EFFECT OF THE ANTIOXIDANT VITAMINS E AND C ON OXIDATIVE STRESS IN TYPE II DIABETES MELLITUS

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Abstract:

Plasma Malondialdehyde and Protein carbonyl were studied in 79 Type II Diabetes mellitus patients before and after supplementation with vitamin E, or vitamin C, or both. The results revealed that both Malondialdehyde and Protein carbonyl were decreased after supplementation and the decrease was most significant in the patients who received both vitamins C and E. These findings suggest that these antioxidant vitamins might have a beneficial role in diabetes by decreasing lipid and protein oxidation products.

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Keywords : Diabetes mellitus, Malondialdehyde, Protein carbonyl, Vitamin E, Vitamin C

Introduction-

Diabetes mellitus is a common metabolic disorder. The involvement of oxidative stress in the various stages of Type II diabetes mellitus is a topic of interest. Type II Diabetes mellitus patients are exposed to increased oxidative stress due to several mechanisms (1). The effect of free radical injury can be measured in the form of products of lipid peroxidation like malondialdehyde (MDA) in the form of Thiobarbituric Acid Reacting Substances (TBARS) (2), or products of protein damage like protein carbonyl assay (3).

MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and also during arachidonic acid metabolism and Prostaglandin H breakdown. Hydro- and endo-peroxides derived from polyunsaturated fatty acid are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds including MDA. MDA is used as a general indicator of oxidative stress in lipid peroxidation (4).

Protein carbonyls can be formed by direct oxidative cleavage of the peptide chain or by oxidation of specific amino acid residues like lysine, arginine, proline and threonine. Carbonyls can also be formed indirectly through modification of lysine, histidine and cysteine residues by alpha,beta-unsaturated aldehydes such as 4-hydroxynonenal in a process called Michael addition, or via Schiff base formation between lysine residues and dialdehydes like MDA (5).In oxidized proteins a variety of amino acids like methionine sulfoxide,3-nitrotyrosine are found(3).A vital sign of oxidative protein damage is carbonyl group incorporation in proteins(6).

Antioxidants are decreased in type II diabetes mellitus due to excess free radicals. The balance between oxidants and antioxidants is therefore altered. Vitamins C and E are important antioxidants and are essential to destroy harmful free radicals (7). Though studies, especially with animals, have been done to find the effect of vitamin E on lipid peroxidation products (8), to our knowledge, no studies have been carried out in humans comparing the effect of administering vitamins E and C together and also separately, on both protein and lipid oxidation products in type II diabetes mellitus. This was done by administering vitamins E and C separately and also together, to different groups of type II diabetes mellitus patients & measuring MDA and protein carbonyl levels.

Materials and methods-

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The study was done in Murshidabad medical college and hospital, Behrampore, West Bengal, India on the patients who attended the OPD. Three groups of type II diabetes mellitus patients were taken, all diagnosed as per WHO criteria, aged above 40 years, taking antidiabetic therapy and were administered vitamins for 6 months. Group I consisted of 28 patients who were given 400 IU vitamin E daily. Group II consisted of 25 patients who were given 250 mg vitamin C daily. Group III consisted of 26 patients who were given both 400 IU vitamin E and 250 mg vitamin C daily. Fasting blood samples were drawn from all subjects at the starting, after 3 months and after 6 months. The study was approved by the Institutional Ethics Committee.

Plasma protein carbonyl group levels were evaluated following the 2,4-dinitrophenylhydrazine (DNPH) assay described by Levine et al (9). DNPH in HCl is added to sample, incubated and washed with tricarboxylic acid. The final protein pellet is dissolved, incubated in guanidine and absorbance read at 355 – 390 nm.

Plasma MDA levels were assayed by TBA reaction described by Esterbauer et al (10). The sample was heated with TBA at 90 –100°C in acid.The MDA-TBA adduct was extracted into butanol and OD read.

All data are expressed as means $\pm$ SD. Within-group changes was tested by Student's 'T' test & those between groups was tested using ANOVA.$P<0.05$ was considered significant & $P<0.001$ highly significant.

Results-

Pr. Carb.

	Group I (n = 28)	Group II (n = 25)	Group III (n = 26)
1. At 0 months	1.12±0.16	1.04±0.18	1.01±0.12
2. At 3 months	1.07±0.19	0.95±0.16*	0.94±0.19*
3. At 6 months	0.96±0.09*	0.83±0.12**	0.69±0.07**

MDA

	Group I	Group II	Group III
1. At 0 months	9.17±1.21	9.01±1.73	8.83±1.49
2. At 3 months	7.62±1.56**	8.17±1.14*	7.59±1.07**
3. At 6 months	6.04±1.02**	6.80±0.83**	4.07±0.66**

Table 1 – Plasma protein carbonyl (nmole/mg) and MDA (nmol/ml) levels in the patients. Data are given as mean ± SD. Levels of significance – *P<0.05, **P<0.001. 'n' denotes the number of subjects.

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Discussion-

In animal models of experimental diabetes mellitus, the tissue content of MDA is increased by different mechanisms. Glucose, like many other simple sugars, has both pro-oxidant and antioxidant properties. In addition, glycation of proteins initiates glyco-oxidative pathways, generating additional free radicals (11). This process of increased glycation is enhanced in the presence of MDA, as the process of MDA modification of proteins is enhanced with glycation and some antioxidants can reduce MDA modification of proteins. Further, increased peroxidation of membranes occurs in diabetics due to their higher polyunsaturated fatty acid content (12). In the present study also, increased MDA levels were found in all the diabetes patients (Table 1).

In diabetes, proteins undergo oxidation and protein carbonyls are formed by several mechanisms. A peptide chain can be cleaved by oxidants, or particular amino acids like cysteine or methionine can be oxidized, because of the relative abundance of free radicals in diabetes. As occurs in lipids, radical propagation can occur in proteins, with formation of additional reactive species (3). Also, glucose can be a pro-oxidant and this effect is increased in hyperglycemia (13). Further, glyco-oxidative pathways (which produce more reactive oxygen species) can be initiated by glycation of proteins (10). Thus, via all these pathways, protein carbonyl levels in diabetes are elevated. Increased protein oxidation

has been demonstrated in diabetics (14). Similarly, our present study too has found elevated protein carbonyl levels in the patients (Table 1).

Vitamin E (alpha-tocopherol) is a chain-breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins. It intercepts peroxyl radicals formed previously and inactivates the radical before a polyunsaturated fatty acid can be attacked. Alpha-tocopherol reacts with the radical, converting it into a hydroperoxide product. The tocopheroxyl radical thus formed is stable and can react with another peroxyl radical getting converted into inactive products (15). Also, before MDA is formed during lipid peroxidation, vitamin E stabilizes the hydroperoxides formed and reduces their further decomposition into MDA (16). Vitamin E might cause decreased incidence of coronary artery disease in diabetes mellitus by stabilization of platelet membranes and reduction in LDL oxidation. LDL is converted to o-LDL through oxidation of its lipid component with subsequent modification of apo-B 100 by MDA (17). So, reduction of MDA by vitamin E diminishes its conversion of LDL to o-LDL. But vitamin E levels are often low in diabetes mellitus due to altered nutritional status because of either poor intake or excessive losses, or overconsumption of vitamin E due to excess free radicals (18). Therefore, supplementation of diabetic patients with vitamin E might benefit the antioxidant status, as reflected by the MDA levels, which were decreased (highly significantly) at 3 months and 6 months, compared to MDA levels at 0 months (Table 1).

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Raective oxygen species can peroxidise lipids. This is inhibited by vitamin E, which in turn, is regenerated by vitamin C. Vitamin C itself also causes reduction of the initiating oxygen species. Therefore, vitamin C decreases lipid peroxidation and reduces MDA levels. This is supported by the fact that both endogenous and exogenous vitamin C decreases lipid peroxidation in animals (19). Vitamin C also inhibits lipid peroxidation by hemoglobin-hydrogen peroxide mixtures. Vitamin C prevents haem breakdown and release of iron (this free iron otherwise would have led to formation of free radicals that can peroxidise lipids). Also, vitamin C maintains glutathione in the reduced state, thereby helping the action of glutathione peroxidase in decomposing peroxides (20). In plasma, antioxidants like superoxide dismutase, glutathione peroxidase are present in only small amounts. Therefore, other (small molecule) antioxidants like vitamin C are important (21). Vitamin C inhibits lipid peroxidation via neutrophils, cigarette smoke, superoxide and hydrogen peroxide, aqueous peroxyl radicals and redox active iron. Under all these conditions, only vitamin C can prevent detectable lipid peroxidation in plasma; so, if vitamin C is depleted, detectable amounts of lipid peroxides are formed, even if vitamins A, E are present (22).

Vitamin C content of platelets and leukocytes are decreased in diabetes mellitus for various reasons. Vitamin C and glucose have similar molecular structures and share the same transport carrier. The active transport of vitamin C is decreased by hyperglycemia. Hyperglycemia also inhibits uptake of dehydroascorbate, the oxidized form of vitamin C. Thus, in hyperglycemia the tissue content of vitamin C can decrease. Also, higher oxidative stress in diabetes mellitus can cause overconsumption of the antioxidant vitamin (23). Therefore, in our present study, vitamin C supplementation to the patients has lowered MDA levels significantly at 3 months and highly significantly at 6 months (Table 1).

The free radicals which are readily scavenged by vitamin C are superoxide, hydroxyl free radical, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitroxide, nitrogen dioxide, hypochlorous acid, etc. (24); some of these are involved in oxidizing proteins and forming protein carbonyl. Therefore, by reducing these radicals, vitamin C decreases formation of protein carbonyl. This is supported by the fact that in animal studies there was decreased protein carbonyl formation in guinea pigs supplemented with vitamin C (25). Vitamin C supplementation of patients with H.pylori gastritis led to significant reduction in nitrotyrosine (one of the oxidized amino acids mentioned earlier in this article) (26). Vitamin C can replenish tetrahydrobiopterin or enhance its affinity for nitric oxide synthase (eNOS) cofactor . Tetrahydrobiopterin oxidation to biopterin alters eNOS function, causing the preferential production of superoxide instead of NO. Therefore, indirectly vitamin C decreases superoxide, which would have otherwise led to increased protein oxidation (27). Possibly, all these reasons have led to decreased protein carbonyl levels in this study at 3 months (significantly) and 6 months (highly significantly) (Table 1).

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Vitamin E scavenges superoxide, hydroxyl free radical and prevents injury to thiols. Vitamin E also activates diacylglycerol (DAG) kinase, preventing intracellular DAG accumulation. Increasing concentrations of DAG cause protein kinase C activation and subsequent enzymatic production of superoxide. Vitamin E therefore inhibits the synthesis or action of all these free radicals, which might have caused more formation of protein carbonyl (28). Thus, vitamin E decreases protein carbonyl levels in diabetes. Vitamin E supplementation has thus lowered protein carbonyl levels in the patients in our study after 6 months (significantly), but nonsignificantly after 3 months (Table 1).

Vitamin C acts as a coantioxidant by regenerating alpha-tocopherol from the alpha-tocopheroxyl radical, produced via scavenging of lipid-soluble radicals. (Vitamin C is itself converted to dehydroascorbic acid, which is partially reduced back to ascorbic acid. Some dehydroascorbic acid is hydrolysed and lost. Thus, vitamin C levels become lower) (2). Vitamin C also prevents LDL oxidation by regenerating LDL-associated vitamin E. Further, it has been shown that in vitro, alpha-tocopherol can act as a prooxidant in absence of coantioxidants (29). So, combination of both vitamins C

and E is quite important. The importance has been indirectly found in studies supplementing both vitamins C and E, where the progression of atherosclerosis was reduced (30). Our present study also found highly significant decreases in both MDA and protein carbonyl levels at 3 months and 6 months in patients given both vitamins (group C), compared to the levels at 0 months. Further, this decrease for both parameters in levels of group C patients was significant also when compared to corresponding levels in group A and B patients (Table1).

Some limitations of this study should be considered. The treatment period was relatively short, though it was enough to establish significant findings. Also, the number of patients studied was less. So, extrapolating the results to other populations should be done cautiously and trials with larger number of patients are needed. Lastly, the patients were on medications for diabetes and associated conditions. However, these medicines are commonly taken in diabetes and were not changed during the study period.

To conclude, diabetes mellitus is associated with increased lipid and protein oxidation products and a reduced antioxidant defense system. As these might lead to complications, and also supplementation with vitamins C and E decreases the levels of oxidation products, our findings suggest that antioxidant vitamins might have a beneficial role in diabetes. Further investigations are needed to shed additional light on dose, duration of therapy and other aspects of supplementation.

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