EVALUATION OF OXIDATIVE STRESS AND CARDIOVASCULAR DISEASE RISK FACTORS IN TYPE II DIABETIC POSTMENOPAUSAL WOMEN

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ABSTRACT

The risk of Ischemic heart disease increases in women after the menopause. Women with type 2 diabetes appear to lose the protection against cardiovascular disease afforded by estrogen. The aim of this study was to examine the relationship between oxidative stress and cardiovascular disease parameters in postmenopausal women with and without diabetes, also to evaluate the association of diabetes mellitus (DM) as a risk for coronary artery disease. This study included 50 postmenopausal women had type 2 diabetes mellitus (mean age, 57.3 ± 8.7 years) and 50 healthy postmenopausal as control (mean age, 56.9 ± 7.9 years). Oxidative stress markers, cardiovascular disease parameters and some micronutrients were measured in both groups. Our results showed that postmenopausal women with diabetes had abnormal lipid profile compared with nondiabetic women. Women with DM had significantly higher plasma levels of glucose, HbA1c, GGT and NO, LPO and lower levels of plasma antioxidant enzymes and total antioxidant capacity compared to women without DM. Also, the subjects with DM had significantly lower levels of plasma uric acid, ascorbic acid, and magnesium in comparison to those without DM. Postmenopausal women with DM had higher values of plasma iron and Cu, but the differences were not significant compared with control group. Plasma GGT was positively correlated with LDL-c level, glucose and HbA1c in postmenopausal women with DM. On other hand, plasma NO level was positively correlated with body mass index, Waist circumference, total cholesterol, triglyceride, glucose and HbA1c and negatively correlated with HDL-c, uric acid and total antioxidant capacity (TAC). Moreover, a significant inverse correlation of plasma TAC with triglyceride, glucose and HbA1c were observed. Furthermore, a significant positive correlation of plasma TAC with HDL-c and uric acid were seen too. In conclusion, there is an association between postmenopausal status, DM and cardiovascular risk parameters. This abnormality was associated with increased oxidative stress and impaired antioxidant defense in particular in type 2 diabetic patients before the development of secondary complications. These alterations contribute to the increased risk for occurrence of vascular diseases in such women.

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Key Words: Postmenopausal women, Type 2 DM, Cardiovascular diseases, Oxidative stress, Antioxidant, Uric acid, Nitric Oxide, GGT.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia together with biochemical alterations of glucose and lipid peroxidation. The oxidative stress in diabetes was greatly increased due to prolonged exposure to hyperglycemia and impaired of oxidant \ antioxidant equilibrium **(Ramakrishna & Jailkhani, 2008).** Diabetes decreases the gender protection against coronary heart disease in postmenopausal women **(Marra et al., 2002).** Assuming hyperglycemia as the only risk factor, women demonstrate a change in oxidative status due to an interaction between nitric oxide (NO) and uric acid production **(Pitocco et al., 2008).**

Aging is related to an increase in systemic oxidative stress. By-products of oxidative modification, such as lipid peroxidation of cellular structures are thought to play an important role in aging, atherosclerosis and late complication of diabetes mellitus. However, the rate of free radical formation has been amplified with aging and this may be at a rate that exceeds even the increased antioxidant capacity of the tissue **(Vincent et al., 2005).** As well, aging is often linked with environmental modifications lead to decreased micronutrient supplied, which is directly linked to antioxidant defense mechanisms. Regarding the main role of antioxidant micronutrients in avoiding accelerated aging, statistics linked to relationship between Oxidative stress and antioxidant status in postmenopausal women are limited **(Bureau et al., 2002).**

There is increasing experimental and clinical evidence showing that gamma-glutamyl transferase (GGT) level is associated with oxidative stress **(Simão et al., 2008).** Recent population-based epidemiological studies have shown a strong association of serum GGT activities within the reference interval with many cardiovascular disease risk factors. In addition, in prospective studies, baseline serum GGT activity predicted future diabetes, hypertension, and myocardial infarction. Among these diseases, serum GGT within the reference interval most strongly predicted incident type 2 diabetes **(Lim et al., 2007).** The objective of this study was to investigate whether postmenopausal diabetic women with short duration of disease and without complications have an altered oxidative status. Also, we evaluate the relationship of oxidative stress parameters with cardiovascular risk factors such as lipid profile and uric acid among diabetic and healthy postmenopausal women.

MATERIALS AND METHODS

A total of 100 Egyptian women ≥ 55 were included in this study. Demographic data was recorded for each subject using self-made questionnaire. An informed consent was obtained from all subjects before they participated in the study, which was approved by the ethical committee. They were selected from the Ain Shams University Hospitals.. The study included 2 groups of subjects; **Group I:** Postmenopausal healthy women (n=50); **Group II:** Postmenopausal women with non-insulin dependent type 2 diabetic mellitus (NIDDM) (n=50). They had not been taking any medicines other than antidiabetic pills for the past 3-5 years. The duration of diabetes ranged between 2-10 years and had no history of diabetic complications, such as nephropathy, neuropathy, ischemic heart disease **(Lau et al., 2005).** We excluded women who had anemia, those with acute or chronic liver illness, malignancy disease, hypertension, renal diseases, those who had obesity according to the Italian BMI charts **(Cacciari et al., 2002)** and those with postmenopausal hormone therapy. A woman was considered postmenopausal if she had not menstruated in the last 2 years (**Soules et al., 2001**).

Body Mass Index (BMI) was calculated as weight/height^2 (kg/m^2) (normal BMI (18.5-24.9) (**Garrow & Webster, 1985**). Waist circumference (WC) was measured while the patient standing up, at the midpoint between the bottom of the rib cage and the top of the lateral border of the iliac crest during minimal respiration. Fasting blood samples (8, 12 hours overnight) have been collected into three vacutainer tubes, one containing EDTA for measurement of blood HbA_{1c} and the others containing sodium fluoride for glucose (8 hours) and lipids (12 hours) measurement. The plasma was separated by centrifugation at 3400 rpm for 10 minutes at 4°C, then subdivided into aliquots and were stored at -80°C until analysis. Plasma glucose (**Barham and Trinder, 1972**), total cholesterol (**Allain, et al., 1974**), triacylglycerols (**McGowan et al, 1973**), HDL-c (**Finley et al, 1978**) and uric acid (**Fossati et al.,1980**) concentrations were determined by enzymatic methods. LDL-c was calculated by the Friedwald formula (**Friedwald et al, 1972**). Atherogenic index was calculated from ratio of total cholesterol / HDL-c (**Wilson, et al., 1980**). Glycosylated hemoglobin ($HbA_{1c)}$ was determined in whole blood using chromatographic–Spectrophotometric methods (**Bisse, et al., 1986**).

The gamma glutamyl transferase (GGT) enzyme activity is determined using kinetic colorimetric method according to **Szasz and Persijn (1974)**. The level of plasma NO was measured spectrophotometrically as nitrite concentration after reduction of nitrate by the method described by **Bories and Bories (1995).** Plasma lipid hydroperoxide (LPO) concentration was determined using commercially available Cayman chemical LPO assay kit according to **Mihaljevic et al., (1996).**

Total antioxidant capacity (**Koracevic et al., 2001)** and antioxidant enzymes like Superoxide dismutase **(Sun et al., 1995),** Catalase **(Johansson and Bory, 1988),** glutathione peroxidase **(Paglia and Valentin, 1967**) and glutathione reductase **(Carlberg and Mannervik, 1985)** concentrations were determined using commercially available Cayman chemical assay kit.

Plasma Iron (**Henry, 1984)**, copper, calcium and magnesium measurements were made by atomic absorption according to **Makino and Takaha (1981)**. Plasma zinc measurement was analyzed by Inductively Coupled Plasma Mass Spectrometry (**Makino and Takahara, 1981)**. Plasma ascorbic acid was assayed colorimetric by the method of **Harris and Ray, 1935**.

Statistical Analysis: Data was expressed as the mean ± S.D. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 10.0, 1999, Chicago, IL, USA). Differences between groups were analyzed by one-way analysis of variance (ANOVA). Pearson's correlation analysis was performed to determine the relationships between nitric oxide, uric acid and other cardiovascular risk variables. *P*-value <0.05 was accepted to indicate statistical significance.

RESULTS

The basic anthropometric and clinical characteristics of the postmenopausal women with and without diabetes are shown in Table 1. The mean age ± S.D of postmenopausal women without diabetes was 56.9 ± 7.9 years; and of postmenopausal women with diabetes, 57.3 ± 8.7 years. There were significant differences between the women with and without diabetes in the levels of fasting plasma glucose and HbA1c. There was no significant difference seen in women with diabetes than in those without in the Age, BMI, Systosolic and diastolic blood pressure (Table 1). Also, our results show that postmenopausal women with diabetes had increased dyslipidemia with significant higher plasma levels of TG, LDL-c and atherogenic index (P<0.001) and lower levels of HDL-c compared with nondiabetic women (Table 2).

Table (3) shows the significantly higher levels of plasma GGT (P < 0.05), LPO (P < 0.001) and NO (P < 0.001) in women with diabetes in comparison to those without, while plasma antioxidant enzymes and total antioxidant capacity were significantly lower in diabetic postmenopausal women than healthy postmenopausal women (P<0.001). Plasma uric acid and ascorbic acid levels were significantly decreased in diabetic postmenopausal women (P < 0.001) compared to healthy postmenopausal women.

Diabetic postmenopausal women had a significantly lower plasma level of Mg than control group (P<0.001). But, no statistically significant difference (P>0.05) was found between the two groups as regards plasma iron, copper, zinc and calcium levels (Table 4)

Pearson's correlation analysis represented in Table (5) revealed that, plasma GGT was positively correlated with plasma LDL-c level (r = 0.25), glucose level (r = 0.25), HbA1c (r = 0.2) in postmenopausal women with diabetes mellitus. On other hand, plasma NO level was positively correlated with BMI (r = 0.28), WC (r = 0.31), TC (r = 0.24), TG (r = 0.52), glucose (r= 0.63) and HbA1c (r= 0.5) and negatively correlated with HDL-c (r= -0.42), uric acid (r = -0.53) and TAC (r = -0.34). Moreover, a significant inverse correlation of plasma TAC with TG(r = -0.45), glucose (r= -0.48) and HbA1c (r = -0.35) were observed. Furthermore, a significant positive correlation of plasma TAC with HDL-c (r = 0.2) and uric acid (r = 0.37) were seen too.

Table (1): Clinical and anthropometric measurements in both groups. (Mean ±S.D).

Variables	Healthy Postmenopausal Women	Diabetic Postmenopausal Women
Subjects (n)	50	50
Age (years)	56.9 ± 7.9	57.3 ± 8.7
Duration of DM (years)		5.6 ± 3.01
Waist circumference (cm)	74.42 ± 9.8	76.7 ±8.58
BMI (Kg/m^2)	22.77 ± 1.6	23.19 ± 2.8
Systolic BP (mmHg)	122.8± 17.9	117 ± 3.7
Diastolic BP (mmHg)	77 ± 8.1	76.7± 5.6
Glucose (mg/dl)	103.7 ± 12.9	218.4 ± 52 *
HbA_{1C} (%)	5.6 ± 1.6	9.4± 2.1*

*P < 0.001

Table (2): Lipid profile of the both studied. (Mean ±S.D).

Group Parameters	Healthy Postmenopausal Women	Diabetic Postmenopausal Women	Normal values
TC (mg/dl)	195.9± 37.9	210 ± 62.2	**<200**
TG (mg/dl)	83.9 ± 17.3	173.5± 41.6 *	**<150**
HDL-c (mg/dl)	52.6 ± 12.7	38.08 ± 9.5 *	**>50**
LDL-c (mg/dl)	129.6 ± 19.8	152.8 ± 39.7*	**<100**
Atherogenic index (TC/HDL-c)	4.17 ± 1.2	5.0 ± 2.3 *	_

*P < 0.001

Table (3): Plasma levels of oxidative stress markers and antioxidant levels in both studied groups (Mean±S.D.)

Groups / Parameters	Healthy Postmenopausal Women	Diabetic Postmenopausal Women
GGT (U /L)	11.22 ±2.34	15.29± 7.5*
NO (µmol/ L)	49.7± 2.32	63.44 ± 12**
LPO (µM)	12.99 ± 3.9	16.83 ± 3.55**
TAC (mmol/L)	1.88 ±0.21	1.43 ± 0.56**
SOD (U/ml)	0.79 ± 0.42	0.54 ± 0.27**
Catalase (nmol/min/ml)	10.5 ± 2.4	4.51±1.15*
Glutathione peroxidase (nmol/min/ml)	51.09 ± 14.6	38.49 ± 8.7**
Glutathione reductase (nmol/min/min)	16.7 ± 6	12.57 ± 3.6**
Uric acid (mg/dl)	5.4 ± 1.2	3.4 ± 0.82**
Ascorbic acid (mg/L)	18.27 ± 1.46	14.1 ± 1.94**

*P < 0.05
**P < 0.001

Table (4): Plasma levels of some micronutrients in both studied groups (Mean ±S.D.)

Groups / Parameters	Healthy Postmenopausal Women	Diabetic Postmenopausal Women
Iron (µg /dl)	115.4 ±38	122.4 ± 38
Cupper (mg/ L)	1.05± 0.18	1.09 ± 0.1
Zinc (µg/dl)	52.8 ± 13.5	48 ± 9.4
Calcium(mg/dl)	7.3 ± 0.22	7.2 ± 0.33
Magnesium (mg/L)	20 ± 1.54	18±1.8*

*P < 0.001

Table (5): The significant Correlation analysis between plasma GGT, NO, TAC and cardiovascular risk factors in diabetic postmenopausal women.

Variables	*GGT*	*NO*	*TAC*
BMI	0.06	0.28*	-0.14
WC	0.04	0.31*	0.04
Systolic BP	-0.0	-0.1	0.11

Diastolic BP	0.01	-0.04	-0.04
TC	0.19	0.24*	0.01
HDL-c	-0.06	-0.42*	0.2*
Triglycerides	0.12	0.52*	-0.45*
LDL-c	0.25*	0.15	-0.18
Glucose	0.25*	0.63*	-0.48*
HbA1c	0.2*	0.5*	-0.35*
Uric acid	-0.17	-0.53*	0.37*
GGT	-	0.18	-0.09
NO	0.18	-	-0.34*
TAC	-0.09	-0.34*	-

Correlation coefficients were calculated using Pearson's Correlation Coefficient.
* *P-value* : Correlation is significant at the 0.05 level

DISCUSSION

Oxidative stress plays a major role in the pathogenesis of type 2 diabetes mellitus. Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and formation in several damage in diabetes mellitus (**Mahboob et al., 2005**). The present study was designed to evaluate the relationships between oxidative stress related parameters and the cardiovascular risk factors, focusing on postmenopausal women with and without diabetes mellitus.

There were highly significant increases in fasting plasma glucose and HbA1c in diabetic postmenopausal women. Higher levels indicate that they have poorly glycemic control. The data obtained were similar to those presented by other authors **(Masding et al., 2003).** In addition to this, **Jain & Lim, 2001** reported that high levels of glucose can produce permanent chemical alterations in proteins and increase lipid peroxidation in a variety of experimental models of hyperglycemia. Hyperglycemia, itself, may stimulate platelet aggregation and auto-oxidation of glucose may also lead to free radical production in diabetics. On the other hand, **Marra et al., 2002** did not find any relationship between metabolic control, as evaluated by HbA1c, antioxidant status, and markers of oxidative stress. This can be explained by the fact that HbA1c is a mean evaluation of glycemic control and reflects, only in part, glycemic fluctuations, such as postprandial hyperglycemia or disglycemia states, which may induce a pro-oxidative status and may play a significant role in the pathogenesis of diabetic complications. Moreover, **Khaw et al., 2001** suggested that HbA1c was related to total mortality in the general population, determining an increased risk even for values more than 5 %.

Lipid profiles were affected by metabolic conditions, and alterations in lipid metabolism have been implicated in atherosclerosis and coronary heart disease (CHD) **(Winder, 1994).** In the present study, TG, LDL-c and atherogenic index were significantly higher ($P < 0.001$) and HDL-c lower ($P < 0.001$) in diabetic postmenopausal women when compared to healthy women. This agrees with the findings of **Gan et al., (1999),** who demonstrated that hyperlipidemia is common in type 2 diabetes mellitus and contributed significantly to the incidence of CHD. The dyslipidemia profile includes a high TG and low HDL-c. The concentrations of LDL-c show less specific changes in diabetics, but when elevated, are an important contributor to the risk of CHD. Also, **Usoro et al., (2006)** confirmed that hormonal changes associated with menopause and age alters the lipid profile in women as evidenced by higher TC, LDL-c, atherogenic index and lower HDL-c seen in postmenopausal women. An increased triglyceride level is a common feature of diabetes mellitus (**Nayak & Roberts, 2006**). Research has suggested that this is a result of reduced action of insulin on adipocytes resulting in suppression of lipolysis.

Postmenopausal women experience more type 2 diabetes and cardiovascular diseases than their premenopausal counterparts. One hypothesis concerning the increased prevalence of type 2 diabetes and cardiovascular diseases in postmenopausal women is that it may be related to age-related changes in sex-

steroid hormones. Although sex hormones do not appear to play a primary role in the etiology of type 2 diabetes, they may be related to other metabolic factors **(Crespo et al., 2002).**

Gamma;-glutamyl transferase (GGT) has been regarded as a biomarker of hepatobiliary disease and alcohol consumption/abuse (**Whitfield, 2001).** However, GGT is elaborated by extrahepatic tissues including the kidney, epididymis, fibroblasts, lymphocytes, and lung **(Karp et al., 2001).** Accumulating experimental evidence suggests an important role for GGT in extracellular catabolism of glutathione, the principal thiol antioxidant in humans. GGT enhances the availability of cysteine to promote intracellular glutathione (GSH) resynthesis, thereby counteracting oxidant stress **(Lee et al., 2007).**

There is evidence that GGT is a potential biochemical marker for the preclinical development of atherosclerosis. GGT was found to play a role in the pathogenesis of atherosclerosis because it was detected in atheromatous plaques of carotid and coronary arteries triggering the oxidation of LDL **(Paolicchi et al., 2004**). In the present study, the plasma GGT was significantly increased (P< 0.05) in diabetic postmenopausal women compared to healthy women.

If serum GGT is a marker of oxidative stress, it might have important implications both clinically and epidemiologically because measurement of serum GGT is easy, reliable, and not expensive. Mechanisms that explain the contribution of GGT to Cardiovascular disease and mortality have not been fully elucidated. Although we observed that the relations of GGT to cardiovascular events and death remained robust after accounting for fasting glucose and components of the metabolic syndrome, it is conceivable that such adjustment incompletely accounts for hepatic insulin resistance **(Lim et al., 2004).**

Nitric oxide (NO) overproduction in diabetes has been documented in several clinical studies **(Chiarelli et al., 2000).** This appears contradictory to the abundant experimental data indicating that nitric oxide is a beneficial endothelium-derived vasodilating factor that is deficient in diabetes. NO production by the endothelium is regulated by endothelial nitric oxide synthase and may respond differently to chronic hyperglycemia than does nitric oxide produced elsewhere **(Hoeldtke, 2003)**.

Furthermore, the diabetic postmenopausal women exhibited higher activities of plasma lipid hydroperoxide (LPO) ($P < 0.001$) than healthy women. This indicates that oxidizability of plasma as measured by LPO was greater in diabetic women as a result of increase plasma peroxide concentration in diabetic **(Heffner et al., 1995).** Our finding is similar to that by **Nourooz zadeh et al., 1997** who found that plasma LPO level was substantially higher in type 2 diabetic patient

Uric acid has been proposed to play a pivotal role in the antioxidant defense systems in humans. The intriguing question of a role for uric acid in the free radical–scavenging processes in diabetes has received little attention. Elevated glucose levels seem to be the principal mechanism, because hyperglycemia has, at least in part, an osmotic diuretic effect, leading to an excessive excretion of uric acid **(Marra et al., 2002).** In our study, the plasma NO level was significantly increased ($P < 0.001$) while uric acid and vitamin C levels were significantly lower in postmenopausal women with diabetes than in healthy control. This fact, together with the positive relationship found between uric acid levels and antioxidant capacity, as shown in Table 5, confirms the importance of uric acid for the antioxidant defenses .

It is likely that uric acid is degraded or metabolized when it scavenges peroxynitrite **(Santos et al., 1999).** Accordingly, the suppression of serum uric acid occurred early, at the first patient evaluation, when there was minimal uricosuria. This indicates that direct effects of peroxynitrite excess are the most important cause of uric acid suppression, at least initially. Indirect renal effects appear to play a contributory role. Although nitric oxide overproduction has been previously reported in patients with diabetes, this is a complex and poorly understood phenomenon. Our simplified interpretation does not take into account the multiple potential mechanisms for formation of reactive oxygen species in patients with diabetes, nor did we address the possibility that eNOS may generate both Superoxide anions and nitric oxide **(Xia et al., 1998).** Nitrosative stress and oxidative stress in concert lead to peroxynitrite formation and lipid peroxidation, which synergistically compromise ATP synthesis and damage mitochondria , decrease cellular viability, and promote apoptosis. Unfortunately, hyperglycemia stimulates the synthesis of reactive oxygen intermediates in multiple tissues and subcellular locations, and it is uncertain which is the most important or suppressible with antioxidants **(Kelso et al., 2002).**

Our data indicate that oxidative stress and nitrosative stress have detectable adverse effects on peripheral nerve function within the first few years of diabetes, and therefore, it may be possible to prevent them with interventions introduced early in those patients who are unable to maintain normoglycemia. we have evidence that nitric oxide overproduction occurs in patients with poorly controlled type 2 diabetes and leads to suppressed uric acid. These metabolic changes are associated with detectable adverse effects on

peripheral nerve function even in patients who have been exposed to hyperglycemia only a few years **(Hoeldtke et al., 2002).**

Teramoto et al., (2004) have found that vitamin C administration is capable of restoring endothelial function in certain high risk groups characterized by oxidative stress, and ongoing research may establish whether this treatment can reduce cardiovascular risk in a clinical setting **(Waring et al., 2006)**. Other studies suggest that people with low vitamin C levels have higher total and harmful LDL cholesterol levels and lower beneficial HDL cholesterol levels. In a study reported in the American Journal of Clinical Nutrition, USDA researchers found that high blood levels of vitamin C were associated with high levels of HDL cholesterol in 316 women and 511 men aged from 19 to 95.11 Vitamin C also helps to protect blood fats and artery walls against oxidative damage by free radicals, and seems to have beneficial effects on clotting **(Hallfrisch et al., 1994).**

Abnormally-high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes. These consequences of oxidative stress can promote the development of complications in diabetes mellitus patients. Antioxidant enzyme-dependent defenses play an important role in scavenging free radicals produced under oxidative stress **(Mahboob et al., 2005).**

Our studies had consistently demonstrated deficiency in the antioxidant enzymes as well as a significantly decrease in total antioxidant capacity (TAC) in diabetic postmenopausal women than in healthy women , suggestive of reduced total antioxidant defense. Previous studies are consistent with our own findings of increased oxidative stress in NIDDM (**Vincent et al., 2004)**. Also, this result agrees with the finding of **Abou-Seif & Youssef, 2004** who explained this decrease could be due to increased oxidative stress and free radical formation in diabetes mellitus which resulted in decreased antioxidant enzymes. Similarly, oxidative stress is linked to preclinical features of disease, such as vascular endothelial activation that can lead to atherosclerosis . The early increase of oxidative stress in diabetes is more pronounced in women and may account for increased cardiovascular disease in female patients (**Pansini et al., 2008**).

Metals play a major catalytic role in the production of free radicals. A disruption in the homeostasis of the latter two redox-active metals is particularly significant in light of the increases in oxidative stress parameters, such as lipid peroxidation, and the oxidative damage to senile plaques and nucleic acids (**Perry et al., 2002).** There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progress of this disease **(Kazi et al., 2008).**

As regards plasma Iron and Cu levels, high mean values were detected in postmenopausal women with type II diabetes versus the nondiabetic women, but the differences found was not significant ($P < 0.05$). These results are consistent with those obtained in other studies, confirming that deficiency and efficiency of some essential trace metals may play a role in the development of diabetes mellitus **(Kazi et al., 2008).** Sex related differences were also reported by **Ruiz et al., (1998)** who observed significant differences in plasma copper levels between diabetic females and non-diabetic females.

Zinc is known for its ability to block Iron-mediated production of free radicals by serving as antioxidant **(Atamna et al., 2002).** In our results, the plasma zinc level was found to be low in diabetic postmenopausal women but did not reach statistically significant level as compared to healthy control. This finding is in agreement with **Galan et al., (2005)** who observed significantly lower zinc status in the elderly population.

Magnesium is known to play an important role in carbohydrate metabolism, and its imbalance has been implicated in diabetes mellitus both as a cause and a consequence. Diabetic patients have additional risk factors for hypomagnesaemia and magnesium status. It may also play a role in the release of insulin and magnesium depletion has an atherogenic potential **(Nasri and Baradaran, 2008).**

In the present study, the plasma calcium level was approximately similar in both groups; meanwhile there was a significant decrease ($P< 0.001$) in the level of magnesium in diabetic postmenopausal women compared to the healthy women. This observation is in agreement with a **Guerrero et al., (2006)** who founded that diabetics had lower levels of magnesium than normal men and

women. These data confirm the results shown in other studies of significantly lower levels of serum magnesium among those with fasting glucose levels equivalent to ADA criteria for diabetes. A similar observation was made by **Afridi et al., (2008)** among nonhypertensive diabetic patients.

Most factors cause a decrease rather than an increase in trace elements concentration. Decreased concentrations are related mainly to decreased nutrition intake, intestinal uptake and altered distribution while increased concentration is reported to result from excessive homeopathic intake, industrial or environmental exposure. However, in the diabetics, ageing and increasing duration of diabetes enhances urinary loss of these elements, while lower serum zinc might be attributed to the hormonal imbalance associated with the diabetic state (**Nsonwu et al., 2006).**

The correlation analysis showed the plasma GGT, NO and TAC levels were closely related to most variables of cardiovascular risk factors. In conclusion, our data suggest that type 2 diabetic women with a short duration of disease and poor metabolic control show an early imbalance in their antioxidant capacity and augmented levels of lipid, even in the absence of complications. GGT is a potential biochemical marker for the preclinical development of atherosclerosis. Reduced uric acid levels and increased nitric acid production seem to contribute to this phenomenon, especially in diabetic women. The severe alteration of the oxidative pattern together with low antioxidant capacity detected in diabetic women may offer one possible pathogenic explanation for the higher incidence of cardiovascular complications observed in diabetics versus non diabetic women. Also, there is a strong direct relationship between oxidative stress markers and cardiovascular risk factors in diabetes mellitus.

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