



## Research Article

**ROLE OF DIETARY ANTIOXIDANTS IN TYPE 2 DIABETIC INDIAN PATIENTS****Dr PJ Hisalkar<sup>1</sup>, Mrs Anupama B Patne<sup>2</sup>, Dr MM Fawade<sup>3</sup>**<sup>1</sup>Associate Professor, Dept of Clinical Biochemistry, ACPM Medical College & Hospital, Dhule-424001 (MS)<sup>2</sup>Assistant Professor, Dept of Biochemistry, JMF's ACPM Medical College, Dhule- 424001 Maharashtra.<sup>3</sup>Associate Professor, Dept of Biochemistry, Dr Babasaheb Ambedkar Marathwada University, Aurangabad. (MS)

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**Abstract** The prevalence of type 2 diabetes increases dramatically in India because of ample food supplies and a sedentary lifestyle. Excess dietary fat and sugar leads to ectopic lipid accumulation, generation of ROS, and cellular dysfunction. Dietary antioxidants can compensate for the lower plasma antioxidant levels often observed in type 2 diabetes and in prediabetic individuals, whether their diabetes is primarily genetic in origin or due to obesity and a sedentary lifestyle. The consumption of fruits and vegetables rich in vitamin and other antioxidants can increase overall antioxidant status and which could be beneficial in reducing insulin resistance and protecting vascular endothelium.

**Key words:** dietary antioxidant, type 2 diabetes

**INTRODUCTION**

Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population. India faces a grave health care burden due to the high prevalence of type 2 diabetes and its sequelae. Epidemiological data from different parts of the country show a rising prevalence of diabetes in the urban areas. Studies conducted in India in the last decade have highlighted that not only is the prevalence of type 2 diabetes high, but also that it is increasing rapidly in the urban population.<sup>1</sup>

If diabetes is not diagnosed early and managed properly, patients are at enhanced risk of microvascular (neuropathy, renal failure and blindness) and macrovascular complications on an atherosclerotic basis. The development of complications is complex and not yet fully understood, but involves the direct toxic effects of high glucose levels, along with abnormal lipid levels, oxidative stress and chronic inflammation.<sup>2</sup> Increasing evidence indicates that hyperglycaemia is the initiating cause of the tissue damage, either through repeated acute changes in cellular glucose metabolism, or through long-term accumulation of glycated biomolecules and ROS.<sup>3</sup> Several studies have shown that elevated blood glucose and an imbalance between free radical formation and antioxidant defence systems coexist in diabetic tissues both playing a pathogenic role in diabetes related complications.<sup>4,5</sup> In the presence of uncontrolled hyperglycaemia, the increased formation of ROS and lipid peroxidation products exacerbate oxidative stress and results in a loss of cell

integrity, disruption in cellular signalling and homeostasis, followed by inflammation, endothelial dysfunction and micro or macro angiopathies.<sup>6,7</sup> Several investigations have focused on antioxidant status and oxidative stress in type 2 diabetes. Dietary antioxidants have been hypothesized to have a protective effect against the development of diabetes by inhibiting peroxidation chain reactions.<sup>8</sup> It seems plausible that a sufficient intake of antioxidants plays an important role in protection against type 2 diabetes. However, little epidemiological evidence is available on the role of dietary antioxidant intake in prevention of type 2 diabetes. In one prospective cohort study, vitamin C intake was significantly lower among incident cases of type 2 diabetes.<sup>9</sup> In three prospective observational studies, serum alpha tocopherol levels were associated with lower risk of type 1 or type 2 diabetes.<sup>10-12</sup> Serum beta carotene and alpha tocopherol concentrations were non significantly associated with a reduced risk in a cohort of Finns.<sup>13</sup> In some case control and cross sectional studies, significantly lower serum levels of alpha tocopherol, carotene, vitamin C have been observed in individuals with diabetes than in control subjects.<sup>14-16</sup> In one American study, alpha tocopherol concentrations were even higher in diabetic patients than in control subjects.<sup>17</sup>

Some prospective studies have shown that higher vegetable and fruit consumption may lower the risk of developing diabetes, suggesting that antioxidants in the diet may have a synergistic effect.<sup>18,19</sup> Hence the present study aimed to

understand the role of dietary antioxidants in type 2 diabetic Indian patients.

## MATERIAL AND METHODS

### Patients and Methodology

This study included 120 type 2 diagnosed patients at ACPM Medical College and Hospital, Dhule-424001 (MS). These patients were compared age wise & sex wise with non diabetic healthy subjects. Here none of the any patients and control subjects was taking dietary supplements such as vitamins or minerals.

Using standard protocols, fasting blood samples were collected from both controls and patients. Fasting & post prandial BGL were measured by using ERBA diagnostics Mannheim GmbH reagent kit. Glycated HbA<sub>1c</sub> was determined by microcolon test system according to Bisse with Sigma diagnostic kit. Zinc level was measured by using Centronic GmbH-Germany kit. Ascorbic acid (Vitamin C) and Vitamin E level were measured by the method of Ayekeyaw (1978) and method of Hansen and Warwick respectively.

### Statistical analysis

Data are expressed as mean  $\pm$  SD. Statistical significance was evaluated by Student's 't' test. Differences were considered significant at  $p < 0.05$ . Correlation between the parameters tested was studied by a regression analysis.

## RESULTS

	Type diabetic patients (n=60)	Controls (n=60)
Sex ( Male / Female )	33/27	33/27
Age (Years) (Mean $\pm$ SD )	44.7 $\pm$ 8.5	43.1 $\pm$ 8.7
FBG (mg/dl)	157.1 $\pm$ 2.6 *	89.1 $\pm$ 3.5
PPBG (mg/dl)	211.7 $\pm$ 2.8 *	139.7 $\pm$ 2.3
HbA <sub>1c</sub> (%)	8.1 $\pm$ 0.1 *	4.9 $\pm$ 1.1
Ascorbic acid (mg/dl)	0.45 $\pm$ 0.04 *	0.76 $\pm$ 0.33
Vitamin E (mg/l)	2.3 $\pm$ 0.4 *	4.6 $\pm$ 0.7
Plasma Zinc ( $\mu$ g/dl)	59.4 $\pm$ 9.6 *	64.6 $\pm$ 7.8

(Data are presented as means  $\pm$  SD, \* - Significant difference compared with controls ( $p < 0.05$ , FBS -fasting blood glucose, PPBG- post prandial blood glucose, HbA<sub>1c</sub> - glycated haemoglobin)

## DISCUSSION

The basic findings of the present study is a significant increase in FBG, PPBG and HbA<sub>1c</sub> in patients of type 2 diabetes. HbA<sub>1c</sub> is a valuable

index of hyperglycaemia and protein glycation of the previous 3-4 months. Second most important observation was significant reduction in ascorbic acid, vitamin E & zinc in the plasma of diabetic patients as compared with normals.

**Ascorbic acid** is an essential constituent of a healthy diet and also a potent antioxidant that raises cellular defences against oxidative stress. It acts as a pro-oxidant in the diabetic state and could be associated with increased free radical formation and lipid peroxidation, which significantly decreased in diabetics.<sup>20</sup> Human have no ability to synthesise vitamin C due to mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin via the glucuronic acid pathway. Thus vitamin C is obtained through the diet. The vitamin is especially plentiful in fresh fruit, in particular citrus fruit and vegetables.<sup>21</sup> Several studies have reported lowered plasma concentrations of ascorbic acid in diabetics compared to healthy subjects.<sup>22,23</sup>

Our result supports the hypothesis that low vitamin C status in diabetes may be due to a higher turnover rate of ascorbic acid, with increased oxidation to the oxidized form dehydroascorbate. Another possible mechanism for the impaired ascorbate status is a competitive inhibition between glucose and ascorbic acid, which both share a close structural homology and possibly occupy common membrane transport sites.<sup>24</sup> Supplementation with ascorbic acid may have a beneficial effect. Ford S.E. et al (2003) suggested the decreased concentration of antioxidant vitamins in metabolic syndrome like diabetes may be attributed to lower intake of fruits and vegetables rich in antioxidants or increased use of antioxidants to counteract oxidative stress.<sup>25</sup> In a study with 27 type 2 diabetics, supplementation with ascorbic acid (2gm/day) improved glycemic control and fasting plasma glucose.<sup>26</sup> If the efficacy of vitamin C supplementation in diabetes is confirmed, a higher vitamin C intake than for the general population may be recommended for patients with diabetes. Increased demand for vitamin C to compensate the increased oxidative stress and impaired transport or dietary deficiency of vitamin C may be contributing to decreased levels of plasma vitamin C levels observed in type 2 diabetic patients.<sup>27</sup> High but physiologic concentrations of ascorbic acid can directly inhibit erythrocyte aldose reductase and provide a rationale for the use of oral vitamin C supplements in diabetes.<sup>28</sup> Ascorbic acid supplementation for diabetic subjects may provide a simple means of preventing and ameliorating the complications of diabetes.

**Vitamin E** is an important micronutrient that also functions as an antioxidant. It acts to

protect polyunsaturated fatty acids (PUFA) from oxidation by interrupting the chain of membrane lipid peroxidation and thus is also referred to as a 'chain-breaking' antioxidant.<sup>29</sup> In our study significantly decreased results clearly indicate that diabetic patients have need of vitamin E supplementation. Effect of vitamin E supplementation on diabetic induced oxidative stress in experimental diabetes in rats demonstrates that that vitamin E supplementation augments the antioxidant defence mechanism in diabetes and provides evidence that vitamin E may have a therapeutic role in free radical mediated diseases.<sup>30</sup>

Prospective epidemiological studies demonstrate that high serum vitamin E levels are associated with decreased risk of NIDDM.<sup>31</sup> Prospective studies of non-diabetic individuals provide evidence that vitamin E supplementation is associated with a protective effect against coronary heart disease.<sup>32</sup> In humans and in animal models of NIDDM, vitamin E reduces vascular oxidative stress and preserves endothelial function, thus inhibiting the development of atherosclerosis. Specifically, vitamin E supplementation of as little as 400 µg/day can make LDL less susceptible to oxidation and consequently less atherogenic. In vascular tissue, the protein kinase C pathway regulates basement membrane turnover, cellular proliferation and endothelial cell permeability. Activation of this pathway by hyperglycemia has been linked to macro- and microvascular dysfunction. Vitamin E supplementation has been demonstrated to prevent the induction of protein kinase C activity in the hyperglycemic aorta, thereby inhibiting the migration and proliferation of vascular smooth muscle cells. This effect of vitamin E can prevent or at least delay many of the vascular complications associated with NIDDM. Natural vitamin E is a mixture of tocopherols and tocotrienols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - tocopherol, and  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - tocotrienol) produced only by plants. Of these,  $\alpha$ -tocopherol has the highest antioxidant potency.<sup>33</sup> Vitamin E is commercially available as either a mixture of naturally occurring tocopherols and tocotrienols, synthetic  $\alpha$ -tocopherol (which consists of the eight possible stereoisomers in equal amounts) or a mixture of the synthetic tocopherol esters.<sup>34</sup> Its presence in biological membranes is thought to represent the major defense system against free radical mediated lipid peroxidation. Vitamin E intake was significantly associated with a reduced risk of type 2 diabetes which supports the hypothesis that development of type 2 diabetes may be reduced by the intake of antioxidants in the diet.<sup>35</sup>

The close relationship between zinc and insulin action was first documented by Scott<sup>36</sup> in early 1930s, when **zinc** was found to be an integral component of crystalline insulin. Over the years,

studies have shown that zinc ions play important roles in the biosynthesis, storage and action of insulin.<sup>37</sup> It binds to metallothionein (MT) under physiological conditions, is a potent inducer of MT.<sup>38</sup> In Indian context zinc is considered as important trace element and it can exert a number of indirect antioxidant functions. Many researchers have found evidence to support this concept by studying mild zinc deficiency in rats. This state produces low resistance to chemically induced liver oxidant injury and it produces high vulnerability of lipoproteins to oxidation.<sup>39</sup> In people with diabetes, the vulnerability to oxidative damage may be partly attributed to deficient antioxidant micronutrient status, including trace elements. Impairment of zinc status has been reported as an aggravating factor in the progression of diabetes. Although zinc may influence the processes associated with oxidant stress, the practical consequences of its status have not been studied extensively. The function of zinc in the body metabolism is based on its enzymatic affinity, such as a zinc-enzyme complex or zinc metalloenzyme. In humans and animals, diabetes may result in disturbance of these vital trace elements.<sup>40</sup> In most mammals, insulin is stored as zinc crystals and is likely that it is secreted in zinc form. Zinc has important role in modulating the immune system and its dysfunction in diabetes mellitus may be related in part to the status of zinc.<sup>41</sup> Lack or inadequate supply of such nutrients produces functional impairment and can result in disease. The clinical significance and evaluation of zinc in regard to different diseases, including diabetes mellitus remains conflicting as well as controversial. Zinc may act in normalizing glycemia and a restored zinc status in patients with type 2 diabetes may counteract the deleterious effects of oxidative stress, helping to prevent many complications associated with diabetes.<sup>42</sup>

The results of this study are in agreement with the results of studies showing that plasma zinc levels in patients with type 2 diabetes are significantly decreased.<sup>43-45</sup> A possible explanation for this is that there is loss of a large amount of zinc from the body via urine. The source of the zinc that being excreted remains unresolved. There is a concurrent hypozincemia and a decrease in tissue zinc stores. However, it is not clear if this results from hyperzincemia or from an independent event, an insulin or hyperglycemia related induced loss of zinc from tissue stores. Zinc would then be released into the plasma and thereafter excreted. This results in a net loss of total body zinc and eventual hypozincemia. Possible sources of elevated free radicals in type 2 diabetes include increased production of radical oxygen species, especially from glycation or lipoxidation processes, auto-oxidation of glucose and oxidizing of glucose and decreased antioxidant defense system. Improvement

of glycemic control and supplementation with zinc appears to be a beneficial factor in decreasing lipid peroxidation in patients with diabetes. Prevention of lipid peroxidation may help to delay the development of diabetic complications.<sup>46</sup>

A recent study examined whether Zinc supplementation could protect against diabetic cardiomyopathy through cardiac MT induction. These results provide evidence that the prevention of diabetic cardiomyopathy by zinc supplementation is predominantly mediated by an increase in cardiac MT and suggest zinc supplementation with cardiac MT induction, as a potential therapeutic approach to prevent diabetic cardiomyopathy. Furthermore, zinc is required cofactor for a variety of antioxidant enzymes, particularly superoxide dismutase. Alterations of zinc metabolism resulting in reduced availability of zinc might be expected contribute to tissue damage observed in diabetes.<sup>47</sup>

## CONCLUSION

Dietary antioxidants like Vitamin C, E and zinc may either block the formation of free radical or scavenge them once they have formed. The study therefore suggests the use of certain antioxidant vitamin and mineral supplements may be beneficial as an adjunct therapy in the management of diabetes and its complications.

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