Effect Of Vitamin On Malondialdehyde And Glutathione Levels In Type 2 Diabetic Nigerians

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Citation

I Nweke, O Ohaeri, C Ezeala. *Effect Of Vitamin On Malondialdehyde And Glutathione Levels In Type 2 Diabetic Nigerians*. The Internet Journal of Nutrition and Wellness. 2008 Volume 7 Number 2.

Abstract

The effect of modest supplementation of vitamin E (-tocopherol) on lipid peroxidation product, malondialdehyde (MDA), and reduced glutathione (GSH), was investigated in type 2 diabetic Nigerian patients. Written and informed consent to participate in this study was obtained from 80 type 2 diabetic patients. 50 randomly selected type 2 diabetic patients were supplemented with vitamin E capsule orally (1000 i.u / day) and 30 age-matched patients to placebo for 2 months. Fasting blood was collected from each patient before and after vitamin E or placebo supplementation. Levels of Reduced glutathione (GSH) and malondialdehyde (MDA) were determined. Hyperglycemia correlated with reduced blood GSH and increased malondialdehyde levels in type 2 diabetes. Vitamin E supplementation significantly increased GSH levels ($P<0.05$) and lowered MDA levels ($p<0.05$) which are markers of oxidative stress and this may reduce the risk of microvascular and macrovascular complications associated with diabetes mellitus.

INTRODUCTION

Elevated lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) levels have been implicated in the pathophysiology of type 2 diabetes mellitus. An exaggerated oxidative stress has been postulated as the link between hyperglycaemia and clinical complications such as cardiovascular diseases[1]. The depletion of defensive endogenous substances (antioxidants) is thought to increase the risk of complications in type 2 diabetes.

These antioxidants include enzymes like superoxide dismutase , catalase, glutathione peroxidase and glutathione reductase, minerals such as selenium, manganese, copper and zinc, vitamins such as vitamins A, C and E and other compounds such as glutathione, uric acid and flavonoids.[2] These antioxidants either protect, prevent or reduce the extent of oxidative destruction of cellular tissues. Increased free-radical production is said to mediate tissue injury in a wide range of diseases including diabetes mellitus and cardiovascular diseases[34]. Elevated levels of lipid peroxidation products and the simultaneous decline of antioxidant defense mechanism has been suggested to be harmful due to its disruption of membrane lipid and damage of cellular organelles resulting in oxidative stress.

Many studies have reported the antioxidant status in type 2 diabetes[5], but reports on the effect of antioxidant vitamins on oxidative stress indices such as malondialdehyde (MDA) and glutathione (GSH) in type 2 diabetic patients are scarce. In this study, we report the results of the effect of vitamin E on markers of oxidative stress such as glutathione and malondialdehyde in type 2 diabetic patients.

MATERIALS AND METHODS

PATIENTS

The study was conducted on patients who are members of the Rivers State Chapter of Diabetes Association of Nigeria (DAN) after informed and written consent was obtained from them and was approved by the Ethical Clearance Committee of the Institution. The study group consisted of eighty (80) adult type 2 diabetic patients, on diet and various hypoglycemic agents. Fifty (50) subjects (36 males and 14 females), were supplemented with -tocopherol, while the remaining thirty (20 males and 10 females) were supplemented with placebo. The age range was between 44 – 70 yrs.

Exclusion criteria included those with history of allergy to the study medication and existence of other illnesses requiring administration of other drugs. Neither diet nor hypoglycemic agent was changed in dose throughout the study. At baseline, their clinical and biochemical characteristics were evaluated. All patients were randomly

assigned to vitamin E supplementation at a dose of 1000 i.u/day (670mg/day, n = 50) or placebo (n =30) orally. The vitamin E capsules were sourced from Korea Etex Inc Manufacturing Company, Korea. Each treatment lasted for 8 weeks at the end of which a complete re-evaluation of the patients was made.

ANALYTICAL METHODS

About 6.0mls of venous blood were obtained from the patients after an overnight fast. Fasting plasma glucose was measured using commercially available kits (Randox Laboratories Manual Procedures,1996)[6]. The glycated hemoglobin concentration was estimated using commercially available kits[7]. MDA was measured as TBARS by the method of Wilbur et al[8]. Erythrocyte reduced GSH levels were determined in whole blood by adopting the method described by Beutler et al[9]. Vitamin E concentration was measured using the reverse phase high pressure liquid chromatography method[10]. All assays were performed in triplicate.

STATISTICAL ANALYSIS

Statistical analyses were performed using statistical package for social sciences (SPSS) software version. Pearson's correlation analysis was used to determine the relationships between variables and the extent of correlation was determined using regression analysis. All results are expressed as means ± SD. A P-value less than 0.05 was considered statistically significant.

RESULTS

The clinical and biochemical parameters of type 2 diabetic patients are summarized in Table 1. The patients had significantly decreased erythrocyte reduced glutathione (GSH) and elevated plasma malondialdehyde (MDA) levels before the vitamin E supplementation. Vitamin E treatment significantly increased the glutathione level from 0. 82 ± 0.43mmol/L to 1.36 ± 0.29mmol/L ($p<0.05$), and reduced the malondialdehyde level from 5.40 ± 0.57ng/ml to 3.99 ± 0.25ng/ml ($p< 0.05$). The glycated hemoglobin level was significantly reduced from 10.35 ± 3.00% to 7.92 ± 1.21% ($p< 0.05$). The levels of glycated hemoglobin, MDA and GSH in type 2 diabetic Nigerians pre- and post-vitamin E treatment are shown in Figure 1. Figures 2a and b show correlation scatter diagrams of glycated HbAIc levels against reduced GSH levels pre- and post-vitamin E treatment respectively. A significant inverse correlation between HbA_{Ic} and GSH was found ($p<0.05$, $r= -0.513$). Figures 3a and 2b show correlation scatter diagrams of glycated hemoglobin (HbAIc) levels against malondialdehyde (MDA) levels pre-and-post vitamin E treatment respectively. A significant positive correlation between HbA_{Ic} and MDA was found pre-vitamin E treatment($p<0.05$, $r = 0.467$) but was no longer significant post-vitamin E treatment ($p>0.023$, $r = 0.321$).

Figure 1

Table 1: Clinical and biochemical characteristics of type 2 diabetic patients

	Placebo (n = 30)		Vitamin E (n = 50)	
	Baseline	Treatment	Baseline	Treatment
Mean age (yr)	58 ± 15	-	58 ± 14	-
Diabetes duration(yr)	9 ± 7	-	9 ± 7	-
SBP (mmHg)	137 ± 17	140 ± 16	136 ± 16	130 ± 14
DBP (mmHg)	88 ± 14	86 ± 16	87 ± 15	84 ± 17
FPG (mmol/L)	8.50 ± 5.65	9.00 ± 5.80	8.00 ± 6.20	8.20 ± 6.0
HbA_{Ic} (%)	10.80 ± 2.40	9.80 ± 3.20	10.35 ± 3.00	7.92 ± 1.21*
MDA (ng/ml)	6.00 ± 0.60	6.20 ± 0.44	5.40 ± 0.57	3.99 ± 0.25 *
Red GSH(mmol/L)	0.74 ± 0.22	0.78 ± 0.30	0.82 ± 0.43	1.36 ± 0.29 *
Vitamin E (ug/ml)	4.04 ± 0.58	3.86 ± 0.63	3.96 ± 0.70	7.49 ± 0.70

All results are the mean ± S.D. SBP – Systolic blood pressure, DBP – Diastolic blood pressure, FPG - Fasting plasma glucose, HbAIc – Glycated hemoglobin, MDA – Malondialdehyde, Red GSH – Reduced glutathione.

* Data significant at $p<0.05$

Figure 2

Figure 1 : Glycated hemoglobin (HbA),malondialdehyde (MDA) and glutathione (GSH) levels in type 2 diabetic patients pre-and post-vitamin E treatment

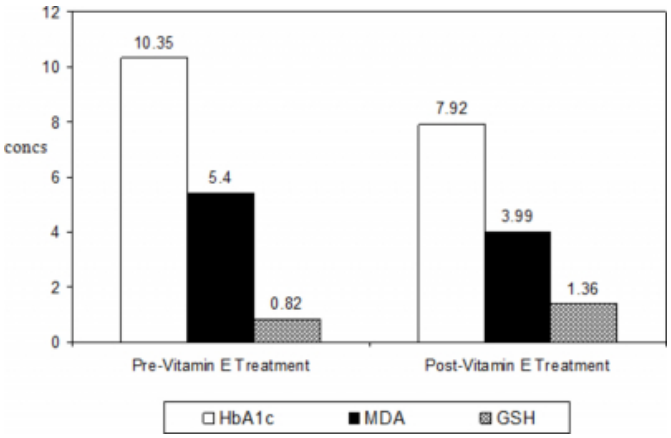


Figure 3

Figure 2a: A correlation scatter diagram of glycated hemoglobin (HbA) levels against glutathione (GSH) levels in type 2 diabetic patients pre-vitamin E treatment.

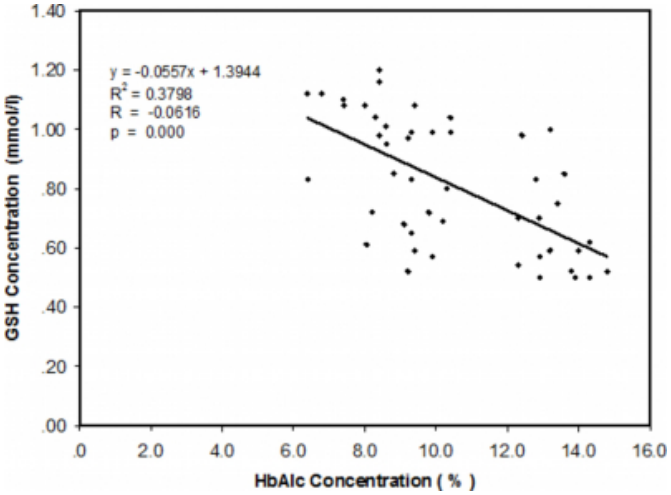


Figure 4

Figure 2b : A correlation scatter diagram of glycated hemoglobin (HbA) levels against glutathione (GSH) levels in type 2 diabetic patients post-vitamin E treatment.

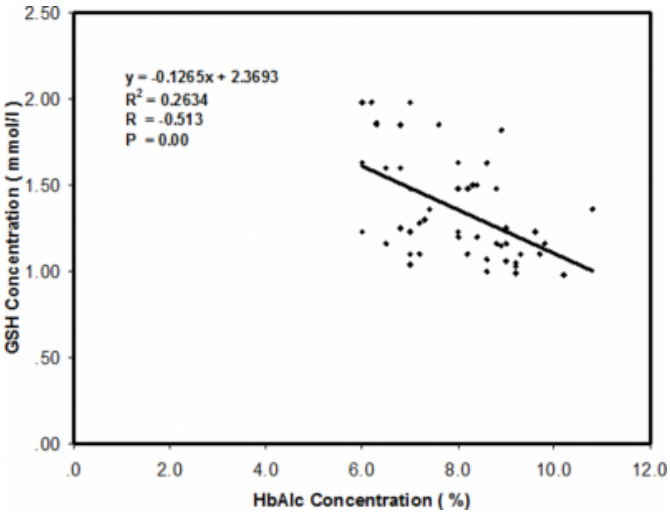


Figure 5

Figure 3a : A correlation scatter diagram of glycated hemoglobin (HbA) levels against malondialdehyde (MDA) levels in type 2 diabetic patients pre-vitamin E treatment

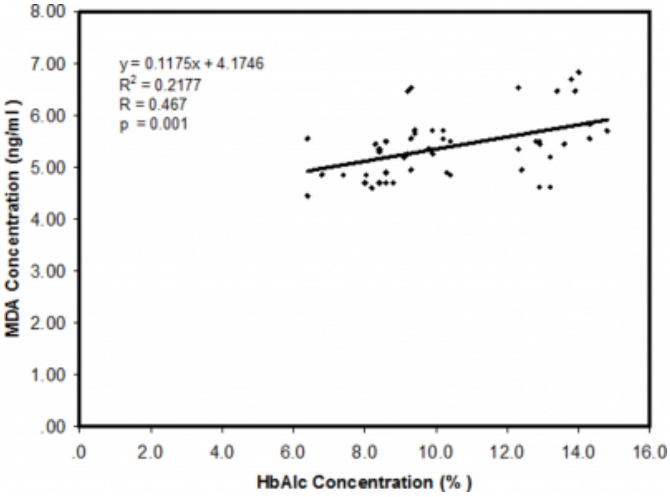
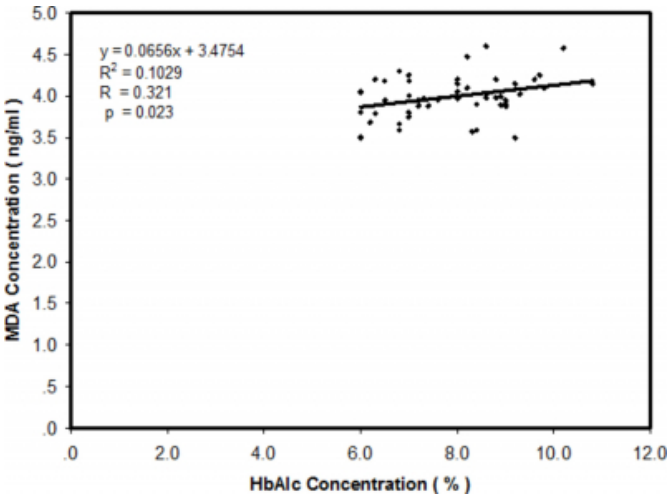


Figure 6

Figure 3b : A correlation scatter diagram of glycated hemoglobin (HbA) levels against malondialdehyde (MDA) levels in type 2 diabetic patients post-vitamin E treatment



DISCUSSION

Increased oxidative stress as measured by markers of oxidative stress has been shown to be increased in type 2 diabetes mellitus[11]. Despite strong experimental evidence indicating that oxidative stress may determine the onset and progression of late-diabetes complications[12], controversy still exists about whether the increased oxidative stress is merely associative rather than causal in DM. This is because measurement of oxidative stress is usually based on indirect measurement of free radicals[11]. The levels of these free radicals are controlled by levels of antioxidant enzymes as well as non-enzymatic scavengers like reduced GSH, vitamins, selenium and others.

Malondialdehyde, one of the lipid peroxidation products is frequently used to determine the oxidant/antioxidant balance in diabetic patients[13]. A study carried out in 467 cases of type 2 diabetes concluded that lipid peroxidation was significantly raised in their plasma and erythrocytes[4]. In our study, we found significantly elevated MDA levels in plasma of these type 2 patients before vitamin E supplementation. This finding is consistent with the results obtained elsewhere [4].

These patients had a poor glycemic control. In this study, there was a significant decrease in HbAIc levels of these patients after vitamin E supplementation. Glycemic control is fundamental to management of diabetes. Hyperglycemia has been shown to cause permanent alteration in proteins and increased lipid peroxidation in a variety of experimentally streptozotocin-induced diabetes[14].

Hyperglycemia, itself may stimulate platelet aggregation and autooxidation of glucose which may result in free radical production[15]. The importance of glycemic control in the prevention of diabetic complications has been confirmed in all types of diabetes.[16]

Reduced glutathione (GSH) and uric acid are physiological free radical scavengers. Thus glutathione plays a central role in antioxidant defense. Reduced glutathione maintains the integrity of the red blood cell membranes and also regenerates the major aqueous and lipid phase antioxidants such as ascorbate and -tocopherol. Furthermore, it has been shown to be a primary agent involved in redox regulation of protein thiols. In hyperglycemic condition, glucose is preferentially used in the polyol pathway that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme[17]. Hyperglycemia is therefore indirectly the cause of GSH depletion and this results in oxidative stress[18]. We have shown that red blood cell GSH levels decreased in our diabetic patients parallel to the increase in MDA levels before vitamin E supplementation. This is also in accordance with the results obtained elsewhere[15]. It is concluded that vitamin E supplementation was able to improve the already existing oxidative stress in type 2 diabetic patients. This observation nevertheless is an indication that long term vitamin E supplementation could reduce the morbidity and mortality rates associated with complications in diabetes mellitus. It is therefore recommended that vitamin E supplementation be introduced as an adjunct therapy in the management of type 2 diabetes mellitus.

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