Vitamin E Levels in Sera of Healthy Human in Relation to Different Types of Cancers Group of Al-Najaf City Population.

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Abstract:
Vitamin E is a fat - soluble vitamin aid as an antioxidant which protects the polyunsaturated fatty acids of cell membranes from free - radical damage. It was measured by the high performance liquid chromatography technique. This technique was of choice in measuring the low levels of vitamin E in sera. In the current study 25 persons (10 persons healthy and 15 patients) from Al-Najaf city enrolled to assess the level of this vitamin in their bodies. The mean level of the vitamin E in healthy persons was 179.49 μg / dl and the level was 43.06 μg / dl in patients. There are highly significant differences between these two groups. The study emphasized on the importance of vitamin E to inhibit carcinogenic nitrosamines in the bladder and other cancers.

1-Introduction:
Vitamin E is a fat – soluble vitamin that exists in eight different forms. Each form has its own biological activity, four tocopherols and four tocotrienols (alpha, beta, gamma, and delta), differences due to placement of CH3 groups on the ring, and unsaturation of the side chain (tocotrienols)(1). Alpha – tocopherol (α – tocopherol) is the name of the most active form of vitamin E in humans. It is also a powerful biological antioxidant (2, 3). Vitamin E is usually sold as alpha–tocopherol acetate, a form of α – tocopherol that protects its ability to function as an antioxidant. The synthetic form is labeled "D, L" while the natural form is labeled "D". The synthetic form is only half as active as the natural form (4).

The main biological function of vitamin E is as antioxidant property, which protects the polyunsaturated fatty acids of cell membrane from free – radicals damage (5). Free – radicals can damage cells and may contribute to the development of cardiovascular disease and cancer (4). Hydro peroxides and peroxides destroy cell integrity, and dystrophic tissue cells membrane leak creatine and transaminases (e.g. SGOT into plasma). Functions as chain – breaking antioxidants, neutralizing free radicals and preventing oxidation of lipids within membranes, antioxidant property ensures erythrocyte stability and capillary integrity (6). As disease resistance, vitamin E protects leukocytes and macrophages during phagocytosis and maximizes immune response, by
neutralizing reactive oxygen species that can damage DNA (7), or by inhibiting the formation of nitrosamines (8),
also plausibly reduce bladder cancer risk (9), by enhancing immune function (8, 9). Vitamin E in cellular and sub-cellular membrane is the first line defense against peroxidation of phospholipids and prevents tissue breakdown (2, 3). Vitamin E provides protection against heavy metals as Cd, Hg, Ag, and As. Richest sources are vegetable oils, wheat germ oil, nuts, green leafy vegetables, eggs, liver, legumes and green plants in general, and fortified cereals are common food sources of vitamin E in the United States (4). The most problem of vitamin E was unstable and destroyed under some conditions that promote oxidation – oxygen, heat, moisture, frozen where low in tocopherol, indicating substantial losses of tocopherol during freezer storage. Oxidizing fats, trace minerals, as ferric chloride, nitrogen trichloride and chlorine dioxide destroy much vitamin E. 80 % of vitamin E lost in rapid dehydration and affected by stage of maturity and time from cutting to dehydration of vegetables.

Recommendations for vitamin E are provided in the Dietary Reference Intakes developed by the Institute Of Medicine (10). Dietary Reference Intakes (DRIs) is the general term for a set of reference values used for planning and assessing nutrient intake for healthy people. In table 1 RDAs for vitamin E are based only on the alpha-tocopherol form of vitamin E. RDAs for vitamin E in International Units (IU) because food and most supplement labels list vitamin E content in International Units (1 mg alpha-tocopherol vitamin E = 1.49 IU) (10).

Table 1: Recommended Dietary Allowances for Vitamin E in Children and Adults.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Children (mg/day)</th>
<th>Men (mg/day)</th>
<th>Women (mg/day)</th>
<th>Pregnancy (mg/day)</th>
<th>Lactation (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 3</td>
<td>6 mg (=9 IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 – 8</td>
<td>7 mg (=10.5 IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 – 13</td>
<td></td>
<td>11 mg =16.5IU</td>
<td>11 mg =16.5IU</td>
<td>15 mg =22.5IU</td>
<td>19 mg =28.5 IU</td>
</tr>
<tr>
<td>14 + UP</td>
<td></td>
<td>15 mg =22.5IU</td>
<td>15 mg =22.5IU</td>
<td>15 mg =22.5IU</td>
<td>19 mg =28.5 IU</td>
</tr>
</tbody>
</table>

The placental transfer of vitamin E is limited, mammary transfer is much more extensive. Thus the serum α-tocopherol level of breast-fed infants is increased more rapidly than that of bottle-fed infants. An Adequate Intake (AI) has been established that is based on the amount of vitamin E consumed by healthy infants who are fed breast milk. Table 2 list the adequate intake for vitamin E for infants in mg α-tocopherol and IUs (1 mg α-tocopherol vitamin E = 1.49 IU) (10).

Table 2: Adequate Intake for Vitamin E for Infants.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Males and Females (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>4 mg (= 6 IU)</td>
</tr>
<tr>
<td>7 – 12</td>
<td>5 mg /day (= 7.5 IU)</td>
</tr>
</tbody>
</table>

The vitamin E content of human blood was found to range from 361 – 412 g / 100 ml (11). A deficiency of vitamin E has been associated with a wide variety of pathological conditions, many of them involving neonates also was likely occur in.
1 – persons who cannot absorb dietary fat due to inability to secret bile or with disorders of fat metabolism are at risk of vitamin E deficiency (12).

A – Crohn’s Disease is an inflammatory bowel disease that affects the small intestine. People with disease often experience diarrhea and nutrient malabsorption.

B – Cystic Fibrosis is an inherited disease that affects the lungs, gastrointestinal tract, pancreas, and liver. Cystic fibrosis can interfere with normal digestion and absorption of nutrients, especially of fat soluble vitamins including vitamin E.

2 – Individuals with rare genetic abnormalities in the α – tocopherol transfer protein are at risk of vitamin E deficiency. Ataxia and vitamin E deficiency (AVED) is inherited disorder. It is caused by a genetic defect in a liver protein that is responsible for maintaining normal α – tocopherol concentration in the blood (13).

3 – Premature, very low birth weight infants (birth weight less than 1500 g, or 3 pounds) are at risk of vitamin E deficiency (3, 14). Necrotizing enterocolitis, a condition sometimes seen in very low birth weight infants that is characterized by inflammation of the lining of intestines (4).

2-M aterial and M ethods:
During 2 months 25 persons, 10 persons healthy as control (5 males and 5 females), and 15 patients with different types of cancer (9 males, and 6 females) were collected, from Al- Najaf city. After with drawing 5ml of veins blood, the blood centrifuged by the centrifuge for 15 mints with 1600 xg. Then 3ml of separated serum was isolated. Half milliliter was used from this serum to determine alpha – tocopherol vitamin E concentration. The serum stored at deep freeze – 20 ºC until the time of analysis, to prevent any unfavorable decomposition. When the time for analysis had arrived the serum was kept at room temperature with suitable time. The HPLC separation works with an isocratic method at 35 ºC and reversible phase column was applied. Chromatograms are detected by UV – detector. The retention time of alpha – tocopherol vitamin E was 10 mints for each run. Results are integration of the peak area. The methanol – water (95:5) was used as eluant solution. Due to the affinity of alpha – tocopherol vitamin E to plastic all steps was performed in glass tubes, preferable pointed – bottom glass tubes (15).

The analytical conditions for vitamin E measurement were as follow:
A – Mobile phase: (95:5) methanol – water.
B – Flow rate : 1 ml /min.
C – Wave length : 270 nm.
D – Column shim pack : MRC – ODS (octadecylslyle).
E – Temperature : room temperature.

3-Results:
The study primarily was intended to adapt a method for measuring vitamin E level in serum samples in order to make a reliable evaluation of this vitamin into two groups of Iraqi population (Al – Najaf city). The HPLC method was the method of choice in this field. The reverse phase HPLC chromatographic method has a very high sensitivity and need only for a very small amount of sample with out any complicated pretreatment for used serum which may be a major source of errors, unlike previous published methods for the quantitation of vitamin E require large sample volumes as well as extensive extraction and concentration procedures (16).

Many points were strictly followed during the measurement of vitamin E for example, any hemolytic samples were excluded from the measured groups, all samples were
collected from all patients and volunteers were, (as they mentioned), did not taken any medicament that may influence vitamin E levels which may alter the final results.

3-1-Normal value of vitamin E in healthy group:

The mean of vitamin E levels in both sexes in healthy group was 179.5 μg / 100ml as shown in table 3 below:

Table 3: Vitamin E Level in Sera of Healthy Males and Females.

<table>
<thead>
<tr>
<th>Group Type</th>
<th>No.</th>
<th>X µg/dl</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5</td>
<td>137.3</td>
<td>±44.84</td>
<td>32.66</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>221.7</td>
<td>±56.95</td>
<td>25.69</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>179.5</td>
<td>±65.71</td>
<td>36.61</td>
</tr>
</tbody>
</table>

This value in fact can be relatively considered as sub – normal value. Nair, (1956)(11), reported that the normal range of serum alpha – tocopherol vitamin E in human was from 361 – 412 µg /dl. 3-2-Vitamin E Level in Relation with Cancer Disease:

Table 4: Vitamin E Levels in Control and Patients with Cancer Disease.

<table>
<thead>
<tr>
<th>Group Type</th>
<th>Control Group (20-65) ys</th>
<th>Patient Group (20-65) ys</th>
<th>CS S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. X±SD(µg/dl)</td>
<td>CV%</td>
<td>No. X ± SD (µg/dl)</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>137.3±44.84</td>
<td>32.66</td>
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<td>36.61</td>
</tr>
</tbody>
</table>

The results shown in table 4 reveals, through using statistical function test, that there is highly significant (P < 0.01), difference between the two means of vitamin E in control group and patient group.

4- Discussion:

Institute Of Medicine (IOM) reported on vitamin E published in 2000, states that intake estimates of vitamin E may be low because low fat intake and the kind and the amount of fat during cooking. Vitamin E in foods which frozen low in tocopherol, indicating substantial losses of tocopherol during freezer stage, as well as during heat. However, (IOM) they do caution that "Low – fat diets can substantionally decrease vitamin E intakes" (10).

Intestinal disorders that often result in malabsorption of vitamin E(3). It is important
to mention that low content vitamin E in whole meal may contribute in decreasing serum vitamin E levels in our society, which is related to decrease in socioeconomic or other causes like the genetic factors which is likely to be involved in this phenomenon. The most cause to decrease vitamin E level in male due to cigarette smoking. Cigarette smoke increases human vitamin E requirement. Alpha – tocopherol disappearance is faster in cigarette smokers. In experimental studies, deficient vitamin E were more damaged by ozone and nitrogen dioxide, which are among the oxidants in polluted air, which may be present in smog – contaminated atmospheres. Vitamin E was originally act as a biological antioxidants, preventing spontaneous oxidation of highly poly unsaturated fatty acid to aggressive free radicals, also when the body was exposed to many external sources of free radicals such as cigarette smoke, and pollutants, as well as formation of carcinogenic nitrosamines (7,8). Vitamin E also may be block the formation of nitrosamines which are carcinogens formed in the stomach from nitrites consumed in the diet. It was also protect against the development of cancers by enhancing immune function (17). It diminishes the toxic action of oxygen species that can damage DNA (7).

Some evidence associates higher intake of vitamin E with decreased incidence of prostate cancer and breast cancer (18). However, an examination of the effect of dietary factors including vitamin E, on incidence of postmenopausal breast cancer in over 18,000 women from New York State associated with greater vitamin E intake with a reduced risk of developing breast cancer (19).

A study of women in Iowa provides evidence that an increased dietary intake of vitamin E may decrease the risk of colon cancers especially in women less than 65 years of age (20).

The American cancer society recently released the results of a long – term study that evaluated the effect of regular use of vitamin E supplements on bladder cancer mortality in almost 1000000 adults in the U.S. The study, conducted between the years 1982 to 1998 found that subjects who regularly consumed a vitamin E supplement for longer than 10 years had a reduced risk of death from bladder cancer. No benefit was seen from vitamin C supplements (21).

Vitamin E has improved immune system preventing cancer, so there was inverse relationship of plasma alpha – tocopherol levels and risk of cancer of breast, lung, stomach, pancreas, and urogenital tract (22).

Owing to its lipophilic properties, vitamin E accumulates in membranes and thereby protects functionally important cell structures, primarily by inhibition of lipid peroxidation and neutralizing free radicals within membranes as first line of defense mechanism. If high doses of supplemental vitamin E do inhibit bladder carcinogenesis, there could be potential implications for bladder cancer treatment as well as for primary prevention. The possibility that vitamin E supplementation could inhibit later stages of carcinogenesis is supported by the ability of vitamin E supplementation (at 800 IU/day) to cause regression of precancerous oral lesions (23).

Unfortunately, at this time human trials and surveys and researches that have tried to associated vitamin E intake and supplements with incidence of cancer have been in general very little, and very limited.

Conclusion:
The most important conclusion from this current work are:

1–Vitamin E can be measure successfully by the HPLC technique with high accuracy and precision with current modified method.
2–The normal values of vitamin E levels in control group in Al – Najaf city in general lower than reported by other investigators.
3 – Serum vitamin E in patients with cancer disease was relatively very low, and must 4- Be given as supplementation of vitamin E in high dose.

Future works:
1– Determination of vitamin E level in infants and pregnant women.
2–To do an epidemiological study for large group of healthy people and give a supplement of vitamin E for long – term even 10 years and study the vitamin E an _S antioxidant to prevent predisposing carcinogenesis factors as pollutant atmosphere, cigarette smoking, and the usage of diet which content low vitamin E or without vitamin E.

References:
9- Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. Cancer Lett 1995;93:17–48. [ISI][Medline].
16- Castle MC, Cooke WJ. Measurement of vitamin E in serum and plasma by high performance liquid chromatography with electrochemical detection.


