

# Supplementation Effects of Vitamin C and Vitamin E on Oxidative Stress in Post Menopausal Diabetic Women

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## ABSTRACT

### Introduction

Menopause is a natural event of women with cessation of menstrual cycle. Menopause is associated with a wide variety of physical and psychological symptoms. The antioxidant enzyme systems seem to be affected in this phase due to deficiency of estrogen, which possesses antioxidant properties. Oxidative stress occurs at menopause because of loss of estrogens, which have an antioxidant effect on low-density lipoproteins. Diabetes is also a degenerative disease, usually accompanied by increased production of free radicals or impaired antioxidant defenses. Keeping these two real facts in mind, the present study has been designed to determine if menopausal women will suffer from the type II diabetes. This problem of high production of free radicals has become very complicated possibly leading to death.

### Objective

The main objective of the study is to assess the supplementation effect of vitamin E and Vitamin C on oxidative stress markers and

antioxidant enzyme levels with and without type II diabetic menopausal women.

### Materials and Methods

Blood samples of menopausal women age group with or without diabetes were collected from different hospitals of Allahabad. Serum antioxidant enzyme-Glutathione reductase, superoxide dismutase, and catalase were estimated. Serum Malondialdehyde was also estimated as stress marker.

### Results

Supplementation of vitamin C and vitamin E reduces the oxidative stress and increases the antioxidant serum enzyme level. Data shows that vitamin E is more effective than vitamin C.

It is concluded from the study that diabetic menopausal women, with supplementation of vitamin E have less risk of developing oxidative stress.

### INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated.<sup>1-5</sup> Worldwide, there

were approximately 194 million adults aged 20–79 years with diagnosed diabetes mellitus (DM) in 2003 (with type 2 diabetes accounting for 90–95% of all diagnosed cases), and that number is expected to increase to 333 million over the next 20 years.<sup>6</sup>

Diabetes is associated with increased coronary artery, cerebrovascular, and peripheral vascular disease, with up to 80% of deaths in people with diabetes caused by cardiovascular disease.<sup>7</sup> Diabetes is usually accompanied by increased production of free radicals<sup>8–11</sup> or impaired antioxidant defenses.<sup>12–14</sup> Menopause is associated with a wide variety of physical and psychological symptoms. It is a gradual three-stage process that concludes with the end of periods and reproductive life. Women experience menstrual bleeding during menopause and perimenopause. When a woman's menstruation has ceased spontaneously at least for a year, it is post menopause.<sup>15</sup> In post-menopause, a woman's ovaries stop making estrogen hormone. The antioxidant enzyme (AOE) system seems to be affected in this phase due to deficiency of estrogen, which has antioxidant properties. The beneficial effects of estrogens might be attributable to their free radical scavenging structures.<sup>16</sup>

Oxidative stress occurs at menopause because due to a loss of estrogens, which have antioxidant effect on low-density lipoproteins. Estrogens confer cardio protection by lowering protein oxidation and antioxidant properties.<sup>17</sup> Diminished antioxidant defense is associated with osteoporosis in post-menopause. Modulation of the estrogen receptors  $\alpha$  and  $\beta$  has been reported to be effected in vitro by oxidative stress.<sup>18</sup> A currently favored hypothesis is that oxidative stress, through a single unifying mechanism of super oxide production, is the common pathogenic factor leading to insulin resistance,  $\beta$ -cell dysfunction, impaired glucose tolerance (IGT) and ultimately to type 2 DM (T2DM).<sup>19</sup> Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications.<sup>20–22</sup> Overproduction

of free radicals can cause oxidative damage to bimoleculars, (lipids, proteins, DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging, and other degenerative diseases in humans.<sup>23</sup> Intake of natural antioxidants has been reported to reduce risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging, There is considerable controversy in this area.<sup>24</sup>

## **MATERIALS AND METHOD**

### **Subjects**

The case group consisted of 130 post-menopausal women with pre-existing type II diabetes. Out of 129 case subjects, 80 subjects are randomly selected for supplementation study with vitamin E and vitamin C, The remaining 49 case subjects are treated as positive control group (group-III). All 80 subjects were divided into two groups: group I consisted of 40 subjects consuming Vitamin E, and group II consisted of 40 subjects consuming vitamin C. Age range for all groups was 55–70 years.

### **Blood Sampling and Biochemical**

#### **Analyses**

Five milliliters of blood were collected between 0800–0900 hr in the morning from postmenopausal women of all groups into nonheparinized bottle for the measurement of biomolecules in serum. The blood was allowed to clot, retract, and the serum was separated by centrifugation at room temperature (200C). The serum was stored at –20oC till needed Then, the blood samples were analyzed for antioxidant enzymes like glutathione reductases,<sup>25</sup> catalase,<sup>26</sup> and superoxide dismutase<sup>27</sup> by auto pack kit method (Span / Diagnostic Ltd.), and MDA was estimated by using the Nadigar et al method with thiobarbituric acid.<sup>28</sup>

#### **Statistical Analysis**

Differences of data between supplementation group and the control group were tested with Student's t-test. A two-sided p value

**Table 1:** showing effect of vitamin C and E on different biochemical parameters.

Types	Control(n=50) Group-I	Supplementation with vitamin C(n=40) Group -II	Supplementation with vitamin E(n=40) Group-III	p value
Age(year)	60.00±10.50	59.86±11.25	60.80±14.90	i-ii NS i-iii NS ii-iii NS
Body weight(kg)	80.20±9.80	81.10±10.90	79.70±8.70	i-ii NS i-iii NS ii-iii NS
BMI(Kg/m <sup>2</sup> )	24.80±4.90	23.86±5.80	24.20±5.90	i-ii < 0.0001 i-iii <0.0001 ii-iii NS
MDA levels (nmol/ dl)	1.9 ± 0.4	1.1 ± 1.04	1.03 ± 1.1	i-ii < 0.0001 i-iii <0.0001 ii-iii NS
Superoxide dismutase (SOD) U/mg	5.09 + 1.94	6.46 + 0.256	6.86 + 1.1	i-ii < 0.0001 i-iii < 0.0001 ii-iii <0.0001
Catalase(CAT) U/mg	3.08 +1.05	3.37+ 2.1	3.98 +1.9	i-ii < 0.0001 i-iii < 0.0001 ii-iii <0.0001
Glutathione redu- catase (Moles of GSSH/ mg)	0.62 + 1.004	0.86 +1.00	0.98 +1.2	i-ii < 0.0001 i-iii < 0.0001 ii-iii <0.0001

<0.0001 was the level of statistically significance. All data were expressed as mean±SD. Statistical computations were calculated using SPSS 9.0 for windows software (SPSS Inc, Chicago, IL, USA).

## RESULTS

In the present study, evaluation of effect of vitamin C and vitamin E on serum oxidative stress marker (MDA) and antioxidant enzymes such as SOD, CAT, and GPX were done in post menopausal women having type II diabetes. Table 1 shows the effect of vitamin C and vitamin E on anthropometry parameters and biochemical parameters of post menopausal women having diabetes. There is no significant difference between the three groups with respect to age, weight, and BMI. Serum MDA of group I significantly differs from group II and group III (p<0.0001). In addition, there is no significant difference between of serum MDA in group II and group III. On the other hand, with reference to antioxidant enzymes, there is also a significant decrease in serum SOD

and GPX and significant increase in CAT enzyme in group I as compared to group II and group III.

Supplementation with vitamin E and vitamin C shows that there is a significant difference between antioxidant enzymes of group II and group III. There is a significant decrease in SOD and GPX and significant increase in CAT enzyme in group II which was supplemented with vitamin E as compare to group II which was supplemented with vitamin C.

## DISCUSSION

The menopausal phase in a woman's life is an important physiological phenomenon associated with the cessation of menstrual cycle due to loss of ovarian function. The deficiency of estrogen in postmenopausal women develops oxidative stress due to release of free radical or reactive oxygen species (ROS), and becomes the cause of various pathologies like the development of hypertension. Nonenzymatic sources of oxidative stress originate from the oxidative

biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate •OH radicals, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of •O<sub>2</sub><sup>-</sup>.

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