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Evaluation of plasma vitamin C concentration in age related cataract patients

Jaskiran Kaur[1]*, Sahiba Kukreja[1], Kawalinder Girgla[2], Amandeep Kaur[1]

[1]Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar, (INDIA)

[2]Department ofPhysiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar, (INDIA)

E-mail : drjaskiran@rediffmail.com

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ABSTRACT

Cataract is a vision-impairing disease characterized by gradual, progressive thickening of the lens. It is one of the leading causes of blindness in the world today. Oxidative agents like ultraviolet (UV) rays are one of the most important causes of cataract. Dietary antioxidant vitamins, in particular vitamin C (Ascorbic acid), can play a role in preventing the onset or progression of age-related cataract. The aim of the study was to find correlation between blood oxidative stress marker malondialdehyde (MDA) and ascorbate (vitamin c) levels in cataractous patients. The patients were subdivided in 3 groups according to age criteria. Group I- 45-55 years, Group II-56-65 years and Group III- 66-75 years. 50 age -matched healthy subjects served as controls. Plasma ascorbic acid level was measured by a colorimetric method using acid phosphotungstate. The mean plasma ascorbic acid level in 3 test groups was 0.69±0.08, 0.70±0.08 and 0.65±0.10 mg/dl respectively, and in control group was 0.86±0.07, 0.84±0.08 and 0.86±1.10 mg/dl respectively. The results were statistically analyzed using student't' test. The decrease in Plasma ascorbic acid level was highly significant in group II and III as compared to control group ($p<0.001$). This study revealed that plasma vitamin C level in patients with senile cataract was lower than normal individuals. The concentration of vitamin C was not related to the type of cataract. Aging was associated with a reduction in plasma ascorbic acid levels. Elderly patients should be advised to take fresh vegetables and fruits rich in vitamin C or other supplements to improve the plasma levels.

KEYWORDS

Oxidative stress;
Age related cataract;
Vitamin C;
Antioxidants.

INTRODUCTION

Age related cataract is the most common variety of cataract. Usually some degree of cataract is present after the age of 50 years[1]. Compared to western countries, population-based studies have reported higher prevalence rates of cataract in India even after differing rates of cataract surgery are taken into account[2-4]. Crystalline lens has life-long crystals and proteins with special structure that are important for the maintenance

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of its transparency. A senile change of crystalline formations of the lens such as oxidation is the cause of their gloom and cataract[5]. Many risk factors are known to cause senile cataract and the most important and preventable ones are ultraviolet (UV) rays especially UV-B rays exposure and nutritional deficiencies[6,7]. Cataract is usually treatable surgically, but a large number of operations impose a great cost. It has been estimated that if cataract development could be delayed by 10 years, the need for cataract extraction and the cost might be diminished by 50 %[8].

Vitamin C is considered the most important antioxidant in extracellular fluids and the only endogenous antioxidant that can completely protect the lipids from detectable peroxidative damage induced by aqueous peroxy radical[9]. The role of vitamin C is said to be beneficial for delaying the onset of cataract development in human beings therapeutically as well as nutritionally[10]. Antioxidants are also said to be associated with decreased rates of all cataract types, but further studies are needed to establish the association[11]. In this view, present study was undertaken in North Indian population to determine the plasma vitamin C levels of cataract patients in different age groups; the levels compared to normal healthy control group and correlation of the type of cataract with plasma vitamin C levels.

MATERIAL AND METHODS

This subjects included in the study were 50 patients suffering from cataract above 45 years of age of either sex. Considering age criteria three groups were formed. Group I- 45-55, Group II- 56-65 and group III – 66-75. The number of patients in each group was 10, 19 and 21 respectively. The patients were selected randomly from outpatient department (OPD) and wards of Ophthalmology Department, Ram Lal Eye hospital attached to Govt. Medical College, Amritsar. 50 healthy subjects served as control group. Patients with history of Diabetes Mellitus, malignancies, Tuberculosis, Hypertension, Coronary Artery Disease and on dietary supplements were excluded from the study.

Before starting the study, approval of institutional ethical committee was obtained. Informed consent was sought individually after full explanation of the purpose and nature of the study. Participants were interviewed for socioeconomic and lifestyle factors (tobacco, alcohol, household cooking fuel, work, and diet); attended a clinical examination including a slit-lamp examination of eyes and provided a blood s ample for vitamin C analysis. Plasma vitamin C was measured manually by a colorimetric method[12]. Serum malondialdehyde (MDA) was estimated by applying the method of Satoh[13]. The values were expressed as Mean±SD. The comparison between groups was done by student 't' test.

RESULTS

The present study was conducted on 50 cataract patients aged 45-75 years, divided into 3 groups. Group I- 45-55 years, Group II- 56-65 years and Group III- 66-75 years. The patients and controls in Group I were 10 and 10 respectively; Group II were 19 and 18 respectively and Group III were 21 and 22 respectively (TABLE 1). The mean plasma ascorbic acid levels in cataract patients were 0.68±0.09, the range was 0.602-0.81. In controls, the mean value of Vitamin C and its range was 0.85±0.09 and 2.72-0.98 respectively (TABLE 2). Ascorbic acid level in the test group was significantly lower than the control group (p value < 0.001). Aging had a reverse relation with plasma ascorbic acid level. TABLE 3 shows comparison of plasma ascorbic acid levels in male and female, amongst control and patients under study. In control male individuals (n=24) the range is 0.72 – 0.98 mg/dl with mean ± S.D of 0.83 ± 0.09 while levels in male patients range from 0.5 – 0.8 mg/dl with mean ± S.D of 0.67 ± 0.08. The difference between the levels was statistically highly significant. In case of female control (n=26) the levels range from 0.72 – 0.98 with mean ± S.D of 0.84 ± 0.14 mg/dl while in female patients (n=22) the levels range from 0.5 – 0.8 with mean ± S.D of 0.69 ± 1.10. The difference between the levels in controls and patients was statistically highly levels in controls and patients was statistically highly significant (p<0.001). The difference between the levels in males and females was statistically insignificant.

It was observed that MDA levels among normal individuals ranged between 1.5–2.8 nmol/ml with mean ± SD of 2.429±0.46 while in comparison the corre-

sponding values amongst patients were 2.3–7.5 nmol/ml with mean ± S.D of 5.43 ± 1.69 nmol/ml. The difference in the levels of MDA in controls and patients were statistically highly significant ($p<0.001$) with the level of MDA significant higher in patients as compared to controls (TABLE 4).

TABLE 5 shows comparison of MDA levels in males and females amongst control and patients under study. In control male individuals (n=24) the range of MDA was 1.5 – 2.8 nmol/ml with mean ± S.D of 2.36 ± 0.42 while the levels in male patients (n=28) range of MDA was 2.3 – 7.3 nmol/ml with mean of S.D of 5.01 ± 1.64. The difference between the levels was statistically highly significant. In case of female controls (n=26) the range of MDA was 1.5 – 3.6 nmol/ml with mean ± S.D. of 2.38 ± 0.58 while in the female patients (n=22) the range of MDA was 4.0 – 8.54 with mean ± S.D of 5.96 – 1.63. The difference between the levels of MDA in controls and patients was highly significant ($p<0.001$) the difference between the levels of MDA in males and females was statistically in insignificant.

TABLE 1 : Age wise distribution of the number of patients and controls

Age Group (years)	Patients (No.)	Mean ± SD	Controls (No.)	Mean ± SD
I (45-55)	10	0.69±0.08	10	0.86±0.07
II (56-65)	19	0.70±0.08	18	0.84±0.08
III (66-75)	21	0.65±0.10	22	0.72-0.98

't' VALUE WITH STATISTICAL SIGNIFICANCE OF PLASMA VITAMIN C LEVELS ON COMPARISON BETWEEN THE PATIENTS AND CONTROLS

Group I	t = 3.29	p<0.01 Significant
Group II	t=6.11	p<0.001 Highly Significant
Group III	t=7.33	p<0.001 Highly Significant

't' VALUE WITH STATISTICAL SIGNIFICANCE OF PLASMA VITAMIN C LEVELS IN PATIENTS AND CONTROLS ON INTER-COMPARISON BETWEEN THE GROUPS

PATIENTS		CONTROLS	
Group I/II	t=0.32,p>0.05 *(NS)	Group I/II	t=0.41,p>0.05 *(NS)
Group I/III	t=1.10,p>0.05 *(NS)	Group I/III	t=0.41,p>0.05 *(NS)
Group II/III	t=1.73,p>0.05 *(NS)	Group II/III	t=0.90,p>0.05 *(NS)

* NS – Not Significant

TABLE 2 : Comparison of plasma ascorbic acid levels in patients and controls

SUBJECTS	NUMBER	MEAN	± SD	SE
PATIENTS	50	0.68	0.09	0.01
CONTROLS	50	0.85	0.09	0.01

t= 9.48; p<0.001* (*Highly significant)

DISCUSSION

Cataract remains the leading cause of visual disability and blindness worldwide[14]. It is estimated that 180 million people are visually disabled globally. Of these 37 million people are blind and this number increases by one to two million every year[15]. At present, the only remedy is surgical removal of the cataractous lens and substituting it with a lens made of synthetic polymers. However, the incidence is so large that the available surgical facilities are unable to cope up with the problem. In addition to these, postoperative complications can occur such as posterior capsular opacification, endopthalmitis and uncorrected residual refractive error[16]. Therefore, there is a search for pharmacoogical intervention that will maintain the transparency of the lens. During the last decades, extensive research inputs have been made to delineate the etiology of cataract. Efforts have been directed to delay the onset and slow down the progression of cataract by various agents especially antioxidants like vitamin C[17].

The potential role of vitamins in preventing cataract is well documented especially vitamin C or ascorbic acid which plays an important part in lens biology, both as an antioxidant and as a UV filter[18]. Dietary deficiency of vitamin C led to reduction in lens concentration of ascorbate[9]. Vitamin C also has potential as an aldose reductase inhibiting bioflavonoid (ARI) with both animal and clinical studies showing that it minimizes the sorbitol levels[20,21].

We measured vitamin C levels in the blood. There are several advantages of blood measurements. They provide an objective measurement of antioxidant status and are not subject to usual problems of diet questionnaires such as recall or biased responses. Calculating dietary vitamin C intake from diet questionnaires requires appropriate food composition tables and information on portion sizes and methods of cooking that may be additional sources of error. In our study, blood measurements were the only marker of this antioxidant intake available, since there is very limited information on antioxidants in the Indian food composition tables.

In several epidemiological studies, cataract patients were shown to have low vitamin C intake and low plasma vitamin C levels[22]. According to the results of this study, the mean concentration of plasma ascorbic

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TABLE 3 : Comparison of plasma ascorbic acid levels in male and female groups of controls and patients under study

	Plasma Ascorbic Acid Levels mg/dl							
	Controls				Patients			
Sex	No. of Subjects	Range mg/dl	Mean ±SD	S.E	No. of Patients	Range mg/dl	Mean ±SD	S.E
Male	24	0.72-0.98	0.83±0.09	0.02	28	0.5-0.8	0.67±0.08	0.02
Female	26	0.72-0.98	0.84±0.14	0.03	22	0.5-0.8	0.69±1.10	0.23

Controls M/F t = 0.30 p>0.05 NS

Patients M/F t = 0.78 p>0.05 NS

Control vs patients
Male control/patients t = 6.85 p<0.001 Highly Significant
Female control/patients t = 4.25 p<0.001 Highly Significant

TABLE 4: Comparison of serum MDA levels in controls and patients under study

Subjects	No. of cases	Range (nmol/ml)	Mean	±SD	S.E
Control	50	1.5-2.8	2.42	0.46	0.07
Patients	50	2.3-7.5	5.43	1.69	0.24

t=12.21; p<0.001*; *Highly Significant

acid in patients and controls were 0.68± 0.09 and 0.85 ± 0.09 respectively. The reason for decreased plasma ascorbate may be its consumption in the body due to increased lipid peroxidation in cataractous patients. It acts as a co- antioxidant by regenerating α- tocopherol from α-tocopheroxyl radical produced during scavenging[9]. The highly toxic reactive oxygen species that are formed by photochemical reactions of oxygen species that are formed by photochemical reactions of oxygen in the presence of electron donors are converted to less toxic hydrogen peroxide via ascorbic- acid-mediated reduction[23,24]. It is proved in animal studies that a diet without vitamin C and exposure to UV- B as an oxidative stress can cause cataract after some weeks[25]. Also vitamin C has antioxidant properties due to which it has the potential to reduce oxidative damages[26]. The available evidence suggests that maintenance of sufficient plasma vitamin C is needed to prevent oxidative damage in the lens[27].

In the study of Jacques and his colleagues, plasma of 77 cases of cataract and 35 controls were examined, they concluded that vitamin C concentration in patients who were at risk of cataract was lower than control group (p< 0.05). The result is in harmony with our study.

A pilot study done by Dherani M et al showed inverse relationships between cataract and the plasma levels of vitamin C and other antioxidants[28].

In our study vitamin C concentration showed variation with age, the vitamin C density decreases as the age increases, but the decrease was not statistically significant. Evidence indicates that vitamin C levels in the eye decreases with age[29] and that supplementing with vitamin C prevents this decrease[30] possibly leading to a lower risk of developing cataracts**[31,32]**. In contrast to our study Birlouez- Aragon et al concluded that vitamin C concentration of plasma was not related to the age[33].

A number of epidemiological studies using cross-sectional data have shown an increased prevalence of cataract in women compared with men[34]. The cause of the gender differences in cataract occurrence is not clear but could be related to the hormonal differences between women and men. Postmenopausal estrogen deficiency may be a factor. Recent epidemiologic data provided some evidence that estrogen and hormone replacement therapy may play a protective role in reducing the incidence of age-related cataract[35]. In our study, the mean vitamin C concentration in male and female patients was 0.67±0.08 and 0.69±1.10 respectively. However, when the vitamin C concentration of plasma was compared in males and females, the difference was not statistically significant (p> 0.05). It may

TABLE 5 : Comparison of serum MDA in male and female groups of controls and patients under study

	Controls				Patients			
Sex	No. of Subjects	Range nmol/ml	Mean ±SD	S.E	No. of Patients	Range nmol/ml	Mean ±SD	S.E
Male	24	1.5-2.8	2.36±0.42	0.09	28	2.3-7.3	5.01±1.64	0.31
Female	26	1.5-3.6	2.38±0.53	0.10	22	4.0-8.54	5.96±1.63	0.35

Controls M/F t = 0.15 p>0.05 NS

Patients M/F t = 2.06 p>0.05 NS

CONTROL Vs PATIENTS
Male control/patients t = 7.77 p <0.001 Highly Significant
Female control/patients t = 10.69 p <0.001 Highly Significant

be that both males and females received similar amount of vitamin C.

Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. Malendialdehyde is major end product of free radical reaction on membrane fatty acids.

In this study, a significant increase in serum MDA level ($p<0.001$) was observed in patients compared to controls (TABLE 3). Serum MDA levels among normal individuals ranged between 1.5-2.8 n mol/ml with mean ±S.D of 2.42±0.46 while in comparison the corresponding values amongst patients were 2.3-7.5 with mean ± SD of 5.43±1.69 nmol/ml. The observations were similar to findings of other investigators[36,37]. Increase in MDA level observed could be due to increased oxidative stress (age related) or decrease in antioxidant defense mechanism and vice-versa. In case of development of age related cataract, LPO may also be the real cause of destruction of the plasma membrane of the lenticular fibres and the subsequent oligmerization of the crystalline lens[38].

Since vitamin C can be made easily available to the elderly population and its beneficial effects popularized through educational and social instructions on the nutritional status of the food we eat, we can make the right decision to prevent or delay the initiation of cataract in developing countries like India. Use of vitamin C supplements has been inversely associated with cataract risk. High intake of fruits and vegetables which are rich sources of ascorbic acid appear to be protective too.

The present study suggests that if educational and social instructions can be popularized regarding the beneficial effects of vitamins and nutritional status of food that we eat, we can make the right decision to prevent or delay the initiation of cataract formation.

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