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Patient Selection in Vitamin E Treatment in Diabetes Mellitus

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Abstract

In diabetes there is an increase in oxidative stress due to elevated glucose levels in the plasma. High glucose promotes glycosylation of both plasma and cellular proteins which particularly affects the endothelial cell lining of the blood vessel wall and interferes with its normal function. Thus diabetes mellitus patients suffer from a higher incidence of cardiovascular complications such as atherosclerosis as compared to the non-diabetic population. Haptoglobin (Hp) is a plasma protein which binds free hemoglobin and prevents heme-iron mediated oxidation. There are three different types of Hp which differ in their antioxidant ability. Several clinical studies have shown that the Hp 2-2 genotype is associated with higher incidence of cardiovascular diseases among diabetics. Vitamin E, a low cost, easy to use antioxidant, was found to decrease the risk of developing cardiovascular diseases in Hp 2-2 diabetic patients. This review summarizes several studies which show the importance of vitamin E supplementation in a specific sub-group of patients consisting of diabetic individuals carrying the Hp 2-2 genotype.

Keywords

haptoglobin; hemoglobin; oxidative stress; diabetes; glycation

Diabetes mellitus

Diabetes mellitus (DM) is a global health problem that results in a greater risk of vascular complications, decreased quality of life, and increased mortality. DM patients suffer from metabolic alterations which affect many organs in the body, especially the cardiovascular system[1]. High plasma glucose levels result in glycosylation of both plasma and cellular proteins. One major target of glycosylation is the blood vessel wall that leads to endothelial dysfunction. Beyond this, hyperglycemia also interferes with nitric oxide (NO) release resulting in impaired vascular tone. Moreover, smoking, hypertension and hyperlipidemia enhance oxidation of lipids and their subsequent accumulation in macrophages, leading to foam cell formation and plaque development. This process of atherosclerosis is the leading cause of morbidity and mortality in DM patients and ultimately leads to myocardial infarction, stroke, and loss of kidney function.

Clinical studies of vitamin E

In DM there is an increase in oxygen radical formation due to glucose auto oxidation, the formation of advanced glycosylation end products, and metabolic stress. Therefore the influence of different antioxidants has been the subject of many studies over the years. Due to the involvement of LDL oxidation in the pathology of atherosclerosis vitamin E, an antioxidant with the ability to react with lipid peroxy radical and terminate the propagation

of free radicals was studied. Several observational epidemiologic studies suggest that vitamin E supplementation might decrease the risk of developing cardiovascular disease (CVD).

Over the past 15 years a number of prospective studies were established to investigate this association. For the purpose of this review, these studies have been divided into two categories. The first category includes primary prevention studies, which involve healthy people with no history of myocardial infarction (MI). The second category comprises secondary prevention studies, which involve people with known cardiovascular complications. It should be noted that none of these studies used genetic markers as a means of determining patient selection, nor were they confined to diabetic patients only (except for the HOPE study) although statistically a significant proportion (approximately 30%) of patients who have or will have CVD are diabetic.

Studies of primary prevention

The first randomized controlled trial (RCT) was published in 1998 by Virtamo and colleagues. In this study almost 30,000 Finnish male smokers, aged 50–69 years, were randomly given beta carotene (20 mg/day) with or without vitamin E (50 mg/day) or placebo, and were followed for 5 to 8 years or until the first MI. A minor decrease that was not statistically significant was noted in the incidence of MI in the people who received vitamin E [2]. Similar results were found in another study that was published by de Gaetano and colleagues in 2001 [3]. In the St. Francis RCT study published in 2005, supplementation of aspirin (81 mg/ml) with or without vitamin E (1000 IU/d), vitamin C (1 g/d) and atorvastatin (20 mg/d) did not affect the progression of calcium deposits in the coronary arteries or improve clinical symptoms [4].

Another group of studies investigated the association between vitamin E and atherosclerosis exclusively. In 2002, a group of men and women were randomly assigned to receive vitamin E (400 IU/d) or placebo for 3 years. Study participants were over 40 years old and had high levels of LDL (> 3.37 mmol/L) but did not show symptoms of CVD. The purpose of this trial was to monitor the changes in the intima-media thickness (IMT) of the common carotid artery. The levels of circulating oxidized LDL in the plasma were also measured. The results showed that although oxidized LDL levels were lower among the recipients of vitamin E, no improvement was noted in IMT in this group [5]. However, in the Anti-Oxidant Therapy in (ATIC) study, the combination of three anti oxidants: vitamin E, homocysteine, and pravastatin, were investigated and a noteworthy decrease in IMT, together with improved albuminuria and endothelial dysfunction were found [6]. In 2005 the Womens' Health Study (WHS), which included 40,000 healthy women, was designed to assess the relevance of supplementation of vitamin E or aspirin in preventing CVD and cancer. The results showed a decrease of 24% (RR, 0.76; P value 0.03) in CV death among the women who received the preventable treatment. However, these results were not sufficient to recommend vitamin E supplementation for CV disease prevention. [7].

In the Physicians Health Study 14,641 males over the age of 50 years were randomized to receive vitamin E (400 IU/d) and C (500 mg/day) for 8 years. In this study, no effect was found on CV death, nonfatal stroke or CVD. However, an increased risk of hemorrhagic stroke among people who received vitamin E was noted [8].

Studies of secondary prevention

In the Gruppo Italiano per lo Studio della Sopravvivenza nell' Infarto miocardico (GISSI)-Prevenzione study, 11,324 patients who had a recent MI were randomized to vitamin E (300 mg/day), polyunsaturated fatty acids (PUFA) (1 g/day), both, or neither of them. They were

followed for 3.5 years after which no effect of treatment with vitamin E was observed [9]. In the Cambridge Heart Antioxidant Study (CHAOS), approximately 2000 patients with known coronary atherosclerosis were divided into two sub-groups: 1035 patients received vitamin E (400 IU/day or 800 IU/day) and 967 received placebo. The purpose of this research was to see if vitamin E supplementation can decrease the risk of developing non-fatal MI or CV death. After a follow up of 510 days the data was evaluated and a statistically significant reduction in the risk of developing non-fatal MI or CV death was found. However, after reevaluating the data for non-fatal MI and CV death separately a disparity was found. Treatment with vitamin E decreased the risk of developing non-fatal MI, but increased the risk of CV death[10].

Similar results were obtained from the Heart and Outcomes Prevention Evaluation (HOPE) in 2000. This study included 6,996 men and 2,545 women with diabetes. The patients were followed for about 4.5 years during which they received vitamin E (400 mg/day) or angiotensin converting enzyme (ACE) inhibitor (Ramipril) or a placebo. The results showed that vitamin E did not influence the risk of developing CVD[11]. In 2005 an extension of this study was performed called the HOPE-TOO study. In this analysis the incidence of CVD, cancer and cancer death was investigated in patients who received vitamin E (400 IU/day) or a placebo. Patients who received vitamin E had a higher risk of heart failure and required hospitalization[12]. The Women's Angiographic Vitamin and Estrogen (WAVE) Trial showed an increased risk of developing coronary heart disease in postmenopausal women who received vitamin E (400 IU) or vitamin C (500 mg), both, or neither daily [13]. One study that showed a beneficial effect of vitamin E was the Secondary Prevention with Antioxidants of Cardiovascular Disease End stage Renal Disease (SPACE) study. Hemo dialysis patients were randomized to vitamin E (800 IU/day) or placebo groups and were followed for 519 days. The results showed a beneficial effect of vitamin E on different cardiovascular complications, although the risk of mortality was not affected [14].

In summary, due to the contradictory results described above regarding the beneficial role of vitamin E in protecting against cardiovascular complications, high dose vitamin E supplementation has not been recommended by the medical community. In fact, a meta-analysis of over 135,000 individuals treated with vitamin E concluded that high dose vitamin E (greater than 400 mg/day) slightly increases the risk of mortality [15]. However, recent investigations into the polymorphic serum protein haptoglobin (Hp) indicate that vitamin E may be beneficial in a genetically defined subgroup of patients, namely, diabetic patients of the Hp 2-2 genotype. The role of Hp as an antioxidant, its importance in diabetes, and the therapeutic role of vitamin E will be discussed below.

Haptoglobin structure and function

Hp is known best as a hemoglobin (Hb) binding protein and antioxidant agent [16]. Binding of free Hb by Hp changes the tertiary structure of Hb so that the heme group within its heme pocket is less accessible and thus less chemically reactive [17]. The affinity of Hb for Hp is very high ($K_d = 10^{-15}$), thus once formed it is very stable. Hp-Hb complex formation creates a unique binding site that is recognized by CD163, a receptor on circulating monocytes and macrophages in the liver. Its function is to clear Hp – Hb complexes from the plasma [16]. Hp is formed in the liver as one long pre-protein that is cleaved into α and β chains which become disulfide bonded. The Hb binding site is located on the β chain and each mole of Hp binds two moles of Hb[16].

In the human population there are two alleles of Hp known as Hp 1 and Hp2, giving rise to three genotypes: Hp 1-1, Hp 2-1 and Hp 2-2. Hp 1 is common to humans and animals; however, Hp2 is unique to humans. Hp2 was formed as a result of a partial duplication of

the Hp1 gene. The duplication site is located in the alpha chain in the multimerization domain. Therefore, Hp2 has two multimerization domains and forms a series of linear proteins in Hp 2-1 individuals or cyclic proteins in Hp 2-2 individuals. Hp 1-1 individuals form dimers only [18]. Hp phenotypes can be identified by using Hb starch gel (or polyacrylamide gel) electrophoresis. Each phenotype results in a unique electrophoretic pattern. Serum from Hp 1-1 individuals reveals one fast migrating band while serum from Hp 2-2 individuals shows a series of slower migrating bands. Serum from Hp 2-1 individuals shows a pattern of intermediate sized bands[19].

The distribution of the different phenotypes in most western populations is 48% for Hp 2-1, 36% for Hp 2-2 and only 16% of Hp 1-1. This distribution differs in some geographical regions, such as southeast Asia where Hp 2 is more common giving rise to a 90% Hp 2-2 population[19].

Hp antioxidant function

Hb can mediate the formation of free radicals. For example, Hb can react with hydrogen peroxide to yield hydroxyl radicals, also known as the Fenton reaction. Hp has been shown to inhibit this reaction [20]. Another Hb derivative is ferryl Hb that is also formed as a result of reacting Hb with high concentrations of hydrogen peroxide. This derivative of Hb is very unstable and can react rapidly with other hydrogen peroxide molecules to yield superoxide anions [21]. Besides its damaging effect as a radical, two molecules of superoxide can dismutate to form one molecule of hydrogen peroxide. This additional source of hydrogen peroxide augments the formation of additional hydroxyl radicals. Hb can undergo spontaneous oxidation to methemoglobin which readily releases its heme moiety to other lipophilic environments such as cell membranes or lipoproteins [22]. At its new site the iron within the heme can react with hydrogen peroxide to produce hydroxyl radicals and superoxide anions that attack lipoproteins and cell membranes to yield lipid peroxides. These reactive molecules have the potential to react with other lipids creating a domino effect route to extensive lipid oxidation. Hp 1-1 has been shown to inhibit oxidation of LDL by Hb with greater efficiency than Hp 2-2[23].

Studies using human macrophage cells (THP-1) showed that after addition of Hp/Hb complexes, more reactive oxygen species were present in cells treated with Hp 2-2/Hb compared to Hp 1-1/Hb. In a separate study using iron chelators it was shown that after chelating all the free iron in the plasma, there was still more plasma labile iron present in serum from Hp 2-2 versus Hp 1-1 individuals[24]. This again suggests that iron present in Hp/Hb complexes is more reactive in Hp 2-2 compared to Hp 1-1 individuals.

Another difference between Hp types is the half-life of the Hp-Hb complex in the bloodstream. It was shown in mice that the half-life of the Hb/Hp 2-2 complex is longer than the Hb/Hp 1-1 complex (50 min versus 20 min, respectively) [25]. A logical reason for differences in half-life is divergent binding to the CD163 receptor. This interaction was studied in cells expressing the CD163 receptor using ¹²⁵I tagged Hb/Hp complexes. It was found that the rate of clearance of Hp 1-1/Hb by CD163 is markedly greater than that of Hp 2-2/Hb [17]. These findings combined with the above data lead to the conclusion that the Hb/Hp 2-2 complex remains in the plasma longer than Hb/Hp 1-1 and oxidizes more plasma and tissue components.

Binding of Hp to HDL

Hp is known to bind to apoA1 present in HDL particles either alone, or complexed to Hb. The binding constant of this interaction is in the micromolar range (unpublished observations). The Hp binding site is on helix 6 of Apo-1, near the binding site of lecithin

cholesterol acetyl transferase (LCAT). This overlapping causes displacement of LCAT and has been shown to result in an inhibition of cholesterol esterification rate in vitro[26]. This presumably negative affect may be outweighed by the protective effect Hp confers to HDL by binding and neutralizing free serum Hb that might associate with HDL and promote its oxidation. As will be discussed below, Hp 2-2 provides less protection than Hp 1-1 to HDL in diabetic patients.

Haptoglobin and diabetes

As mentioned above, during diabetes the high levels of glucose promote endothelial dysfunction and hemolysis, leading to increased levels of free Hb relative to the non-diabetic population[27]. In this setting Hp becomes critically important. In addition to elevated Hb levels in the plasma, the diabetic condition changes other parameters that are important for Hb clearance from the plasma. For example, in diabetic individuals there is a reduction in concentration of CD163 on monocytes due to decreased mRNA expression causing Hb/Hp complexes to remain in the plasma longer. Also, under normal conditions, Hp prevents the oxidation of LDL by Hb. However glycosylated Hb is not neutralized to the same extent by Hp (10% inhibition of glycosylated Hb versus >90% inhibition of non-glycosylated Hb), resulting in greater LDL oxidation by Hb in the diabetic state [17].

Human epidemiologic studies

Although there is no difference in the distribution of Hp alleles among diabetes patients, recent studies have established that Hp genotype can predict the risk of developing CVD [28]. In 2002 an analysis of Hp phenotypes from the Strong Heart Study was published. In this study the Hp phenotype of 206 diabetic patients and 206 non-diabetic controls were determined and the association between Hp phenotype and CVD was examined. The results of the study showed that the Hp 2-2 phenotype was associated with an almost fivefold increase in risk of developing CVD compared to the Hp 1-1 phenotype[29]. In the Ram bam Myocardial Infarction Outcomes in Diabetes Study, the Hp 1-1 phenotype was associated with a smaller infarct size as compared to the Hp 2-2 phenotype in individuals with diabetes. Furthermore, the diabetic patients with Hp 2-2 phenotype suffered from an increased rate of cardiac events [30]. An additional study followed 935 type 2 diabetes patients that were treated with insulin and/or an oral agent, within one year after percutaneous trans luminal coronary angioplasty, and investigated the occurrence of cardiovascular complications in this group according to Hp phenotypes. The results showed that the Hp 2-2 phenotype was associated with an increased risk of cardiovascular events as compared to the Hp 1-1 phenotype [31]. The largest study conducted to date was the Israel Cardiovascular Vitamin E study(ICARE) trial that included 3,054 type 2 diabetics. The participants were followed for a year and half and the incidence of cardiovascular disease (stroke, MI, and cardiovascular death) was recorded. The results from this trial were consistent with those from previous studies and showed a twofold increased risk in Hp 2-2 individuals of developing CV complications as compared to Hp 1-1 or Hp 2-1 individuals [32].

The association of Hp phenotype in type 1 diabetics with cardiovascular complications was also investigated in two studies. In the Pittsburgh Epidemiology of Diabetes Complication (EDC) study there was an increased rate of developing coronary artery disease in Hp 2-2 type 1 diabetes patients (34.6%) as compared to Hp 1-1 (15.4%) or Hp 2-1 type 1 diabetes patients (28.3%) [33]. The Coronary Artery Calcification in type 1 Diabetes (CACTI) study examined 436 diabetics and 526 control subjects. None of the participants had any calcification in their coronary arteries when the study was started. After six years of follow up, the Hp 2-2 phenotype was found to predict the development of new coronary artery calcification in diabetic patients only. In this study, as well as the one above, the number of

Hp2 alleles was found to have a linear relationship with the incidence of coronary artery calcification and coronary heart disease [34].

Functional studies were designed to test the effect of Hp binding to HDL. Serum was taken from humans with different Hp phenotypes, with or without diabetes. HDL function was measured by its ability to promote cholesterol efflux from macrophages (J447 cells). The results showed that serum from Hp 2-2 diabetics interfered with cholesterol efflux to a greater extent than serum from Hp 1-1 diabetics. No association between Hp phenotype and cholesterol efflux was found in serum from non-diabetic patients [25]. This study also showed that dysfunctional HDL led to the formation of lipid peroxidation, probably due to decreased antioxidant capability [35]. These results might explain the reason why some individuals with elevated HDL levels suffer from an increase in cardiovascular complications, instead of a decrease [36].

Clinical studies: haptoglobin, diabetes and vitamin E

The following studies investigated the effect of vitamin E in diabetic patients of known Hp phenotype. The rationale behind this strategy was that Hp 2-2 diabetics represent a subgroup of patients with high levels of oxidative stress that is inadequately alleviated by Hp 2-2 proteins. A high dose of vitamin E may therefore be beneficial in this genetically defined subgroup.

In the Heart Outcomes Prevention Evaluation (HOPE) study that was published in 2000 the influence of supplementation of vitamin E (400 IU/day) or placebo on 9,541 men and women was investigated. Study participants were known to have cardiovascular disease, diabetes and an additional risk factor. The end point of the study was myocardial infarction, stroke or cardiovascular death. After follow up of approximately 4.5 years the results showed that Hp 2-2 individuals with diabetes that received vitamin E benefited from this treatment and showed an approximately 50% statistically significant reduction in the risk of myocardial infarction and cardiovascular death [11], [37]. In 2008 in the ICARE study subjects were randomized to vitamin E (400 IU/ day) or placebo for 18 months. The results showed a significant 50% decrease in cardiovascular events in Hp 2-2 diabetic individuals. This reduction brought the incidence of cardiovascular events in Hp 2-2 diabetics to the level of events in Hp 1-1 diabetics [32]. It was also found that dual therapy with statins and vitamin E is superior to statins alone in decreasing the risk of cardiovascular disease in individuals with both DM and the Hp 2-2 genotype [38].

Additional studies have focused on the effect of vitamin E on HDL dysfunction that was observed in Hp 2-2 diabetic individuals. Two trials have shown that supplementation with vitamin E improved the ability of HDL to promote cholesterol efflux from human macrophage cells (THP-1) [25, 39]. Similar to previous studies, no effect was seen in non diabetic individuals. Moreover, in the first study [25], subjects were followed for several months after cessation of vitamin E supplementation. Within two months they suffered again from HDL dysfunction. In the second study [39] it was found that supplementation of vitamin E to diabetic individuals with the Hp 2-1 phenotype impaired their HDL function. This unusual finding indicates that vitamin E supplementation may be detrimental to non-Hp 2-2 diabetics and may explain the lack of benefit seen in previous studies of vitamin E and non genetically defined participants.

Results from the WAVE study provide additional limitations on vitamin E therapy. In this study vitamin E and vitamin C were given to postmenopausal women with one or more 15–75% coronary stenoses. The surprising results were that the improvement in the minimum luminal diameter (MLD) was seen only in Hp 1-1 diabetic individuals. Moreover, MLD was worse in Hp 2-2 diabetic individuals who received this treatment. A similar result was

obtained in another study which showed opposite effects of vitamin C and vitamin E on HDL function among diabetics of known Hp genotype. While vitamin E treatment improved cholesterol efflux from macrophages and prevented HDL oxidation, vitamin C treatment did not. It is possible that combining vitamin C and vitamin E interferes with the shielding effect of vitamin E on HDL function [40, 41].

In summary, Hp 2-2 diabetic individuals define a subgroup of patients who stand to benefit dramatically from treatment with vitamin E. Further prospective studies are needed to confirm these findings. In addition, the mechanism of vitamin E action in diabetics requires further investigation so that appropriate treatments can be devised for non Hp 2-2 individuals as well.

In conclusion

CVD is the leading cause of death in individuals with DM. Yearly analysis indicates that DM affects approximately 10% of the population and its incidence is increasing. It is known that in the US over \$174 billion dollars are being spent each year to cover the cost of care due to diabetic cardiovascular complications[42, 8]. The need for an inexpensive and readily obtainable treatment is paramount and now appears to be available in the form of vitamin E. However, only the diabetic population carrying the Hp 2-2 phenotype appears to benefit from vitamin E treatment. Diabetic individuals of non Hp 2-2 phenotype may require lower doses of vitamin E or other antioxidant treatments. These results underscore the importance of personalized medicine in determining the future of medical care.

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Key issues

- To date vitamin E treatment of cardiovascular disease has not focused on genetically defined patient groups and has not been effective.
- Haptoglobin is a polymorphic serum protein which binds to Hb and prevents hemeiron induced oxidation.
- Two major alleles for Hp termed Hp1 and Hp2 give rise to three common genotypes: Hp 1-1, Hp 2-1, and Hp 2-2.
- Hp 2-2 diabetic individuals have an increased risk of developing cardiovascular complications compared to non Hp 2-2 diabetics.
- Several clinical studies have shown that vitamin E supplementation decreases CVD complications in Hp 2-2 diabetic patients.
- Vitamin E supplementation in non Hp 2-2 diabetics may be harmful.