through the production of free radicals and weaken antioxidant defense mechanism (Maragnon *et al*., ١٩٩٨; Zhao & Yu, ١٩٩٨; James *et al*., ٢٠٠٠; Al-Meshhadani, ٢٠٠٣). Therefore, smoking slightly increases level of MDA in serum of diabetic smokers than diabetic non-smokers.

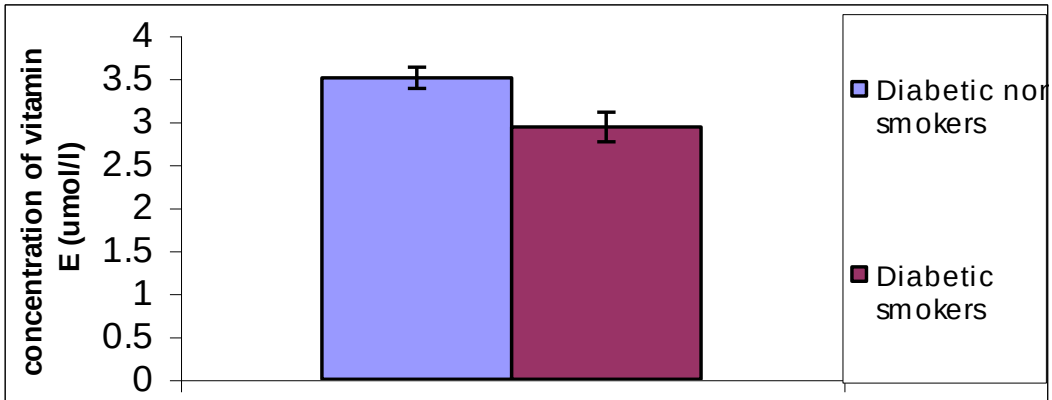


Figure (3-18): Level of vitamin E in serum of smokers and non-smokers patients with type2 diabetes mellitus (DM) disease. The values are the mean ± SE

٢. Some toxic components of tobacco smoke could interact with thiol rich compounds leading to structural and functional changes of these molecules (Zappacosta *et al*., ٢٠٠٢). Therefore, smoking could be slightly depletion level of GSH and accumulation of tHcy in serum of diabetic smokers than diabetic non-smokers, or decreased level of GSH may be due to increase GSH utilization in neutralizing smoking induced free radicals.

٣. Cigarette smoke contains an extremely high concentration of various free radicals (Shurch & Pryor, ١٩٨٥), which are increased

susceptibility of –SH groups of CK enzyme to be oxidized and inhibition or probably due to the interaction between smoke aldehydes and –SH groups; the active sites of enzyme molecules and reduced its activity (Zappacosta *et al.*, ٢٠٠٢), therefore smoking could be slightly decreased level of CK activity in serum of diabetic smokers than diabetic non-smokers.

٤. Smoke contains $O_2^{\cdot-}$, and in response to the increase amounts of substrate of $O_2^{\cdot-}$, this results in an increase in Ec-Cu/Zn SOD activity probably through induction (Gregorg *et al.*, ١٩٧٩). In addition nicotine (٥ X $١٠^{-٦}$ to ٥ X $١٠^{-١١}$M) in cigarette was found to inhibit of $O_2^{\cdot-}$ production from polymorphonuclear cells (Sasagawa *et al.*, ١٩٨٥). But because $O_2^{\cdot-}$ considered as a stimulant to increase synthesis of SOD (Lunec *et al.*, ١٩٨١), therefore this explanation could be the cause behind the slight decrease in Ec-Cu/Zn SOD level in diabetic smokers, also lead (Pb) and cyanide is considered as a component of cigarette smoke (Mawin-Mateo *et al.*, ١٩٩٧) and the copprozinc enzymes are inhibited by cyanide (Borders & Fridovich, ١٩٨٥), while Pb causes non-competitive inhibition of a covalent bond at the enzyme and decrease in Vmax of enzyme reaction (Mawin-Mateo *et al.*, ١٩٩٧).

٥. Increased CAT enzyme utilization in neutralizing smoking induced free radicals (Muhlhauser *et al.*, ١٩٩٦; Ritz et al., ١٩٩٦; Al-Delaimy, ٢٠٠١) lead to slightly decreased levels of CAT activity in serum of diabetic smokers than diabetic non-smokers.

٦. Presence of toxic compounds in the cigarette smoke such as semiquinones (Kasai & Nishimura, ١٩٨٦) induced accumulation of GST enzyme by increase its synthesis to play a protective role, therefore smoking may be slightly increases level of GST activity in serum of diabetic smokers than diabetic non-smokers.

٧. Utilizing high level of vitamin C in diabetic smokers to neutralize free radicals generation through smoking results in slightly reduced

levels of vitamin C in serum of diabetic smokers than non-smokers, while vitamin A levels were slightly reduced in serum of smokers with type ٢ DM than diabetic non-smokers, but is reduced significantly in serum of smokers with type ١ DM, these results are due to that gas phase of cigarette smoke is a complex oxidizing milieu processing an array of free radical species including peroxy radicals ($ROO^{\cdot}$), and $O_2^{\cdot-}$ (Pryor & Ston, ١٩٩٣), which has been shown to deplete lipid phase. Furthermore, vitamin E levels were slightly reduced in serum of smokers with type ١ DM than diabetic non-smokers, while are reduced significantly in serum of smokers with type ٢ DM. This may be due to increase production of catecholamines hormone in smokers with type ٢ DM and act as an antagonist to insulin action (Targher, ١٩٩٧), thus increase free radicals production under hyperglycemia that require high level of vitamin E to be neutralization.

٨. Smoking is increasing oxidative stress and weakening defense system, therefore it increases disturbance in trace elements levels in diabetic smokers than diabetic non-smokers, thus there was a slightly increase in levels of copper and iron and slightly decrease in levels of zinc, selenium and chromium in serum of diabetic smokers than diabetic non-smokers.

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References

Adachi, T.; Yamada, H.; Yamada, Y.; Morihara, N.; Hirano, K.; Murakami, T.; Futenma, A.; and Kato, K. Substitution of Glycine for Arginino-٢١٣ in Extracellular-Superoxide Dismutase Surface. Biochemical Journal, ١٩٩٦; ٣١٣(١): ٢٣٥-٢٣٩.

Aebi, H. "Methods of Enzymatic Analysis", ed. New York, Academic Press, ١٩٧٤(٢): ٦٧٤-٨٤.

Akkus, I.; Kalak, S.; Vural, H.; Caglayan, O.; Menekse, E.; Can, G., and Durmus, B. Leukocyte Lipid Peroxidation, Superoxide Dismutase, Glutathione Peroxide, and Serum and Leukocyte Vitamin C Levels of Patients with Type II Diabetes Mellitus. Clinica Chimica Acta., ١٩٩٦; ٢٤٤: ٢٢١-٢٢٧.

Al-Delaimy, W.K. Smoking and Mortality among Women with Type ٢ Diabetes: The Nurse Health Study Cohort. Diabetes Care. ٢٠٠١; ١٢: ٢٠٤٣-٨.

Al-Meshhedani, Q.H. "Blood Markers in Relation to Atherosclerosis in Iraq Smokers" (Thesis), Ph.D., Iraq, Al-Mustansiriya University, ٢٠٠٣.

Al-Meshhadani, W.M.S. "A Study on the Attenuation of Vascular Respone in Non-Insulin Dependent Diabetic Patients with Microalbuminuria." (Thesis), Ph.D., Iraq, Al-Mustansiriya University, ٢٠٠٠.

Al-Zamely, Oda.M.Yasser. "Ischemic Heart Disease Via Oxidative Hypothesis" (Thesis), Ph.D., Iraq, Mustansiriya University, ٢٠٠١.

Anderson, R.A.; Cheng, N.; Bryden, N.A.; Polansky, M.M.; Chi, J.; Feng, J. Beneficial Effects of Chromium for People with Diabetes. Diabetes, ١٩٩٧; ٤٦: ١٧٨٦-١٧٩١.

Anderson, G.W.; Gowri, M.S.; Turner, J.; Nichols, L.; Diwadkar, V.A.; Chow, C.K., and Oettgen, P.R. Antioxidant Supplementation Effects on Low-Density Lipoprotein Oxidation for Individuals with Type ٢ Diabetes Mellitus. J. Am. Coll. Nutr., ١٩٩٩; ١٨(٥): ٤٥١-٦١.

Atalay, M.; and Laaksones, D.E. Diabetes, Oxidative Stress and Physical Exercise. J. Sports Science and Medicine, ٢٠٠٢; ١: ١-١٤.

Atalay, M.; Laaksones, D.E.; Khanna, S; Kaliste-Korhnen, E.; Hanninen, O., and Sen, C.K. Vitamin E regulates changes in tissue antioxidants induced by fish oil and acute exercise. Medicine and science in sports and Exercise, ٢٠٠٠; ٣٢:٦٠١-٦٠٧.

Atalay, M., and Sen, C.K. Physical exercise and antioxidant defenses in the heart. Annals New York Academy of Sciences, ١٩٩٩; ٨٧٤:١٦٩-١٧٧.

Bagchi, K.; and Puri, S. Free Radicals and Antioxidants in Healthy and Disease. Estran Mediterrean Health Journal, ١٩٩٨; ٤(٢): ٣٥٠-٣٦٠.

Banerjee, D.; Kumar, P.A.; Kumar, B.; Madhusoodanan, U.K.; Nayak, S., and Jacob, J. Determination of Absolute Hydrogen Peroxide Concentration by Spectrophotometric Method. Current Science. ٢٠٠٢; ٨٣(١٠): ١١٩٣-١١٩٤.

Baynes, J.W. Role of Oxidative Stress in Development of Complication in Diabetes. Diabetes, ١٩٩١; ٤٠: ٤٠٥-٤١٢.

Becker, A.; Smnlders, Y.M.; Guldener, C.V., and Stehouwer, C.D.A. Epidemiology of Homocysteine as a Risk Factor in Diabetes. Metabolic Syndrome and Related Disorders, ٢٠٠٣; ١: ١٠٥-١٢٠.

Beckg, M.A. The Influence of Antioxidant Nutrient in Non-Viral Infection. Nutr. Rev., ١٩٩٨; ٥٦: ٥١٤٠-٥١٤٦.

Bedwal, R.S.; Nair, N.; Sharma, M.P., and Mathur, R.S. Selenium and its Biological Perspective. Med. Hypotheses, ١٩٩٣; ٤١: ١٥٠-١٥٩.

Belinda, S.O., and Connell, M.S. Select Vitamins and Minerals in the Management of Diabetes. Diabetes Spectrum., ٢٠٠١; ١٤: ١٣٣-١٤٨.

Berg, J.M.; Tymoczko, J.L.; and Stryer, L. "Biochemistry" ٣rd ed. New York, Freeman W.H. & Company, ٢٠٠٢: ٢٢٣-٢٢٨.

Bishop, M.L.; Engelkirk, J.L.D.; and Fody, E.P. "Clinical Chemistry" ٤th ed. Philadelphia. Lippincott Williams & Wilkins Company, ٢٠٠٠: ٢٢٠-٢٣٠.

Blom, H.J. Consequences of Homocysteine Export and Oxidation in the Vascular System Semin. Thromb. Hemost., ٢٠٠٢; ٢٦: ٢٢٧-٢٣٢.

Boers, G.H. Mild Hyperhomocysteinemia is an Independent Risk Factor of Arterial Vascular Disease. Semin. Thromb. Hemost., ٢٠٠٠; ٢٦: ٢٩.

Borders, C.L.J.R., and Fridovich, I. A Comparison of the Effects of Cyanide Hydrogen Peroxide and Phenylglyxal on Eukaryotic and Prokaryotic Cu/Zn SOD. Arch. Biochem. Biophys. ١٩٨٥; ٢٤١: ٤٧٢-٤٧٦.

Bork, C. " antioxidant and Cancer" SCI. Med. ١٩٩٧: ١١٢: ٥٢-٦١.

Brownlee, M. “The Role of Non-Enzymatic Glycosylation in the Pathogenesis of Diabetic Angiopathy In: Draznin, B.; Melmed, S.; LcRoith, D. eds. “Molecular and Cellular Biology of Diabetes Mellitus.” Vol.III, New York, Alan R Liss, pp.: ٩-١٧.

Buettner, G.R.; Oberley, L.W. Buthionine Sulfoximine: Glutathione Depleter : Free Radicals in Biology and Medicine. Lowa University. Lowa, ٢٠٠١; ١-١٠.

Bursell, S.E.; Clermont, A.C.; Aiello, L.P.; Aiello, L.M.; Schlossman, D.K.; Feener, E.P.; Laffe, L., and King, G.L. High-Dose Vitamin E Supplementation Normalizes Retinal Blood Flow and Creatinine Clearance in Patients with Type ١ Diabetes. Diabetes Care, ١٩٩٩; ٢٢(٨): ١٢٤٥-١٢٥١.

Burtis, C.A., and Ashwood, E.R. “Tietz Textbook of Clinical Chemistry,” ٣rd ed., W.B. Saunders Company, Tokyo, ١٩٩٩, pp.: ١٠٣٤-١٠٥٤.

Cameron, N.E.; Cotter, M.A. “Neurovascular Dysfunction in Diabetic Rats: Potential Contribution of Autoxidation and Free Radicals Examined Using Transition Metal Chelating Agents. J. Clin. Invest. ١٩٩٥; ٩٦: ١١٥٩-١١٦٣.

Cedeberg, J. “Oxidative Stress, Antioxidative Defense and Outcome of Gestation in Experimental Diabetic Pregnancy,” (Thesis), Ph.D., Sweden, UpPsala University, ٢٠٠١.

Ceriello, A. Hyperglycemia: The Bridge between Non-Enzymatic Glycation and Oxidative Stress in the Pathogenesis of Diabetic Complications. Diabetes. Nutr. Metab., ١٩٩٩; ١٢(١): ٤٢-٦.

Cerutti, P.A. “Oxy-Radicals and Cancer.” Lancet, ١٩٩٤; ٣٤٤: ٨٦٢-٨٦٣.

Champe, P.C., and Harvey, R.A. “Lippincott’s Illustrated Reviews: Biochemistry.” ٢nd. Ed. Philadelphia, Lippincott Williams & Wilkins Company, ١٩٩٤: ٢٩٥-٣٠٢.

Chase, H.P. Cigarette Smoking Increases the Risk of Albuminurea among Subjects with type ١ Diabetes. JAMA. ١٩٩١; ٢٦٥(٥): ٦١٤-٦١٧.

Chelchowska, M.; Laskowska-Klita, T., and Szymbork, J. Level of Retinol and Beta-Carotene in Plasma of Smoking and Non-Smoking Women. Wiad-Lek., ٢٠٠١; ٥٤(٥-٦): ٢٤٨-٥٤.

Chow, C.K. Cigarette Smoking and Oxidative Damage in the Lung. Ann. N.Y. Acid Sci. ١٩٩٣; ٦٨٦: ٢٨٩-٩٨.

Chow, F.I., and Omaye, S.I. “Lipids,” ١٩٨٣; ١٨: ٨٣٧-٨٤١. In: Salman A.M.H. “Clinical Biochemical Study of Oxidation and Antioxidants in Patients with Non-Insulin-Dependent Diabetes Mellitus” (Thesis), Ph.D., Iraq, Baghdad University, ٢٠٠١.

Church, D.F., and Pryor, W.A. Free Radical Chemistry of Cigarette Smoke and its Toxicology Implication. Environ. Health Perspect., ١٩٨٥; ٦٤: ١١١-١٢٦.

Collier, A.; Rumley, A.; Rumely, A.G.; Paterson, J.R.; Leach, J.P.; Lowe, G.D.O, and. Small, M. Free Radical Activity and Homostatic Factors in NIDDM Patients with and without Microalbuminuria. Diabetes, ١٩٩٢; ٤١: ٩٠٩-٩١٣.

Colman, P.G.; Wang, L.I., and Lafferty, K.J. Molecular Biology and Autoimmunity of Type ١ Diabetes Mellitus, ١٩٨٩; In: Drazini, B.; Melmed, S.; Lepoith, D., eds. Molecular and Cellular Biology of Diabetes Mellitus. Insulin Secretion. New York, Alan R. Liss Inc., pp.: ١٢٥-١٣٧.

Corbett, J.A.. and McDaniel, M.L. Intraislet Release of Interleukin ١ Inhibits B-Cell Expression of Inducible Nitric Oxide Synthesis. J. Exp. Med., ١٩٩٥; ١٨١: ٥٥٩-٥٦٨.

Cordoba, P.A.; Sanches, J.L.Q.; Gonzalez, F.S; Ordone, J.L., and Blanco, F.V. Susceptibility of Plasma Low- and High-Density Lipoproteins to Oxidation in Patients with Severe Hyperhomocysteinemia. J. Mol. Med., ١٩٩٦; ٢٤: ٧٧١-٧٧٦.

Cunningham-Rundles, S. Zinc Modulation Immune Function: Specificity and Mechanism of Interaction. J. Lab. Clin. Med., ١٩٩٦; ١٢٨: ٩-١١.

Delmas-Beanvieux, M.C.; Peuchant, E.; Couchouron, A.; Constans, J.; Sergeant, C.; Simonoff, M.; Pellegrin, J.L.; Leng, B.; Corni, C., and Clerc, M. The Enzymatic Antioxidant System in Blood and Glutathione Status in Human Immunodeficiency Virus (HIV)-Infected Patients: Effects of Supplementation with Selenium or Beta-Carotene. Am. J. Clin. Nutr., ١٩٩٦; ٦٤: ١٠١-١٠٧.

Domingues, C.; Ruiz, E.; Gussinye, M., and Carrascosa, A. Oxidative Stress at Onset and Early Stages of Type ١ Diabetes in Children and Adolescents. Diabetes Care, ١٩٩٨; ١٢(١٠): ١٧٣٦-١٧٤٢.

Draper, H.H.; Squires, E.J.; Mahmoodi, H.; Wu, J.; Agarwal, S., and Hadley, M. A Comparative Evaluation of Thiobarbituric Acid Methods for the Determination of Malondialdehyde in Biological Materials. Free Radical Biology and Medicine. ١٩٩٣; ١٥: ٣٥٣-٣٦٣.

Dzeja, P.P., and Terizc, A. Phosphotransfer Networks and Cellular Energetics. Journal of Experimental Biology., ٢٠٠٣; ٢٠٦: ٢٠٣٩-٢٠٤٧.

Edwards, C.R.W., and Bouchier, J.A.D. "Disease of Liver and Biliary System: Principles and Practice of Medicine" Eds. Churchill Press. Livingstone. ١٩٩١, pp. ٤٩١-٤٩٢.

Elgawish, A.; Glomb, M.; Friendlander, M., and Monnier, V.M. Involvement of Hydrogen Peroxide in Collagen Cross-Linking by Glucose In Vitro and In Vivo. J. Bio. Chem. ١٩٩٥; ٢٧١: ١٢٩٦٤-١٢٩٧١.

Ewadh, M.J., and Jabir, F.A. Homocystine Induce Endothelial Cells Damage Due to Glutathione Level Depletion. Journal of Al-Qadisiya, Pure Sciences, ٢٠٠٢; ٦(٤):

Faure, P.; Benhamou, P.Y.; Perard, A.; Halimi, S., and Roussel, A.M. Lipid Peroxidation in Insulin-Dependant Diabetic Patients with Early Retina Degenerative Lesions: Effects of an Oral Zinc Supplementation. Eur. J. Clin. Nutr., ١٩٩٥; ٤٩(٤): ٢٨٢-٨.

Fernandez-Real, J.M.; Lapez-Bermejo, A., and Ricart, W. Cross-Talk between Iron Metabolism and Diabetes. Diabetes, ٢٠٠٢; ١٥: ٢٣٤٨-٢٣٥٤.

Fiskerstrand, T.; Refsum, H.; Kvalheim, G., and Ueland, P.M. Homocysteine and other Thiol in Plasma and Urine: Automated Determination and Sample Stability. Clin. Chem., ١٩٩٣; ٣٩: ٢٦٣-٧١.

Fodinger, M.; Mannhalter, C.; Wolf, G.; Pabinger, I.; Muller, E.; Schmid, R.; Horl, W.H., and Sunder-Plassman, G. Mutation (٦٧٧C to T) in the Methylenetetrahydrofolate Reductase gene Aggravates

Hyperhomocysteinemia in Hemodialysis Patients. Kidney Int., ١٩٩٧; ٥٢: ٥١٧-٥٢٣.

Frank, S.; Kämffer, H.; Podda, M.; Kaufmann, R., and Pfeils-Chifter, P. Identification of Copper/Zinc Superoxide Dismutase as a Nitric Oxide-Regulated Gene in Human (Ha CaT) Keratinocytes: Implication for Keratinocyte Proliferation. Biochem. J., ٢٠٠٠; ٣٤٦: ٧١٩-٧٢٨.

Franz, M.J., and Bantle, J.P. "American Diabetes Association Guide to Medical Nutrition Therapy for Diabetes" Eds., Alexandria Va. Press, American Diabetes Association, ١٩٩٩.

Franz, M.J., and Bantle, J.P.; Beebe, C.A.; Brunzell, J.D.; Chiasson, J.L.; Garg, A.; Holzmeister, L.A.; Hoogwerf, B.; Mayer-Davis, E.; Moordian, A.D.; Purnell, J.Q., and Wheeler, M. Evidence Based Nutrition Principles and Recommendations for the Treatment and Prevention of Diabetes and Related Complications. Diabetes Care, ٢٠٠٢; ٢٥: ١٤٨-١٩٨.

Fujimoto, S.; Kawakami, N., and Ohara, A. Non-Enzymatic Glycation of Transferrin: Decrease of Iron-Binding Capacity and Increase of Oxygen Radical Production. Biol. Pharm. Bull., ١٩٩٥; ١٨: ٣٩٦-٤٠٠.

Fuller, C.J.; Chandalia, M.; Gary, A.; Grundy, S., and Jialal, I. RRR-alpha-tochopheryl acetate supplementation at pharmacologic doses decreases low-density-lipoprotein oxidative susceptibility but not protein glycation in proteins with diabetes mellitus. A. J. Clin. Nutr. ١٩٩٦; ٦٣(٥): ٧٥٣-٩.

Ganong, W.F. “Review of Medical Physiology” ١٨th ed. Hall International Com. USA., ١٩٩٧, pp. ٣١٣-٣١٧.

Gazzaruso, C.; Garzaniti, A.; Giordanetti, S.; Falcone, C., and Fratino, P. Silent Coronary Artery Disease in Type ٢ Diabetes Mellitus: The Role of Lipoprotein (a), Homocysteine and apo(a) Polymorphism. Cardiovascular Diabetology, ٢٠٠٢; ١: ٥.

Gerbitz, K.D. Does the Mitochondrial DNA Play a Role in the Pathogenesis of Diabetes?, Diabetologia, ١٩٩٢; ٣٥: ١١٨١-١١٨٦.

Gillham, B.; Papachristodoulou, D.K., and Thomas, J.H. “Free Radical in Health and Disease In: Will’s Biochemistry Basis of Medicine” ٣rd ed., Butterwort Heinemann Press. ٢٠٠٠; pp. ٣٤٦-٣٤٧.

Glugliano, D.; Ceriello, A., and Paolisso, G. Diabetes Mellitus, Hypertension, and Cardiovascular Disease: Which Role for Oxidative Stress. Metabolism, ١٩٩٥; ٤٤: ٣٦٣-٣٦٨.

Glugliano, D.; Eriello, A., and Paolisso, G. Oxidative Stress and Diabetic Vascular Complication. Diabetes Care, ١٩٩٦; ١٩: ٢٥٧-٢٦٧.

Goughlin, S.S., and Hall, I.J. Genetics in Medicine: Glutathione S-transferase Polymorphisms and Risk of Ovarian Cancer. Atlanta. ٢٠٠٢; ٤(٤): ٢٥٠-٥٧.

Gunst, J.J.; Langlois, M.R., and Delanghe, J.R. Serum Creatin Kinase Activity is not a Reliable Marker for Muscle Damage in Conditions Associated with Low Extracellular Glutathione Concentration. Clinical Chemistry, ١٩٩٨; ٤٤(٥): ٩٣٩-٩٤٣.

Habig, W.H.; Pabst, M.J., and Jakoby, W.B. Glutathione S-transferase, the First Enzymatic Step in Mercapturic Acid Formation. J. Biol. Chem. ١٩٧٤; ٢٤٩: ٧١٣٠-٧١٣٩.

Hadwan, M.H. "Diabetes Correlation between Creatine Kinase Activity and Antioxidants in Diabetes Mellitus Type ١ and Type ٢" (Thesis), M.Sc., Iraq, Babylon University, ٢٠٠٥.

Halliwell, B., and Gutteridge, J.M.C. "Free Radicals in Biology and Medicine" ٣rd ed., Oxford University Press. UK., ١٩٩٩.

Halliwell, B., and Aruoma. O.I. DNA Damage by Oxygen-Derived Species. Its Mechanism and Measurement in Mammalian System. FEBS Lett., ١٩٩١; ٢٨: ٩-٩١.

Handelman, G.J. The Evolving Role of Carotenoid in Human Biochemistry. Nutrition. ٢٠٠١; ١٧(١٠): ٨١٨-٢٢.

Harris, E. The Iron-Copper Connection: The Risk of Ceruloplasmin Grows Stronger. Nutr. Rev., ١٩٩٥; ٥٣: ١٧٠-١٧٣.

Hayden, M.R., and Tyagi, S.C. Homocysteine and Reactive Oxygen Species in Metabolic Syndrome, Type ٢ Diabetes Mellitus, and Atherosclerop athy: The Pleiotropic Effect of Folate Supplementation. Nutr. J., ٢٠٠٤; ٣: ٤.

Hayden, M.R., and Tyagi, S.C. Myocardial Redox Stress and Remodeling in Metabolic Syndrome, Type ٢ Diabetes Mellitus and Congestive Heart Failure. Med. Sci. Monit., ٢٠٠٣; ٩: SR ٣٥-٥٢.

Hayden, M.R., and Tyagi, S.C. Islet Redox Stress: The Manifold Toxicities of Insulin Resistance, Metabolic Syndrome and Amylin Derived Islet Amyloid in Type ٢ Diabetes Mellitus. JOP. ٢٠٠٢; ٣: ٨٦-١٠٨.

Hayden, M.R., and Tyagi, S.C. Intimal Redox Stress: Accelerated Atherosclerosis in Metabolic Syndrome and Type ٢ Diabetes Mellitus. Atheroscleropathy. Cardiovasc. Diabetol. ٢٠٠٢; ١: ٣.

Hayden, M.R., and Tyagi, S.C. Is Type ٢ Diabetes Mellitus a Vascular Disease (Atheroscleropathy) with Hyperglycemia a Late Manifestation? The Role of NOS, NO and Redox Stress. Cardiovasc. Diabetol., ٢٠٠٣; ٢: ٢.

Hayes, A.W. "Metals In: Principles and Methods of Toxicology" ed. Tylor and Francis. ٢٠٠١; pp. ٦٦١-٦٦٣.

Hermes-Lima, M.; Willmore, W.G., and Story, K.B. Quantification of Lipid Peroxidation in Tissue Extracts Based on Fe(III) Xylenol Orange Complex Formation. Free Radical Biology and Medicine. ١٩٩٥; ١٩: ٢٧١-٢٨٠.

Heury, R.J. "Clinical Chemistry Principles and Techniques" Ed. New York, Harper & Row Press, ١٩٦٨; p. ٣٨٦.

Higgis, P.J., and Bunn, H.F. Kinetic Analysis of the Non-Enzymatic Glycosylation of Hemoglobin. J. Biol. Chem., ١٩٨١; ٢٥٦: ٥٢٠٤-٥٢٠٨.

Hoogeveen, E.K.; Kostense, P.J., and Jakobs, C. Hyperhomocysteinemia Increase Risk of Death, Especially in Type ٢ Diabetes: ٥ Years Follow-Up of the Hoorn Study. Circulation, ٢٠٠٠; ١٠١: ١٥٠٦-١٥١١.

Hoogeveen, E.K.; Kostense, P.J., and Eysink, P.E. Hyperhomocysteinemia is Associated with the Presence of Retinopathy in Type ٢ Diabetes Mellitus: The Hoorn Study. Arch. Intern. Med., ٢٠٠٠; ١٦٠: ٢٩٨٤-٢٩٩٠.

Howard, J.A. “Advances in Free Radical Chemistry.” ١st ed., Academic Press. New York, ١٩٧٢.

Hristozova, T.; Rashera, T.; Nedeva, T., and Kujumdzieva, A. Superoxide Dismutase During Glucose Repression of Hansenula Polymorpha. CBS٤٧٣٢. Z. Naturforsch., ٢٠٠٠; ٥٧: ٣١٣-٣١٨.

Imlay. J.A., and Linn, S. DNA Damage and Oxygen Radical Toxicity. Science, ١٩٨٨; ٢٤٠: ١٣٠٢-١٣٠٩.

Inal, M., and Kanbak, G. Antioxidant Status and Lipid Peroxidation in Red Blood Cells of P-Aminophenol-Treated Rats. Med. Sci. Res., ١٩٩٧; ٢٥: ٣٢٣-٣٢٥.

Ioannides, C.; Barnett, C.R.; Irizar, A., and Flatt, P.R. Drug Metabolism and Chemical Toxicity in Diabetes In: “Drugs, Diet and Disease” Eds., Vol.٢. Ionnides, C.; Flatt, P.R.; Hemel Hempstead Press, UK. Ellis Horwood, ١٩٩٥; pp. ٣٣٠-٣٥٣.

Jacobs, R.L.; House, J.D.; Brosnan, M.E., and Brosnan, J.T. Effects of Sterptozotocin-Induced Diabetes and of Insulin Treatment on Homocysteine Metabolism in the Rat. Diabetes, ١٩٩٨; ٤٧: ١٩٦٧-١٩٧٠.

Jain, S.K., and McVie, R. Effect of Glycemic Control, Race (White versus Black) and Duration of Diabetes on Reduced Glutathione Content in Erythrocytes of Diabetic Patients. Metabolism, ١٩٩٨; ٤٢(١): ٢٠-٢٣.

James, R.W.; Levier, I., and Righetti, A. Smoking is Associated with Reduced Serum Paraoxonase Activity and Concentration in

Patients with Coronary Artery Disease. Circulation, ٢٠٠٠; ١٠١: ٢٢٥٢-٩.

Javanovic, L.; Gutierrez, M., and Peterson, C.M. Chromium Supplementation for Women with Gestational Diabetes Mellitus. J. Trace Elem. Exp. Med., ١٩٩٩; ١٢: ٩١-٩٧.

Jennings, P.E.; Mclaren, M.; Scott, N.A.; Saniabadi, A.R., and Blech, J.J. The Relationship of Oxidative Stress to Thrombotic Tendency in Type ١ Diabetic Patients with Retinopathy. Diabetic Medicine, ١٩٩١; ٨: ٨٦٠-٨٦٥.

Juckett, M.R.; Balla, J.;Balla, G.; Jessurun, J. Jacob, H.S., and Vercellotti, G.M. Ferritin Protects Endothelial Cells from Oxidized Low Density Lipoprotein In Vitro. Am. J. Pathol., ١٩٩٥; ١٤٧: ٧٨٢-٧٨٩.

Kanbak, G.; Inal, M., and Baycu, C. The Role of Free Radicals in P-Aminophenol-Induced Nephrotoxicity Does Reduced Glutathione Have a Protective Effect. Clinical Chimica Acta., ١٩٩٦; ٢٥٢: ٦١-٧١.

Kaneto, H.; Fujji, J.; Myint, T.; Miyazawa, N.; Islam, K.N.; Kawasaki, Y.; Suzuki, K.; Nakamura, M.; Tatsumi, H.; Yamasaki, Y., and Taniguchi, N. Reducing Sugars Tigger Oxidative Modification and Apoptosis in Pancreatic Beta-Cells by Provoking Oxidative Stress through the Glycation Reaction. Biochemical Journal, ١٩٩٦; ٣٢٠: ٨٥٥-٨٦٣.

Kannel, W.B. Cigarettes Coronary Occlusion and Myocardial Infrction. J. AMA. ١٩٨١; ٢٤٦: ٨٧١-٢.

Kasai, H., and Nishimura, S. Hydroxylation of Guanine in Nucleosides and DNA at the C-٨ Position by Heated Glucose and Oxygen

Radical Forming Agents. Environmental Health Perspective. ١٩٨٦; ٦٧: ١١١-١١٦.

Kashiwagi, A.; Asahina, T., and Ikebuchi, M. Abnormal Glutathione Metabolism and Increased Cytotoxicity Caused by H_2O_2 in Human Umbilical Vein Endothelial Cells Cultured in High Glucose Medium. Diabetologia, ١٩٩٤; ٣٧: ٢٦٤-٢٦٩.

Keith, R.E. Antioxidant and Health. Nutrition and Food Science, ١٩٩٩; ١: ١-٥.

Kelley, D.E.; He, J.; Menshikova, E.V., and Ritov, V.B. Dysfunction of Mitochondria in Human Skeletal Muscle in Type ٢ Diabetes. Diabetes, ٢٠٠٢; ١٥: ٢٩٤٤-٢٩٥٠.

Kelly, S.A.; Havrilla, C.H.M.; Brady, T.C.; Abramo, K.H., and Levin, E.D. Oxidative Stress in Toxicology: Established Mammalian and Emerging Piscine Model Systems Review. Environmental Health Perspective, ٢٠٠٥; ١١٣(٧).

Khanna, S.; Atalay, M.; Laaksonen, E.E.; Roy, S., and Sen, C.K. Alpha-lipoic acid supplementation: tissue glutathione hemeostasis at rest and after exercise. Journal of Applied Physiology, ١٩٩٩; ٨٦:١١٩١-١١٩٦.

Kim, H.S., and Miller, D.D.: Proline-Rich Proteins Moderate the Inhibitory Effect of Tea on Iron Absorption in Rats. J. Nutr., ٢٠٠٥; ١٣٥: ٥٣٢-٥٣٧.

Kinlaw, W.B.; Levine, A.S., and Morley, J.E. Abnormal Zinc Metabolism in Type II Diabetes Mellitus. Am. J. Med.,١٩٨٣; ٧٥: ٢٧٣-٧.

Knight, G.A. “Oxidative Stress and Free Radical Antioxidants Aging and Disease” ed. American Association for Clinically Chemistry. ١٩٩٩; pp. ٣٠, ٣١٥.

Langseth, L. Oxidants, Antioxidants and Disease Prevention. Belgium, International Life Science Institute, ١٩٩٦.

Leahy, J.L., and Weir, G.C. Non-Insulin-Dependant Diabetes Mellitus: Current Concepts of Pathogenesis In: Draznin, B.; Melmed, S. , and LeRoith, D. eds. Molecular and Cellular Biology of Diabetes Mellitus. Insulin Secretion. New York, Alan, R. Liss Inc., ١٩٨٩; pp.: ١٤٩-١٥٨.

Lehninger, A.L. “Biochemistry: The Molecular Basis of Cell Structure and Function” ٢nd ed. Worth Press. New York, ١٩٧٥.

Lim, P.S.; Chan, E.C.; Lu, T.C.; Yu, Y.C.; Kuo, S.Y.; Wang, T.H., and Wei, Y.H. Lipophilic Antioxidants and Iron Status in ESRD Patients on Hemodialysis. Nephron, ٢٠٠٠; ٨٦: ٤٢٨-٤٣٥.

Lokitch, G.; Godophin, W., and Pendray, M. R. Serum Zinc, Copper, Retinol-binding, Prealbumin, and Ceruloplasmin Concentrations in infant Receiving intravenous Zinc and Copper supplementation. J. Pediat., ١٩٩٨; ١٠٢: ٣٠٤.

Lunec, J.; Halloran, S.P.; White, A.C., and Domrmandy, T.L. Free Radical Oxidation (Peroxidation) Products in Serum and Synovial Fluid in Rheumatoid Arthritis. J. Rheumatol., ١٩٨١; ٨: ٢٣٣-٢٤٥.

Lunec, J.: Review Articale. Ann. Clin. Biochem., ١٩٩٠; ٢٧: ١٧٣-١٨٢.

Luo, M.; Kannar, K.; Wahlqvist, M.L., and O’Brien, R.C.: Inhibition of LDL Oxidation by Green Tea Extract. Lancet, ١٩٩٧; ٣٤٩:٣٦٠-١.

Luxton, R. “Clinical Biochemistry” ١st ed. Butterworth-Heinemann Press. Oxford, ١٩٩٩.

MacRury, S.M.; Gordon, D.; Wilson, R.; Bradley, H.; Gemmell, C.G.; Paterson, J.R.; Rumley, A.G., and MacCuish, A.C. A Comparison of Different Methods of Assessing Free Radical Activity in Type ٢ Diabetes and Peripheral Vascular Disease. Diabetic Medicine, ١٩٩٣; ١٠: ٣٣١-٣٣٥.

Malchoff, C.D. Diagnosis and Classification of Diabetes Mellitus. Conn. Med., ١٩٩١; ٥٥(١١): ٦٢٥.

Mannervik, B. , and Banielson, V.H. Glutathione Transferase S- Structure and Catalytic Activity. Crit. Rev. Biochem., ١٩٨٨; ٢٣(٣): ٢٨٣-٣٣٧.

Marangon, K.; Herbeth, B.; Lecomte, E.; Paul-Dauphin, A.; Grolier, P.; Chancerlle, Y., and Artur, Y. Diet-Antioxidant Status and Smoking Habits in French men. Am. J. Cln. Nutr., ١٩٩٨; ٦٧: ٢٣١-٢٣٩.

Mari, M., and Cederbaum, A.I. Induction of Catalase, Alpha and Microsomal Glutathione S-Transferase in CYP٢E١ Overexpressing HepG٢ Cells and Protection against Short-Term Oxidative Stress. Hepatology., ٢٠٠١; ٣٣: ٦٥٢-٦٦١.

Marra, G.; Cotroneo, P.; Pitocco, D.; Manto, A.; Dileo, M.A.S.; Rnotolo, V.; Caputo, S.; Giardino, B.; Ghirlanda, G., and Santini, S.A. Early Increase of Oxidative Stress and Reduced Antioxidant Defense in Patients with Uncomplicated Type ١ D.M. Diabetes Care, ٢٠٠٢; ٢٥: ٣٧٠-٣٧٥.

Mayesca, J.B., and Holden, H.E. Genetic Effects of P-Aminophenol in Chinese Hamster and Mouse Lymphoma Cells: Results of a Multiple Endopoint Test. Environ. Mol. Mutagen., ١٩٩٥; ٢٦: ١٦٣-١٧٠.

Mawim-Mateo, M.C.; Molpeceres, L.M., and Gamos, G. Assay for Erythrocyte Superoxide Dismutase Activity in Patients with Lung Cancer and Effects on Population and Smoke Trace Elements. Biological Trace Element Research, ١٩٩٧; ٦٠: ٢١٥-٢٢٦.

McCord, J.M. Is Iron Sufficiently a Risk Factor in Ischemic Heart Disease. Circulation, ١٩٩١; ٨٣: ١١١٢-١١١٤.

Meister, A. Glutathione Metabolism. Methods in Enzymology, ١٩٩٥; ٢٥١: ٣-٧.

Mekhfi, H.; Veksler, V.; Mateo, P.; Maupoil, V.; Rochette, L., and Ventura-Clapier, R. Creatine Kinase is the Main Target of Reactive Oxygen Species in Cardiac Myofibrils. Circulation Research, ١٩٩٦; ٧٨: ١٠١٦-١٠٢٧.

Meneghini, R. Iron Homeostasis, Oxidative Stress, and DNA Damage. Free Radic. Biol. Med., ١٩٩٧; ٢٣(٥): ٧٨٣-٧٩٢.

Mezzetti, A.; Lappenna, D., and Pierdomenico, S.D. Vitamin E, C, and Lipid Peroxidation in Plasma and Arterial Tissue of Smokers and Non-Smokers. Atherosclerosis, ١٩٩٥; ١١٢: ٩١-٩٩.

Michiels, C.; Raes, M.; Toussan, O., and Remacie, J. Importance of Se-Glutathione, Peroxidase, Catalase, and Cu-Zn-SOD for Cell Survival against Oxidative Stress. Free Radical Biological Medicine, ١٩٩٤; ١٧: ٢٣٥-٢٤٨.

Miro, O.; Alonso, J.R., and Jarreta, D. Smoking Disturbs Mitochondrial Respiratory Chain Function on Human Circulating Lymphocytes. Carcinogenesis, ١٩٩٩; ٢٠(٧): ١٣٣-٦.

Morrow, J.; Frei, B., and Longmire, A. Increase in Circulating Products to Lipid Peroxidation ($F_{٢}$-Isoprostanes) in Smokers. N. Engl. J. Med., ١٩٩٥; ٣٣٢: ١١٩٨-٢٠٣.

Mudd, S.H.; Finkelstein, J.D.; Refsum, H.; Ueland, P.M.; Malinow, M.R.; Lentz, S.R.; Jacobsen, D.W.; Brattström, L.; Wilecken, B.; Wilckon, D.E.; Blom, H.J.; Stabler, S.P.; Allen, R.H.; Selhub, J., and Rosenberg, I.H. Homocysteine and its Disulfide Derivatives: A Suggested Consensus Terminology. Arterioscler. Thromb. Vase. Biol., ٢٠٠٠; ٢٠: ١٧٠٤-١٧٠٦.

Mueller, S.; Riedel, H.D., and Stremmel, W. Determination of Catalase Activity at Physiological Hydrogen Peroxide Concentrations. Analytical Biochemistry, ١٩٩٧; ٢٤٥: ٥٥-٦٠.

Mulhauser, I. Cigarette Smoking and Progression of Retinopathy and Nephropathy in Type ١ Diabetes Mellitus. Diabetes Med., ١٩٩٦; ١٣: ٥٣٦-٥٤٣.

Murray, R.K.; Granner, D.K.; Mayes, P.A., and Rodwell, V.W. “Harper’s Biochemistry” ٢٥th ed. Appleton & Lange Press, Lebanon, ٢٠٠٠; ٦٤٠-٦٤١.

National Diabetes Data Group. Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Tolerance. Diabetes, ١٩٧٩; ٢٨: ١٠٣٩-١٠٥٧.

Neumann, D.; Schlattner, V., and Walliamann, T. A Molecular Approach to the Concerted Action of Kinase Involved in Energy Homoestasis. Biochem. Soc. Trans., ٢٠٠٣; ٣١: ١٦٩-١٧٤.

Noto, R.; Alicata, R.; Sfoglian, L.; Neri, S. , and Bifarella, M. A Study of Cupermia in a Group of Elderly Diabetics. Acta. Diabet. Lat., ١٩٨٣; ٢٠: ٨١-٥.

Nourooz-Zadeh, J.; Rahimi, A.; Jajaddini,-Sarmadi, J.; Tritschler, H.; Rosen, P.; Halliwell, B., and Betteridge, D.J. Relationship between Plasma Measures of Oxidative Stress and Metabolic Control in NIDDM. Diabetologia, ١٩٩٧; ٤٠: ٦٤٧-٦٥٣.

Oberley, L.W. Free Radical and Diabetes. Free Radical Bio. Med., ١٩٨٨; ٥: ١١٣-١٢٤.

Okada, E.; Oida, K., and Tada, H. Hyperhomocysteinemia is a Risk Factor for Coronary Arteriosclerosis in Japanese Patients with Type ٢ Diabetes. Diabetes Care, ١٩٩٩; ٢٢: ٤٨٤-٤٩٠.

Ou, P., and Wolff, S.P. Erythrocyte Catalse Inactivation (H_2O_2 Production) by Ascorbic Acid and Glucose in the Presence of Aminotriazol: Role of Transition Metals and Relevance to Diabetes. Biochemical Journal, ١٩٩٤; ٣٠٣: ٩٣٥-٩٣٩.

Palmer, H.J., and Paulson, E.K. Reactive Oxygen Species and Antioxidants in Single Transduction and Gene Expression. Nutr. Rev., ١٩٩٧; ٥٥: ٥٣٥-٦١.

Paolisso, G.; Balbi, V.; Volpa, C. Varricchio, G.; Gambardella, A. Saccomanno, F.; Ammendola, S.; Varricchio, M., and D'Onofrio, F. Metabolic Benefits Deriving from Chronic Vitamin C Supplementation in Aged Non-Insulin-Dependant Diabetics. J. Am. Coll. Nutr., ١٩٩٥; ١٤(٤): ٣٨٧-٩٢.

Paolisso, G.; Tagliamonte, M.R.; Barbieri, M.; Zito, G.A.; Gambardella, A.; Varricchio, G.; Ragno, M., and Varricchio, M. Chronic

Vitamin E Administration Improves brachial Reactivity and Increases Intracellular Magnesium Concentration in Type II Diabetic Patients. Journal of Clinical Endocrinology and Metabolism, ٢٠٠٠; ٨٥(١): ١-١٧.

Pastore, A.; Massoud, R.; Motti, C.; LoRusso, A.; Fucci, G.; Cortese, C., and Federici, G. Fully Automated Assay for Total Homocysteine, Cysteine, Cysteinglycine, Glutathione, Cysteamine, and ٢-Mercaptopropionylglycine in Plasma and Urine. Clinical Chemistry, ١٩٩٨; ٤٤(٤): ٨٢٥-٨٣٢.

Percival, S. Neutropenia Caused by Copper Deficiency Possible Mechanisms of Action. Nutr. Rev., ١٩٩٥; ٥٣(٣): ٥٩-٦٦.

Peuchant, E.; Delmas-Beauvieux, M.C.; Couchouron, A.; Dubourg, L.; Thomas, M.J.; Perromet, A.; Clerc, M., and Gin, H. Short-Term Insulin Therapy and Normoglycemia. Effects on Erythrocyte Lipid Peroxidation in NIDDM Patients. Diabetes Care, ١٩٩٧; ٢٠: ٢٠٢-٢٠٧.

Popovich, B.K.; Sayen, M.R., and Dillmann, W.H. Insulin Responsiveness of Ck-M and Ck-BmRNA in the Diabetic Rat Heart. Am. J. Physiol. Endocrinol. Metab., ١٩٩١; ٢٦١(٣): ٣٧٧-٣٨١.

Popovich, B.K.; Boheler, K.R., and Dillmann, W.H. Diabetes Decreases Creatine Kinase Enzyme Activity and mRNA Level in the Rat Heart. Am. J. Physiol. Endocrinol. Metab., ١٩٨٩; ٢٥٧(٤): ٥٧٣-٥٧٧.

Pryor, W.A. , and Ston, K. Oxidant in Cigarette Smoke. Radicals, Hydrogen Peroxide, Peroxynitrate and Peroxynitrite. Ann NY Acad. Sci., ١٩٩٣; ٦٨٦: ١٢-٢٧.

Rabini, R.A.; Fumelli, P.; Galassi, R.; Dousset, N.; Tans, M.; Ferretti, G.; Mazzanti, L.; Curatola, G.; Solera, M.L., and Valdiguie, P. Increased Susceptibility to Lipid Oxidation of Low-Density Lipoproteins and Erythrocyte Membranes from Diabetic Patients. Metabolism, ١٩٩٤; ٤٣: ١٤٧٠-١٤٧٤.

Rahbani-Nobar, M.E.; Rahimi-Pour, A.; Rahbani-Nobar, M.; Adi-Beig, F., and Mirhashemi, S.M. Total Antioxidant Capacity Superoxide Dismutase and Glutathione Peroxidase in Diabetic Patients. Medical Journal of Islamic Academy of Sciences, ١٩٩٩; ١٢(٤): ١-٧.

Rajpathak, S.; Rimm, E.B.; Tricia, L.; Morris, J.S.; Stampter, M.J.; Willett, W.C., and Hu, F.B. Lower Toenial Chromium in Men with Diabetes and Cardiovascular Disease Compared with Healthy Men. Diabetes Care, ٢٠٠٤; ٢٧: ٢٢١١-٢٢١٦.

Rasmussen, K. , and Moller, J. Total Homocysteine Measurement in Clinical Practice. Ann Clin. Biochem., ٢٠٠٠; ٣٧: ٦٢٧-٦٤٨.

Ravina, A.; Slezak, L.; Mirsky, N.; Bryden, N.P., and Anderson, R.A. Reversal of Corticosteroid-Induced Diabetes with Supplementation Chromium. Diabet. Med., ١٩٩٩; ١٦: ١٦٤-١٦٧.

Raza, H.; Robin, M.A.; Fang, J.K., and Avadhani, N.G. Multiple Isoforms of Mitochondrial Glutathione S-Transferases and their Differential Induction under Oxidative Stress. Biochem. J., ٢٠٠٢; ٣٦٦: ٤٥-٥٥.

Raza, H.; Prabu, S.K.; Robin, M.A., and Avadhani, N.G. Elevated Mitochondrial Cytochrome P٤٥٠ ٢El and Glutathione S-Transferase A٤-٤ in Streptozotocin-Induced Diabetic Rats. Diabetes, ٢٠٠٤; ٥٣: ١٨٥-١٩٤.

Reddy, S.; Jones, A.D.; Cross, C.E.; Wong, P.S-X., and Van der Vlient, A. Inactivation of Creatine Kinase by S-Glutathionylation of the Active-Site Cysteine Residue. Biochem. J., ٢٠٠٠; ٣٤٧: ٨٢١-٨٢٧.

Reif, D.W. Ferritin as a Source of Iron for Oxidative Damage. Free Rad. Biol. Med., ١٩٩٢; ٢: ٤١٧-٤٢٧.

Rice, J. "Principles of Pharmacology for Medical Assisting" ٣rd ed. Canada, International Thomson Publishing Company Ltd., ١٩٩٩; ٣٣٨-٣٤٢.

Ritz, E.; Keller, C., and Bergis, K. Nephropathy of Type II Diabetes Mellitus. Nephrol. Dial. Transplant, ١٩٩٦; ١١(٩): ٣٨-٤٤.

Ros, R.C., and Nahrwold, D.L. Anal. Biochem., ١٩٨١; ١١٤: ١٤٠-١٤٥. In: Salman, A.M.H. "Clinical Biochemical Study of Oxidation and Antioxidants in Patients with Non-Insulin-Dependant Diabetes Mellitus" (Thesis), Ph.D., Iraq, Baghdad University, ٢٠٠١.

Sahin, U.; Unlu, M.; Ozguner, F.; Stucu, R.; Akkaga, A., and Delibas, N. Lipid Peroxidation and Glutathione Peroxidase Activity in Chronic Obstractive Pulmonary Disease Exacerbation: Prognostic Value of Malondialdehyde. J. Basic Clin. Physiol. Pharmacol., ٢٠٠١; ١٢(١): ٥٩-٦٨.

Salman, A.M.H. "Clinical Biochemical Study of Oxidation and Antioxidants in Patients with Non-Insulin-Dependant Diabetes Mellitus" (Thesis), Ph.D., Iraq, Baghdad University, ٢٠٠١.

Samman, S.; Sandström. B.; Toft, M.J.; Bukhave, K.; Jensen, M.; Sorensen, S.S., and Hansen, M. Green Tea or Rosemary Extract Added to Foods Reduces Nonhemeiron Absorption. Clinical Nutrition, ٢٠٠١; ٧٣(٣): ٦٠٧-٦١٢.

Sasagawa, S.; Suzuki, K.; Sakatani, T., and Fujikura, T. Effects of Nicotine on the Function of Human Polymorphonuclear Leukocyte In Vitro. Journal of Leukocyte Biology, ١٩٨٥; ٣٧: ٤٩٣-٥٠٢.

Sedlak, J., and Lindsay, R.H. Annal. Biochem., ١٩٦٨;٢٥:١٩٢.

Sen, C.K.Glutathione homeostasis in response to exercise training and nutritional supplements. Mol. Cell Biochem., ١٩٩٩; ١٩٦:٣١-٤٢.

Sen, C.K., and Hanninen, O. Physiological Antioxidants In: Exercise and Oxygen Toxicity. Ed. Sen, C.K.; Packer, L.; Hanninen, O. Amesterdam. Elsevier, ١٩٩٤; pp. ٨٩-١٢٦.

Sen, C.K.; Packer, L., and Hanninen, O. "Handbook of oxidants and antioxidants in exercise" ١st ed., Elsevier Press. Amsterdam, ٢٠٠٠.

Shanshel, A.A.; Hamash, M.H.; Hamid, A.W.R., and Abid, F.M. Determination of Homocysteine and Other Aminothiols in Plasma from Healthy Subjects and Patients with Ischemic Stroke. National Journal of Chemistry, ٢٠٠٣; ٩: ١٨٢-١٩٠.

Sharma, R.N.; Deva, D.; Dehera, D., and Khandja, K.L. Reactive Oxygen Species Formation in Peripheral Blood Neutrophils in Different Types of Smokers. Indian, J. Med. Res., ١٩٩٧; ١٠٦: ٤٧٥-٨٠.

Sinclair, A.J.; Barnett, A.H., and Lunec, J. Free Radicals and Antioxidant System in Health and Disease. Br. J. Hosp. Med., ١٩٩٠; ٤٣: ٣٣٤-٣٤٣.

Singh, R., and Pathak, D.N. Lipid Peroxidation and Glutathione Peroxidase, Glutathione Reductase, Superoxide Dismutase, Catalase, and Glucose-٦-Phosphate Dehydrogenase Activities in FeCl٣-Induced

Epileptogenic Foci in the Rat. Epilepsia, ١٩٩٠; ٣١(١): ١٥-٢٥.

Smulders, Y.M.; Rakic, M., and Slaats, E.H. Fasting and Posmethionine Homocysteine Levels in NIDDM. Determinants and Correlations with Retinopathy, Albuminuria, and Cardiovascular Disease. Diabetes Care, ١٩٩٩; ٢٢: ١٢٥-١٣٢.

Soinio, M.; Marniemi, J.; Laakso, M.; Lehto, S., and Ronnemaa, T. Elevated Plasma Homocysteine Level is an Indepenant Predictor of Coronary Patients with Type ٢ Diabetes Mellitus. Ann. Intern. Med., ٢٠٠٤; ١٤٠: ٩٤-١٠٠.

Stadtman, E.R., and Berlett, B.S. Reactive Oxygen-Mediated Protein Oxidation in Aging and Disease. Chem. Res. Toxicol., ١٩٩٧; ١٠: ٤٨٥-٤٩٤.

Starkebaum, G, and Harlan, J.M. Endothelial Cell Injury Due to Copper-Catalyzed Hydrogen Peroxide Generation from Homocysteine. J. Clin. Invest., ١٩٨٦; ٧٧: ١٣٧٠-١٣٧٦.

Suarez, L., and Barrett-Connor, E. Interaction between Cigarette Smoking and Diabetes Mellitus in the Prediction of Death Attributed to Cardiovascular Disease. Am. J. Epdemiol., ١٩٨٤; ١٢٠: ٦٧٠-٦٧٥.

Szaleezky, E.; Prechl, J.; Feher, J., and Somogyi, A. Alterations in Enzymatic Antioxidant Defense in Diabetes Mellitus – A Rational Approach. Postgrad Med. J., ١٩٩٩; ٧٥: ١٣-١٧.

Targher, G. Cigarette Smoking and Insulin Resistance in Patients with Non-Insulin-Dependant Diabetes Mellitus. J. Clin. Endocrinol. Metab., ١٩٩٧; ٨٢: ٣٦١٩-٣٦٢٤.

Terpilowsks, S., and Zaporowska, H. The Role of Chromium in Cell Biology and Medicine. Przegl. Lek., ٢٠٠٤; ٦١(٣): ٥١-٤.

Tesfamarian, B. Free Radicals in Diabetic Endothelial Cell Dysfunction. Free Radic. Bio. Med., ١٩٩٣; ١٦: ٣٨٣-٣٩١.

Tesfamarian, B. Free Radicals in Diabetic Endothelial Cell Dysfunction. Free Radical Biology and Medicine, ١٩٩٤; ١٦: ٣٨٣-٣٩١.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, ٢٠٠٣; ٢٦: ٥٥-٦٠.

Thornalley, P.J.; McLellan, A.C.; Lo, T.W.; Benn, J., and Sonksen, P.H. Negative Association between Erythrocyte Reduced Glutathione Concentration and Diabetic Complication. Clinical Science, ١٩٩٦; ٩١: ٥٧٥-٥٨٢.

Tietz, N.W.; Caraway, W.T.; Freier, E.F.; Kachmar, J.F. , and Rawnsley, H.M. “Fundamentals of Clinical Chemistry” ١st ed. W.B. Saunders Company, Canada, ١٩٧٩; pp. ٥٤٣-٥٤٤.

Tihant, T.; Ghiba, P., and Eher, R. Influence of Cell Cycle on Selenium Dependant Glutathione Peroxidase Carcinogeneses, ١٩٨٩; ١٠(٩): ١٧٠٩-١٢.

Tousoulis, D.; Antoniades, C.; Tountas, C.; Bosinakou, E.; Kotsopoulou, M.; Toutouzas, P., and Stefanadis, S. Vitamin C Effects Thrombosis/Fibrinolysis System and Reactive Hypermia in Patients with Type ٢ Diabetes and Coronary Artery Disease. Diabetes Care, ٢٠٠٣; ٢٦: ٢٧٤٩-٢٧٥٣.

Tripathi, K.D. “Essentials of Medical pharmacology” ٢nd ed., Jaypee Brothers Press, ١٩٨٨, pp: ٢٣٧-٢٤٣.

Trow, L.G.; Lewis, J.; Greenwood, R.H.; Sampson, M.J.; Self, K.A.; Grews, H.M., and Fairweather-Tait, S.J. Lack of Effect of Dietary Chromium Supplementation on Glucose Tolerance, Plasma Insulin and Lipoprotein Levels in patients with Type ٢ Diabetes. Int. J. Vitam. Nutr. Res., ٢٠٠٠; ٧٠: ١٤-١٨.

Tutuncu, N.B.; Bayraktar, M., and Varli, K. Reversal of Defective Nerve Conduction with Vitamin E Supplementation in Type ٢ Diabetes: A Preliminary Study. Diabetes Care, ١٩٩٨; ٢١(١١): ١٩١٥-٨.

Uchiama, M. , and Mihara, M. Determination of Malondialdehyde Precursors in Tissues by Thiobarbituric Acid Test. Anal. Biochem., ١٩٨٧; ٨٦: ٢٧١-٢٧٨.

Ulusu, N.N., and Turan, B. Beneficial Effects of Selenium on Some Enzymes of Diabetic Rat Hearts. Biol. Trace Elem. Res., ٢٠٠٥; ١٠٣(٣): ٢٠٧-١٦.

Valk Marx, J.J. Iron, Atherosclerosis, and Ischemic Heart Disease. Arch. Intern. Med., ١٩٩٩; ١٥٩: ١٥٤٢-١٥٤٨.

Van-Antwerpan, V.L.; Theron, A.J. , and Richard, G.A. Vitamin E, Pulmonary Function and Phagocyte-Mediated Oxidative Stress in Smokers and Non-Smokers. Free Radic. Biol. Med., ١٩٩٥; ١٨(٥): ٩٣٥-٤١.

Van-Guldener, C.; Stom, F., and Stehouwer, C.D. Homocysteine Metabolism in Renal Failure. Kidney Int., ٢٠٠١; ٥٩(٧٨): S٢٣٤-S٢٣٧.

Vasankari, T.; Ku, U., and Heinonen, O. Measurement of Serum Lipid Peroxidation During Exercise Using Three Different Methods: Diene Conjugation, Thiobarbutric Acid Reactive Material and Fluorescent Chromolipids. Clin Chim Acta., ١٩٩٥; ٢٣٤: ٦٣-٦٩.

Vatassery, G.T.; Johnson, G.T., and Krezcwski, A.M. Am. Cou. Nutr. ١٩٨٣; ٤: ٣٦٩. In: Salman, A.M.H. "Clinical Biochemical Study of Oxidation and Antioxidants in Patients with Non-Insulin-Dependant Diabetes Mellitus" (Thesis), Ph.D., Iraq, Baghdad University, ٢٠٠١.

Vijayalingam, S.; Parthibam, A.; Shanmugasundaram, K.R., and Mohan, V. Abnormal Antioxidant Status in Impaired Glucose Tolerance and Non-Insulin-Dependant Diabetes Mellitus. Diabetic Medicine, ١٩٩٦; ١٣: ٧١٥-٧١٩.

Vincent, J.B. Recent Advances in the Nutritional Biochemistry of Trivalent Chromium. Proc. Nutr., Soc., ٢٠٠٤; ٦٣: ٤١-٧.

Walter, R.M.; Uriu Hare, J.Y.; Olin, K.L.; Oster, M.H.; Anawalt, B.D.; Critchfield, J.W., and Keen, C.L. Copper, Zinc, Manganese, and Magnesium Status and Complications of Diabetes Mellitus. Diabetes Care, ١٩٩١; ١٤: ١٠٥٠-١٠٥٦.

Wang, H.; Zhang, Z.B., and Wen, R.R. Reduction of Erythrocyte Sorbitol by Ascorbic Acid in Patients with Diabetes Mellitus. Chung Hua I Hsueh Isa Chih., ١٩٩٤; ٧٤(٩): ٥٤٨-٥١, ٥٨٣.

Will, J.C., and Byers, T. Does Diabetes Mellitus Increase the Requirement for Vitamin C? Nutr. Rev., ١٩٩٦; ٥٤: ١٩٣-٢٠٢.

Winterboun, C.C.; Hawking, R.E.; Brain, M., and Carrel, R.W. Determination of Superoxide Dismutase. J. Lab. Clin. Med., ١٩٧٥; ٢: ٣٣٧-٣٤١.

Wolff, H.P. Cigarette Smoking Increases the Risk of Albuminurea among Subjects with Type ١ Diabetes. JAMA, ١٩٩١; ٢٦٥(٥): ٦١٤-٦١٧.

Wolff, S.P., and Dean, R.T. Glucose Autoxidation and Protein Modification. The Potential Role of Autoxidative Glycosylation in Diabetes. Biochem. J., ١٩٨٧; ٢٤٥: ٢٤٣-٥٠.

World Health Organization. Diabetes Mellitus Report of a WHO Study Group. Geneva: WHO. (Technical Report Series ٧٢٧), ١٩٨٥.

Yildiz, D., and Kurman, C.I. Recovery of ١-chloro-٢, ٤-dinitrobenzene Detoxification by N-acetyl-L-cysteine in Glutathione Predepleted Human Erythrocytes. Turk. J. Med. Sci., ٢٠٠٤; ٣٤: ٢٣٣-٢٣٨.

Young, D.S. , and Hicks, J.M. Method for Automatic Determination of Serum Iron. J. Clin. Pathol., ١٩٦٥; ١٨: ٩٨-١٠٢.

Zappacosta, B.; Persichilli, S.; Mordente, A.; Minucci, A.; Lazzaro, D.; Meucci, E., and Giardina, B. Inhibition of Salivary Enzymes by Cigarette Smoke and the Protective Role of Glutathione. Hum. Exp. Toxicol., ٢٠٠٢; ٢١(١): ٧-١١.

Zargar, A.H.; Shah, N.A., and Massodi, S.R. Copper, Zinc, and Magnesium Levels in Non-Insulin-Dependant Diabetes Mellitus. Postgrad Med. J., ١٩٩٨; ٧٤: ٦٦٥-٨.

Zhao, Y.X., and Yu, R.J. Effect of Smoking on the Lipid Peroxidase Antioxidation and Alpha ١-Antitrypsin. Zhongha Jie He Hc Hu Xi Za Zhi, ١٩٩٨; ٢١(٤): ٢١٨-٢٢٠.

Zhao, L. Effects of Free Radicals in Diabetes: Offered by the Free Radicals and Radiation Biology Graduate Program, Iowa University, Iowa, ٢٠٠١; ٧٧: ٢٢٢.

Zinneman, H.H.; Nuttall, F.Q., and Goetz, F.C. Effect of Endogenous Insulin on Human Amino Acid Metabolism. Diabetes, ١٩٦٦; ١٥: ٥-٨.