

## Evaluation of lipid peroxidation , lipid profile and antioxidant status in patients with non insulin dependent diabetes mellitus in Najaf / Iraq .

Abdulhussain J. M. Shamsa

Department of Biochemistry , College of Medicine , University of Kufa .

### تقييم مؤشرات اكسدة الدهون ، البروتينات الدهنية و مضادات الاكسدة عند المرضى المصابين بالسكري النوع ٢ غير المعتمد على الانسولين في النجف/ العراق

يعرف داء السكري النوع الثاني بوجود خلل في خلايا بيتا – في البنكرياس مما يؤدي الى نقصان في كمية الانسولين وكثرة العطش وتكرار التبول وهذا يؤدي الى خلل في النظام العام للمواد المضادة للاكسدة في مرضى داء السكري ويعتبر تولد الأوكسجين الحر ROS ونواتج اكسدة الدهون والبروتينات الدهنية المصاحبة لهذا الخلل من اهم المشاكل في مرضى داء السكري . إن الهدف من هذه الدراسة هو تقييم مؤشرات اكسدة الدهون والبروتينات الدهنية مثل المالون ثنائي الادهاييد MDA الناتج من اكسدة الدهون والكوليسترول الكلي TC والكليسيريدات الثلاثية TG والبروتينات الدهنية عالية الكثافة HDL-C واطئة الكثافة LDL-C واطئة الكثافة جدا VLDL-C ومضادات الاكسدة مثل انزيم كلوتاتايون بيروكسيداز GSH-Px والكوتاتايون المختزل GSH, GR والكاتاليز CAT وتركيز حامض البوليك UA . وقد اجريت هذه الدراسة على ٦٠ مريضاً يعانون من مرض السكري النوع الثاني تتراوح اعمارهم من (٣٥ – ٧٠) سنة و٤٠ شخصاً اصحاء كمجموعة ضابطة وتم تقييم مستويات مضادات الاكسدة المذكورة اعلاه و كذلك المالون ثنائي الادهاييد في مصل الدم وتأثيرها على الدهون والبروتينات الدهنية وارتباطها بزيادة السكر في مصل الدم ومقارنتها مع المجموعة الضابطة. اظهرت الدراسة بوجود زيادة معنوية في تراكيز المالون ثنائي الادهاييد والكوليسترول الكلي والكليسيريدات الثلاثية والبروتينات الدهنية واطئة الكثافة واطئة الكثافة جداً مقدارها  $P < 0.001$  مقارنة بالمجموعة الضابطة بينما لوحظ وجود نقصان في مستويات البروتينات الدهنية عالية الكثافة  $P < 0.001$  . وقد لوحظ وجود انخفاض معنوي ملحوظ ذو دلالة احصائية في مستويات مضادات الاكسدة GSH ، GSH-Px ، GR  $P < 0.001$  وزيادة بسيطة في مستوى الكاتاليز CAT وحامض البوليك UA  $P < 0.001$  مقارنة بمجموعة السيطرة . ونستنتج من هذه الدراسة انخفاض مستوى مضادات الاكسدة وزيادة المالون ثنائي الادهاييد كمؤشر لأكسدة الدهون ويشير الى اجهاد تأكسدي يؤدي الى زيادة السكر في مصل الدم، واصبح من الضروري تحديد كيفية حدوث الاجهاد التأكسدي والمسؤول عن هذا الخلل واستخدام مضادات الاكسدة كعلاج مثل VIT-C ، VIT-E .

**Keywords: Diabetes mellitus, Lipid profile, Glutathion, Oxidative stress (Malondialdehyde), Catalase and Uric acid**

### **Abstract:**

#### **Background:**

Diabetes mellitus arises when insufficient insulin is produced, or when the available insulin does not function correctly. Without insulin, the amount of glucose in the blood stream is abnormally high, causing unquenchable thirst and frequent urination. The body's inability to store or use glucose causes hunger and weight loss<sup>(1)</sup>. Type 2 diabetes – occurs when there is a severe lack of insulin due to the destruction of most or all of the beta ( $\beta$  – cells) in the islets of Langerhans. Diabetes mellitus is considered to be one of a rank of free radical diseases which propagates complications with increased free radical formation. Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms. Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus . Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus. The study was designed to find out the relationship between lipid peroxidation, and complication of diabetes mellitus and to estimate the mutual relationship between serum lipoproteins levels and diabetes severity<sup>(2)</sup>.

### **Introduction**

Diabetes Mellitus Is caused by on absolute or relative insulin deficiency . It has been defined by the World Health Organization (WHO) , on the basis of laboratory findings, as a fasting venous plasma glucose concentration more than or equal to 7.0 mmol/L (on more than one occasion or once in the presence of diabetes symptom ) or random venous plasma glucose concentration more than or equal to 11.1mmol/L<sup>(3)</sup> . Non-insulin-dependent type 2 diabetes – occurs when the body does not produce enough insulin, and the insulin that is produced becomes less effective. This type of diabetes usually appears in people over the age of 40, and tends to have a more gradual onset. In most cases, glucose levels in the blood can be controlled by diet, or diet and tablets, although sometimes insulin injections may be needed. About 90 per cent of diabetics are non-insulin dependent<sup>(4)</sup>. There are probably 100

million people in the world with diabetes mellitus and incidences of diabetes are on the rise. As diabetes progress patients are at increased risk of developing coronary disease <sup>(4)</sup>. Insulin deficiency causes excessive metabolisation of free fatty acids, this may lead to a disorder in lipid metabolism. Insulin is a hypoglycemic hormone secreted from  $\beta$ -cell of the islet of pancreas. Insulin also has an effect on lipid metabolism <sup>(5)</sup>.

Type 2 diabetes (non-insulin-dependent diabetes) is a multi-causal disease which develops slowly and in a stepwise order <sup>(6-8)</sup>. Initially it commences with insulin resistance, which progress gradually with time until the body fails to maintain glucose homeostasis resulting in glucose intolerance. Systemically these perturbations are accompanied with changes in a variety of biochemical processes such as obesity, an altered lipid profile and lipid peroxidation <sup>(9)</sup>.

Oxidative damage to unsaturated lipids is a well-established general mechanism for oxidative stress-mediated cellular injury <sup>(10)</sup>, in addition to increased lipid peroxidation <sup>(11)</sup>. The occurrence of free-radical-induced lipid peroxidation causes considerable changes in the cell membrane <sup>(12)</sup>. Peroxidation of the lipid membrane has been related to the pathogenesis of many degenerative diseases, such as atherosclerosis, aging, carcinogenesis and diabetes mellitus <sup>(13)</sup>. Evidence suggests that oxidative stress is increased in diabetes, because of excessive production of reactive oxygen species (ROS) and an impaired antioxidant defence mechanism<sup>(14,15)</sup>. It has been suggested that ROS induce membrane lipid peroxidation and that the toxicity of the generated fatty acids peroxides are important causes of cell malfunction <sup>(16)</sup>. The most widely used assay for lipid peroxidation involves the measurement of malondialdehyde (MDA) due to its simplicity. Thus, the lipid peroxide in the blood provides useful information for the prognosis of diabetes in which secondary disorders are often fatal <sup>(17)</sup>. Antioxidants can be defined as substances whose presence in relatively high concentration significantly inhibits the rate of oxidation of lipids, proteins, carbohydrates and DNA. Antioxidants such as uric acid (UA) and glutathione (GSH) act as potent electron donors; they donate hydrogen atoms to pair up with unpaired electrons on free radicals. Thus, they convert reactive free radicals into inactive substances <sup>(18)</sup>. The determination of the oxidative stress and antioxidants require sometimes invasive techniques such as taking blood samples.

The aim of the present study was to determine the levels of serum glucose ,lipid peroxidation marker (MDA), lipid profile; total cholesterol (TC), high density lipoprotein (HDL), Triglyceride(TG), Low density Lipoprotein(LDL) and very Low Lipoprotein(VLDL) and make a statistical study on these parameters were done by the methods based on enzymatic determination and antioxidant parameters Glutathione peroxidase(GPx), Reduced Glutathione(GSH), Glutathione redactase(GR), Catalase(CAT) and Uric acid(UA) in patient with Diabetes mellitus.

## **Material and Method**

### **Chemical and Apparatus**

All laboratory chemical and reagents were of analar grade . Trichloroacetic acid ,was obtained from Hopking Williams, Thiobarbituric acid from Merek Germany Co. ltd, reduced glutathione from Biochemical's Co. Ltd, Hydrogen peroxide from Merek CO. Ltd, (di-potassium hydrogen phosphate, potassium dihydrogen phosphate and di-sodium hydrogen phosphate from Merek Germany Co. ltd) were used during our study , lipid profile and uric acid from BIOLABO SA laboratories Ltd. France

### **Patients and control subjects**

Sera of 100 subject were collected, , out of which 40 apparently healthy individuals of age group (35-70 years) were taken as control with normal plasma glucose include (20) male and (20) female . (60) elderly with non insulin dependent (NIDDM) subjects of age (35-70 years) were taken as cases include (30 ) males and (30 ) females were obtained from Al-Najaf Center for diabetes and Endocrine Department in Al - Sader Medical Teaching City / Najaf/ Iraq and the results were compared with healthy individuals with comparable age . The (NIDDM) Patients were not taking any medicines other than oral anti- diabetic pills for the past four years . Patients suffered from other disease interferes with data excluded in the current study . The study was carried out at the Department of Biochemistry . College of Medicine , University of Kufa.

### **Blood specimens**

Disposable syringes and needles were used for blood collection . Blood samples were obtained from patients and control group by vein puncture. Sample were allowed to clot at 37 °C then centrifuged at 3000 Xg for 10 minutes. Sera were removed and stored at -20 °C until analysis time.

## Methods

Both cases and controls were subjected to estimation of biochemical parameters like Fasting plasma Glucose (FPG) <sup>(19)</sup>, Total cholesterol (TC), Triglyceride (Tg), HDL-Cholesterol, LDL-Cholesterol and VLDL-Cholesterol<sup>(20,21)</sup>. GSH-Px was assayed according to the procedure of Rotruck et al with some modification<sup>(22)</sup> while reduced Glutathione was assayed as described by Burits et al <sup>(23)</sup>. (by spectrophotometric assay based on 5,5- dithiobis - nitrobenzoic acid ) on the other hand Glutathion reductase (GR) was assayed by enzymatic method by Horn <sup>(24)</sup> Catalase CAT activity was assayed as described by Aebi H<sup>(25)</sup> and uric acid also was assayed by enzymatic method<sup>(26)</sup>. Malondialdehyde was assayed using thiobarbituric reactive substances method described by Guidet B and Shah <sup>(27)</sup>.

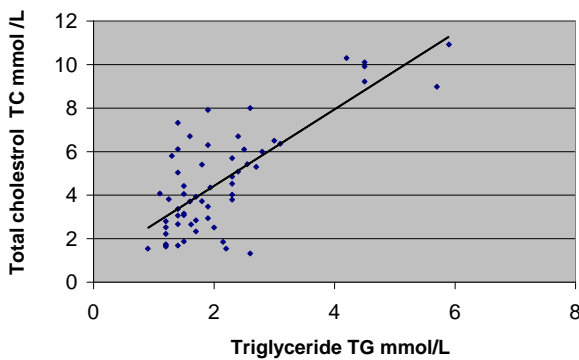
Type 2 diabetes mellitus patients were diagnosed on the basis of history, physical examination and biochemical investigations and according to the biochemical criteria laid down by the National Diabetes Data Group (NDDG) of the National Institute of health in 1985/WHO criteria <sup>(28)</sup>. The diagnosis of NIDDM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes mellitus (2000) <sup>(29)</sup>.

## Biostatistics analysis

The result were expressed as mean  $\pm$  SD. Students t-test was used for comparison of results of patients and the control group. Significant variation was considered when p value was less than  $< 0.05$ . The correlate between the values of oxidative stress parameters and various factor were performed by the liner regression analysis Table(3) and Figures (1- 5).

## Results

In the current study we observed a significant increase  $p < 0.001$  in the lipid profile was observed except HDL cholesterol, which was decreased Table(1) and Figures(1,2), Also significant decrease  $p < 0.001$  in antioxidant enzymes such as glutathione peroxidas, reduced glutathione, glutathione reductase, except uric acid and catalase were seen as compared to the control subjects Table(2). Other findings observed was that the level of lipid peroxide (MDA) increased as per the increase in concentration of blood glucose. Our findings indicate that the increase in the lipid peroxidation product MDA together with uric acid and catalase and decline in glutathione-dependent antioxidant defenses may appear early in non insulin dependent type 2 diabetes mellitus patients (NIDDM). Serum MDA had a weak positive relation with serum UA and serum CAT.



fig(1):Correlation between Total cholesterolTC and Triglyceride TG

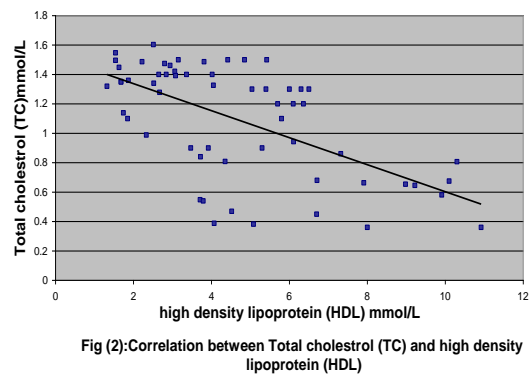


Fig (2):Correlation between Total cholesterol (TC) and high density lipoprotein (HDL)

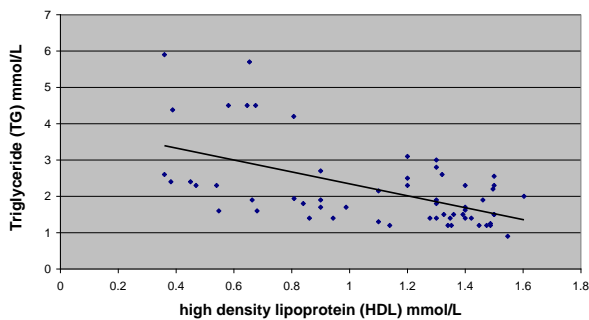


Fig (3) :Correlation between Triglyceride (TG) and high density lipoprotein (HDL)

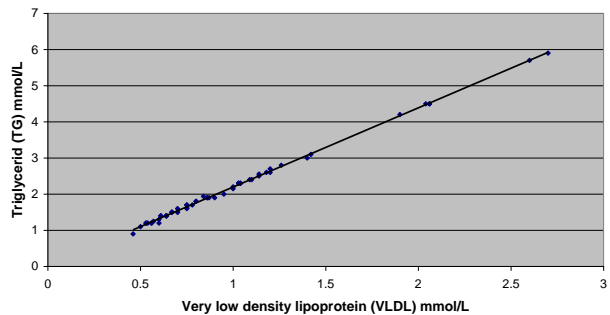


Fig (4) :Correlation between triglycerid (TG) and very low density lipoprotein (VLDL)

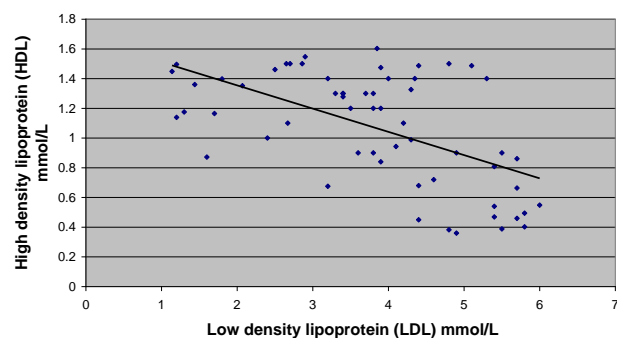


Fig (5) :Correlation between High density lipoprotein (HDL) and Low density lipoprotein (LDL)

Table (1) .Serum value of lipid profile in type 2 diabetes mellitus and the control group

Parameters	Control		Patients		P- value
	Mean $\pm$ SD N = 40	Range	Mean $\pm$ SD N = 60	Range	
TC(mmol/L)	3.91 $\pm$ 0.66	1.6 – 9.4	6.138 $\pm$ 1.2	2.45– 14.9	< 0.001
TG(mmol/L)	1.68 $\pm$ 0.3	0.53–4.72	2.05 $\pm$ 0.51	0.98- 5.9	< 0.001
HDL-(mmol/L)	1.89 $\pm$ 0.36	0.32– 2.29	1.2 8 $\pm$ 0.26	0.78 – 2.2	< 0.001
LDL-C(mmol/L)	2.42 $\pm$ 0.36	1.3 - 5.5	3.84 $\pm$ 1.15	1.34 – 8.6	< 0.001
VLDL–C(mmol/L)	0.36 $\pm$ 0.1	0.47– 1.79	0.93 $\pm$ 0.26	0.34 – 2.7	< 0.001

TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein .

Table (2). Serum value of the status of antioxidant enzymes and Malodialdehyde (MAD) in type 2 diabetes mellitus and control group .

Parameters	Control		Patients		P- value
	Mean $\pm$ SD N = 40	Range	Mean $\pm$ SD N = 60	Range	
GSH ( $\mu$ mol/L)	284 $\pm$ 58	113 – 553	139 $\pm$ 34	52 – 287	< 0.001
GPx (U/ml)	3.92 $\pm$ 1.16	1.4–8.8	2.48 $\pm$ 0.74	0.47 - 6.13	< 0.001
GR-(U/ml)	1.68 $\pm$ 0.33	0.65 –4.02	1.3 8 $\pm$ 0.27	0.5 – 3.2	< 0.001
CAT (K/ml)	60 $\pm$ 6.6	26.7 -133.2	65.6 $\pm$ 19	23.3 -169.2	< 0.001
U.A–(mmol/L)	0.29 $\pm$ 0.039	0.126 –0..66	0.35 $\pm$ 0.08	0.135 –0..86	< 0.001
MDA ( $\mu$ mol/L)	5.64 $\pm$ 0.58	2.53 – 12.4	10.66 $\pm$ 3.2	3.24 - 20.5	< 0.001

GSH, Glutathione; GPx, Glutathione peroxidase; GR, Glutathione reductase ; CAT , Catalase ; UA, Uric acid ; MDA, Malondialdehyde.

Table ( 3). Correlation among serum estimation of lipid profile in patients with diabetes mellitus

Parameters	R	P – value
TC with TG	0.768	< 0.001
TC with HDL- C	- 0.6	< 0.001
TC with LDL- C	0.71	< 0.001
TC with VLDL-C	0.548	< 0.01
TG with HDL- C	- 0.55	< 0.01
TG with VLDL-C	0.932	< 0.001
HDL with LDL-C	- 0.565	< 0.01
HDL with VLDL-C	- 0.422	< 0.05

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein TC, total cholesterol; TG, triglycerides cholesterol; VLDL-C, very low-density lipoprotein .

## Discussion

In diabetes mellitus, abnormally increased levels of lipids, lipoproteins and lipid peroxides in plasma may be due to the abnormal lipid metabolism<sup>(13)</sup>. Patients with type 2 diabetes frequently have an abnormal blood lipid profile consisting of moderately elevated LDL-C, moderately decreased HDL-C, and high TC and triglycerides (Table 1). Thus, inadequate levels of HDL-C, in conjunction with more atherogenic forms of LDL-C may contribute to atherogenesis<sup>(30)</sup>. The results of the present study showed approximately a two-fold increase in serum levels of all lipid fractions (except for HDL-C) for diabetic group when compared with control group Table (1).

Hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL-C, protein glycation and glucose auto oxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects<sup>(31)</sup> Table (2). Enhanced oxidative stress was indicated by increased free radicals production<sup>(32)</sup>. Lipid peroxidation and reduced antioxidant status<sup>(33)</sup>. Several studies have reported an increased susceptibility to lipid peroxidation in patients with diabetes mellitus<sup>(34)</sup>. The generation of free radicals may lead to lipid peroxidation and the formation of several types of damage in diabetes mellitus. In the present study, we have observed that MDA levels, a lipid peroxidation product and a marker of oxidative stress, were elevated significantly in diabetic patients<sup>(35)</sup>.

Uric acid UA is the end product of purine metabolism; it can act as a pro-oxidant, particularly at increased concentration Table (2) and may thus be a marker of oxidative stress<sup>(36-37)</sup>. but it may also have a therapeutic role as an antioxidant<sup>(38,39)</sup>. Thus, it is unclear whether the increased concentration of UA in diseases associated with oxidative stress, such as diabetes mellitus, are a protective response or a primary cause. It is worth noting that hyperuricemia has been found to be associated with obesity and insulin resistance and consequently with type 2 diabetes mellitus<sup>(40-42)</sup>. Chen et al, (2008)<sup>(43)</sup>.

Sailaja et al (2003)<sup>(44)</sup>. reported that diabetic humans have shown increased lipid peroxidation and decreased levels of glutathione peroxidase, reduced GSH and glutathione reductase Table (2). These data suggest that the oxidative stress in these pathologies does not depend on a loss of GSH or a lack of GSH synthesis alone, but a misbalance in the oxidant/reduction cycle of GSH<sup>(45)</sup>.

Reznick et al (2006)<sup>(46)</sup>. have shown that oxidative stress exists in diabetic patients as evidenced by the increased total antioxidant capacity in the blood of patients. Indeed, there is evidence that suggests that endogenous antioxidant capacity is eroded in diabetes, due to several factors, including the impact of non-enzymatic glycation on key enzymes, the polyol pathway and its consumption of reducing power, as well as the constant demands of oxidative stress<sup>(47-52)</sup>. The present study showed that elevated serum endogenous antioxidant activity among diabetics is a response to the damaging effect of free radicals release due to increased oxidative stress. Indeed, the detection of increases in UA levels should therefore alert clinicians to the commensurately increased vulnerability of the diabetic patient to life-threatening cardiovascular complications.

GSH is a ubiquitous tri- peptide that presents in red cells and participates in GPx reaction. When H<sub>2</sub>O<sub>2</sub> is detoxified by GPx, the GSH is simultaneously converted to the oxidized form (GSSG). In the present study, found that GSH levels in type 2 DM patients were significantly lower than that in their same age-matched control subjects. These results are in good agreement with other studies<sup>(53-55)</sup>. As already mentioned GSH serves as an essential cofactor for the enzyme GPx and formed oxidized glutathione (GSSG) during the enzyme processes. Thus, increasing in GPx activities imply higher consumption of GSH. Other mechanisms that may explain the GSH reduction in diabetes are that the

GSH is regenerated by the enzyme glutathione reductase, using reducing equivalents from NADPH. The NADPH is generated in red blood cells through the pentose phosphate pathway, which is stimulated by insulin<sup>(56)</sup>. NADPH production in DM may be sluggish, probably resulting in lowered glutathione reductase activity and reduced GSH recycle.

The enzyme glutathione reductase was found to be decreased in type 2 diabetic patients as reported by Dincer et al (2002)<sup>(57)</sup>. Moreover in diabetes mellitus, the increased sorbitol synthesis via the polyol pathway occurred. This elevated sorbitol production caused the NADPH depletion that was required by aldose reductase enzyme in this pathway. This deficiency will also limit the GSH recycle<sup>(57)</sup>. There is still a controversial view regarding alteration in the activity of catalases in diabetic subjects. According to some scientist increase in level of catalase is compensatory for the removal of free radical, in the absence of glutathione peroxidase in type II diabetes mellitus<sup>(58)</sup>. We found in our study increased catalases level which is in agreement of other reports.

In conclusion, the assay of oxidative stress parameter has brought substantial insight into the pathogenesis and evolution of diabetes. Thus, subjects at high risk of developing hyperlipidemia may benefit from treatment with antioxidants such as vitamin E and C, which might assist endogenous antioxidant capacity and reduce peroxidation rates. Whether such supplementation might possibly delay or even prevent complications of this disease is an area of ongoing investigation.

## References

- 1- Waeber GP and Vollenweider PP. Prevention of type 2 diabetes: Where do we stand? *Med France* 2007; 55.
- 2- Chatrejee C C Human physiology (vol I). Role of endocrine in lipid metabolism. (Editor- Medical allied agency) s 1992; 546-550, Calcutta-INDIA .
- 3- Martin A and Crook. Clinical chemistry and Metabolic medicine. Hyperglycemia and diabetes mellitus. 7<sup>th</sup> ed.2006:182.
- 4- World Health Organization Expert committee on prevention and treatment of diabetes mellitus. WHO technical series No 844.1994;Geneva. World Health Organization
- 5- Godkar P and Godkar D Text book of medical laboratory technology. Ed.2 chemistry of carbohydrates (Bhalani publishing house) s.2003;176-233, New Delhi-India
- 6- Stumvoli M, Goldestain B and Timon WH. Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet* 2005; 65: 333–346.
- 7- Waeber GP and Vollenweider PP. Prevention of type 2 diabetes: Where do we stand? *Med France* 2007; 55.
- 8- Buguslaw L. Pathophysiology of oxidative stress in diabetes mellitus. *J Diabetes Complications* 2001; 15: 203–210.
- 9- Maharjan BR, Jha JC, Adhikeri D, Wishwanath P, Baxi J, Alurkar VM, et al. A study of oxidative stress, antioxidant status and lipid profile in diabetic patient in Western region of Nepal. *Kathmandu Univ Med J* 2008; 6: 16–21.
- 10- Yagi K. Lipid peroxides and altered radicals in clinical medicine. *Adv Exp Med Biol* 1994; 366: 1–15.
- 11- Syryawansh NP, Bhutey AK, Nagdeote AN, Jadav AA and Manoorker GS. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian J Clin Biochem* 2006; 21: 126–130.
- 12- Agrawal S, Banerjee S and Chatterjee SN. Effects of oxygen on ferrous sulphate-induced lipid peroxidation in liposomal membrane. *Indian J Biochem Biophys* 1985; 21: 331–334.
- 13- Nair U, Bartch H, Nair J. Lipid peroxidation- induced DNA damage in cancer prone inflammatory diseases: a review of published adduct types and levels in humans. *Free Radic Biol Med* 2007; 43: 1109–20.
- 14- West IC. Radicals and oxidative stress in diabetes. *Diabet Med* 2000; 17: 171–180.
- 15- Antoine L, Karine L, Jerome G and Anne-Marie P. Value of sperm thiobarbituric acid reactive substance in fertile men. *Clin Chem Acta* 2002; 325: 113–115.
- 16- Sanocka D and Kurpisz M. Reactive oxygen species in sperm cells. *Reprod Biol Endocrinol* 2004; 224: 2–12.
- 17- Tappel AL. Lipid peroxidation damage to cell components. *Clin Pathol Fed Proc* 1973; 32: 1870–1874.
- 18- Bagchi K and Puri S. Free radicals and antioxidants in health and disease. *East Mediterr Health* 1998; 14: 350–360.
- 19- Trinder P. Determination of glucose using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin Bio Chem* 1969; 6: 24-7.

- 20- Burstein M, Scholnick HR, Morfin R. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanion. *J Lipid Res* 1970; 11: 583-95.
- 21- Friedwald WT, Levy R, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
- 22- Rotruck, J.T., Pope, A.L. and Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*: 1973; 588- 590, 179.
- 23- Burtis CA, Ashwood ER. *Tietz. Text Book of clinical Chemistry* 3<sup>rd</sup> ed. Philadelphia WB SAUNDERS. (1999): 45.
- 24- Horn HD. Glutathione Reductase. In: Bergmayer HU ed. *Method in enzymatic analysis* New York :Academic press 1963; 875-9.
- 25- Aebi H. *Methods of enzymatic analysis*, ed. New York Academic press 1974 ; 2 : 674 .
- 26- Burtis CA, Ashwood ER. *Tietz. Text Book of clinical Chemistry* 3<sup>rd</sup> ed. Philadelphia WB SAUNDERS. (1999): p. 1245- 1250
- 27- Guidet B, Shah S V: Enhanced in vivo H<sub>2</sub>O<sub>2</sub> generation by rat kidney in glycerol- induced renal failure. *Am J physiol* 1257 : 1989; F440 - 444 .
- 28- Diabetes Mellitus reports of WHO study group. *Tech Rep Ser* 1985; 727: 1-113.
- 29- 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* 2000; 23 Suppl 1: S4-19.
- 30- Libby P. Reducing the risk of atherosclerosis: the role of high density lipoprotein cholesterol. *Br J Cardiol* 2004; 11(Suppl. 2): s3-s6.
- 31- Yang R L, Shi YH, Hao G, Li W and Le GW. Increasing oxidative stress with progressive hyperlipidemia in human: relation between malondialdehyde and atherogenic index. *J Clin Biochem Nutr* 2008; 43: 154-158.
- 32- Hiramatsu K and Arimori S. Increased superoxide production by mononuclear cells of patients with hypertriglyceridemia and diabetes. *Diabetes* 1998; 37: 832-837.
- 33- Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-412.
- 34- Gallu G, Ruelland A and Legras B. Plasma malondialdehyde in type 1 and type 2 diabetic patients. *Clin Chim Acta* 1993; 214: 227-234.
- 35- Mahboob M, Rashman MF and Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. *Singapore Med J* 2005; 46: 322-324.
- 36- Becker BF. Towards the physiological function of uric acid. *Free Rad Biol Med* 1993; 14: 615-631.
- 37- Strazzullo P and Puig JG. Uric acid and oxidative stress: relative impact on cardiovascular risk? *Nutr Metab Cardiovasc Dis* 2007; 17: 409-414.
- 38- Becker BF, Reinholz N, Leipert B, Raschke P, Permanetter Band Gerlach E. Role of uric acid as an endogenous radical scavenger and antioxidant. *Chest* 1991; 100(3 Suppl.): 176S-181S.
- 39- Hayden MR and Tyagi SC. Uric acid: A new look at the old risk marker for cardiovascular, metabolic syndrome, and type 2 diabetes mellitus: The Urate redox shuttle. *Nutr Metab* 2004; 1: 10.
- 40- Tuomilehto J, Zimmit P, Wolf E, Taylor R, Ram P and King H. Plasma Uric Acid level and its association with diabetes mellitus and some biological parameters in biracial population of Fiji. *Am J Epidemiol* 1988; 127: 321-36.
- 41- Causevic A, Semiz S, Macic-Dzankovic A, Cico B, Dujic T, Malenica M, et al. Relevance of Uric Acid in progression of type 2 diabetes mellitus. *Bosnian Medical Science* 2010; 10: 55-99.
- 42- Dehghan A, von Hoek M, Sijbrands EJ, Hofman A and Witteman JC. High serum uric acid as a novel risk factor for type 2 diabetes mellitus. *Diabetes Care* 2008; 31: 361-362.
- 43- Chen K I, Chen MF, Hsu HC, Chang WT, Su TC and Hu FB. Plasma uric acid and risk of type 2 diabetes in Chinese community. *Clin Chem* 2008; 54: 310-316.
- 44- Sailaja YR, Beskar R and Saralakumari D. The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. *Free Rad Biol Med* 2003; 35: 133-139.
- 45- Arana C, Cutando A, Ferrera MJ, Gomez-Moreno G, Worf CV, Bolanos MJ, et al. Parameters of oxidative stress in saliva from diabetic and parenteral drug addict patients. *J Oral Pathol Med* 2006; 35: 554-559.
- 46- Reznick AZ, Shehadeh N, Shafir Y and Naglar RM. Free radicals related effects and antioxidants in saliva and serum of adolescents with type 1 diabetes mellitus. *Arch Oral Biol* 2006; 51: 640-648.
- 47- Rodney RC and Roger MDB. Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. *J Am Coll Nutr* 2001; 20: 363-369.

- 48- Abdollahi M, Ranjbar A, Shadnia S, Nikfar S and Rezaiee A. Pesticides and oxidative stress: a review. *Med Sci Monit* 2004; 10: RA144–RA147.
- 49- Nazirogilu M and Butterworth P. Protective effects of moderate exercise with dietary vitamin and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Can J Appl Physiol* 2005; 30: 172–185.
- 50- Johansen JS, Harris AK, Rychly DJ and Ergul A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc Diabetol* 2005; 4: 5.
- 51- Chung SSM, Ho ECM, Lam KSL and Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. *J Am Soc Nephrol* 2003; 14: S233–S236.
- 52- Lee AYW and Chung SSM. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 1999; 13: 23–30.
- 53- Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease : which role for oxidative stress? *Metabolism* 1995; 44: 363-8.
- 54- Ceriello A, Bortolotti N, Pirisi M, Crescentini A, Tonutti L, Motz E, et al. Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. *Diabetes Care* 1997; 20: 1589-93.
- 55- Seghrouchni I, Drai J, Bannier E, Riviere J, Calmard P, Garcia I, et al. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. *Clinica Chimica Acta* 2002; 321: 89-96.
- 56- Weber G, Convery HJ. Insulin: inducer of glucose 6-phosphate dehydrogenase. *Life Sci* 1966; 5:1139-46.
- 57- Dincer Y, Alademir Z, Ilkova H, Akcay T. Susceptibility of glutathione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effect of glycemic control. *Clin Biochem* 2002; 35: 297-30
- 58- Inal ME, Kanbak G, Sunal E Antioxidant enzyme activities and Malondialdehyde levels related to aging. *Clinica Chimica Acta* 2001. 305 : 75- 80.