

## Original Research Article

# Estimation of serum ascorbic acid (vitamin C) in various morphological types and clinical stages of age related (senile cataract)

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## ABSTRACT

**Background:** The aim was to estimate the serum ascorbic acid (vitamin C) in the various morphological types and clinical stages of age related (senile) cataract, a common cause of curable blindness globally, especially in India.

**Methods:** This was a study of estimation of serum ascorbic acid (vitamin C) in 100 cases of senile (age related) cataract with 60 cases of senile cortical type (30 cases of immature and 30 cases of mature) and 40 cases of senile nuclear type (20 cases of grades I and II and 20 cases of grades III and IV) in the age group of 45 to 70 years.

**Results:** Vitamin C mean plasma level was significantly decreased in senile cataract ( $0.54 \pm 0.23$  mg/dl or  $30.68 \pm 13.07$   $\mu$ mol) and in its morphological types of cortical and Nuclear, especially in the later stages or grades of maturity when compared with the earlier stages or grades of immaturity i.e. The mean level of Serum Ascorbic acid was  $0.55 \pm 0.23$  mg/dl ( $31.25 \pm 13.07$   $\mu$ mol) in Senile Cortical cataract with  $0.58 \pm 0.24$  mg/dl ( $32.96 \pm 13.64$   $\mu$ mol) in its Immature stage and  $0.52 \pm 0.20$  mg/dl ( $29.55 \pm 11.36$   $\mu$ mol) in its mature stage and the mean level in Senile Nuclear cataract was  $0.54 \pm 0.23$  mg/dl ( $30.68 \pm 13.7$   $\mu$ mol) with  $0.56 \pm 0.29$  mg/dl ( $31.82 \pm 16.48$   $\mu$ mol) in its Grades I and II and  $0.52 \pm 0.22$  mg/dl ( $29.55 \pm 12.5$   $\mu$ mol) in its Grades III and IV.

**Conclusions:** So, our study showed significantly decreased levels of serum vitamin C in all the morphological types and clinical stages of senile cataract especially in the later stages or grades of maturity compared to the earlier clinical stages or grades of immaturity.

**Keywords:** Age related (senile cataract), Ascorbic acid (vitamin C), Blindness, Cortical, Nuclear, Serum

## INTRODUCTION

According to WHO, about 180 million people worldwide are disabled visually, in which about 45 million are blind.<sup>1-4</sup> Blindness and visual impairment have many social implications, as 80% of this visual disability is avoidable.<sup>1-3</sup> In India, with 20% of the global blindness, cataract is the principal cause.<sup>5</sup> About 20 million are blind in India due to cataract with another 4 million turning blind due to cataract every year, with a backlog of about 12 million Cataract cases.<sup>6</sup>

Population-based studies, though showed high prevalence rates of cataract in India compared to western populations with the environmental, nutritional, and genetic factors being the important factors for these high rates, but limited information about these, particularly on antioxidants (especially vitamin C) considered to play an important role in protecting the lens from oxidative damage.<sup>7-10</sup> Inverse associations between cataract and

plasma levels of vitamin C and other antioxidants were reported.<sup>11</sup>

Cataract clinically is the lens opacity from the smallest dot to complete opacity interfering with vision and anatomically is the opacity in the lens or its capsule either developmental or acquired and is classified etiologically into congenital and acquired types. Age related (Senile) cataract, which is the commonest clinical presentation in the acquired cataract has a complex etiology and pathophysiology due to the combined effects of genetic and environmental factors. The prevalence of senile cataract increases with age with 65% in the age group of 50 to 59 yrs and in all the people over 80 yrs. Morphologically, senile cataract is classified into cortical (Soft) and Nuclear (Sclerotic or Hard) types. Cortical type has the clinical stages of immature, mature and hyper-mature and nuclear types has the grades of I (yellowish), II (Amber colour), III (Brown) and IV (Black).

Many people may remain blind from cataracts due to inadequate surgical facilities or high surgery costs, though cataracts can be removed surgically with replacement by an artificial intraocular lens to restore vision.<sup>12</sup> The ocular lens exposed continuously to UV light and ambient oxygen is at a high risk of photo-oxidative damage with generation of Free radicals in the normal routine metabolic activities of the lens causing the oxidation of the lens proteins leading to lens opacity.<sup>13</sup> Body defence mechanisms especially the antioxidants such as, GST, Vitamin C, and Vitamin E etc. dispose, scavenge and suppress the formation or oppose the actions of the free radicals to minimize this photo oxidative damage to the ocular lens.<sup>13</sup> Among the antioxidants, vitamin C is an effective antioxidant at low partial pressures of oxygen, due to conditions found in the lens.<sup>14</sup>

Human studies showed that elevated intake or enhanced serum concentrations of antioxidant vitamins has a protective role against the degeneration of the ageing lens after oxidative damage.<sup>15-17</sup> Increased ocular lens protein modification due to chronic oxidative damage with the lack of optimum intake of antioxidants by the lens and surrounding fluids are the important factors in cataract development.<sup>18-20</sup> Vitamin C and ascorbic acid, which can neutralize the oxygen free radicals generated in the normal biochemical activity of the lens may reduce oxidative DNA damage and genetic mutation.<sup>21</sup>

There is strong biological importance of vitamin C in the lens due to its concentrations in lens or aqueous of about 20- to 30 times than that of the plasma and even higher in the vitreous.<sup>22,23</sup> Earlier Indian studies showed that vitamin C concentrations were higher in the normal lenses compared with mature cataracts.<sup>24</sup> Vitamin C, as a powerful reducing agent, protects the lens from oxidative damage and acts synergistically with vitamin E, and both vitamins C and E to maintain the antioxidant activity of glutathione.<sup>25</sup>

The aim was to estimate the Serum Ascorbic Acid (Vitamin C) in the various morphological types and clinical stages of Age Related (Senile) Cataract, a common cause of curable blindness globally, especially in India.

## METHODS

This study was conducted in the depts. of Biochemistry and Ophthalmology, SV Medical College (Govt.) and SVRR Government General Hospital, Tirupati, AP, India in 100 patients with a clinical diagnosis of age related (senile) Cataract with 60 cases of Senile Cortical type (30 cases of Immature and 30 cases of Mature) and 40 cases of Senile Nuclear type (20 cases of Grades I and II and 20 cases of Grades III and IV) in the age group of 45 to 70 years. Informed consent was taken from all the patients of senile cataract. A provisional diagnosis of age related cataract, its morphological types and clinical stages was assigned based on the clinical ophthalmic examination. The patients with a history of Diabetes Mellitus, Hypertension, systemic diseases, trauma etc., were excluded. The study was approved by the institute ethical committee. The results were analyzed by simple statistical methods.

### Samples collection

10ml of fasting blood sample of age related (senile) cataract in the morning was drawn from a peripheral vein especially from the antecubital vein. For the separation of the serum, 5 ml of blood was taken into a plain vial first and then allowed to clot. Then this clotted blood was centrifuged at 3000 rpm for 5 minutes. This separated serum was used to estimate vitamin C on the same day. 1 ml of blood was taken into a fluoride vial to estimate blood glucose to exclude diabetes mellitus.

Assay of Ascorbic Acid: S.T.O May J. O. Turnbull, 1979. Determination after devitalisation with 2, 4 dinitrophenylhydrazine).

### Principle

Ascorbic acid is oxidized by copper to form dihydroascorbic acid and diketogulonic acid. These products are treated with 2, 4 DNP to form a derivative of bis 2,4 dinitrophenylhydrazine. This compound in conc. H<sub>2</sub>SO<sub>4</sub> undergoes a rearrangement to form a product with an absorption band that is measured at 520nm. This reaction is seen in the presence of Thiourea to provide an unduly reducing reaction which helps to produce an inter substance from non ascorbic acid chromogens.

### Reagents

- TCA (Trichloroacetic Acid) 5% and 10% in distilled water,
- 2, 4 dinitrophenylhydrazine/Thiourea/Copper solutions. (Add 0.4g Thiourea, 0.05g CuSO<sub>4</sub> 5H<sub>2</sub>O

and 30grams 2, 4 dinitrophenylhydrazine and bring to a total volume of 100ml with 9NH<sub>2</sub>SO<sub>4</sub>),

- 65% H<sub>2</sub>SO<sub>4</sub>.

### Procedure

1ml of plasma and 1ml of Ice cold 10% TCA were mixed well and centrifuged for 20 minutes at 3500rpm. 0.5ml of supernatant and 1ml of DTC were taken and incubated for 3hrs at 37 degrees centigrade. To convert this into a inter arrange product, 0.75ml of Ice cold 65% H<sub>2</sub>SO<sub>4</sub> was added. Then mixed well and cooled. The final product was read with 520nm. Instruments used were Bio-systems-BTS 320 Photometer.

### RESULTS

The 100 cases of Senile (Age Related) cataract with 60 cases of Senile Cortical type (30 Immature and 30 Mature) and 40 cases of Senile Nuclear type (20 Grades I and II and 20 Grades III and IV) in the age group of 45-70years were evaluated using the simple statistical methods. Ascorbic acid was measured in mg/dl (umol/L). These are summarized below.

**Table 1: Distribution of morphological types of senile cataract.**

Type	Number	%
Senile cortical	60	60
Senile nuclear	40	40
Total	100	100

**Table 2: Distribution of clinical stages of senile cortical and nuclear types.**

	Senile cortical		Senile nuclear		Total
	Immature	Mature	Grades I & II	Grades III & IV	
Number	30	30	20	20	100
%	30	30	20	20	100
Total	60		40		100

**Table 3: Serum Vitamin C in senile cataract -100 cases.**

Range		Mean		SD	
Mg/dl	umol/l	Mg/dl	umol/l	Mg/dl	umol/l
0.2 -1.1	11.36 -62.5	0.54	30.68	±0.23	±13.07

**Table 4: Serum vitamin C in senile cortical cataract - 60 cases.**

Mean		SD	
Mg/dl	umol/l	mg/dl	umol/l
0.55	31.25	± 0.23	± 13.07

- The mean level of serum ascorbic acid (Vitamin C) in Senile Cataract is 0.54±0.23 mg/dl (30.68±13.07 umol).

- The mean level of Serum Ascorbic acid is 0.55±0.23 mg/dl ((31.25±13.07umol) in Senile Cortical cataract with 0.58±0.24 mg/dl (32.96±13.64 umol) in its Immature stage and 0.52±0.20 mg/dl (29.55±11.36 umol) in its mature stage.
- The mean level in Senile Nuclear cataract is 0.54±0.23 mg/dl (30.68±13.7 umol) with 0.56±0.29 mg/dl (31.82±16.48 umol) in its Grades I and II and 0.52±0.22 mg/dl (29.55±12.5 umol) in its Grades III and IV.

**Table 5: Serum vitamin C in senile Nuclear cataract-40 cases.**

Mean		SD	
Mg/dl	umol/l	mg/dl	umol/l
0.54	30.68	± 0.23	±13.07

When the Difference between the means of 1) Senile Cortical and Senile Nuclear cataract 2) Senile cortical immature and mature stages and 3) Senile Nuclear Grades I and II and III and IV are compared statistically, there is a significant decrease in plasma vitamin C levels in senile cataract and also in the morphological types of Cortical and Nuclear, especially in the later clinical stages or grades of maturity when compared to immature stages.

### DISCUSSION

Age related (senile) cataract is not only the most common type of Acquired Cataract, but also the most common cause of curable blindness in the developing nations and the important visual problem of old people with a substantial health care cost in many countries. The aetiology of age-related cataract is multifactorial.

Vitamin C is a physiological antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress and is associated with better scavenging properties in vivo than the other antioxidants, because of its presence both in intracellular and the extracellular fluid.<sup>26</sup>

Plasma Ascorbic acid is the only endogenous antioxidant that can completely protect the lipids from the peroxidative damage induced by aqueous peroxy radicals.<sup>21</sup>

Vitamin C also acts as a co-antioxidant by regenerating alpha-tocopherol from alpha-tocopheroxyl radical produced during scavenging of oxygen free radicals.

A number of epidemiologic studies were published exploring the relationship of vitamin C and the risk of cataract with inconsistent results. For serum ascorbate, 10 studies data (three prospective studies, six cross-sectional studies and one case-control study) were used including 7305 cataract cases with seven studies in United States, two in India and one in Spain. As the main difficulty in

all the studies in the evaluation of the antioxidant status in the development of the senile cataract was the inability to measure the antioxidants directly in ocular lens in vivo,

many investigators used the plasma or serum RBC's of the patients to evaluate the antioxidants in the senile cataract.<sup>15,17,27-29</sup>

**Table 6: Serum vitamin C in senile cortical type-30 (immature) + 30 (mature) = 60 cases.**

Type	Range		Mean		SD	
	mg/dl	umol/l	mg/dl	umol/l	mg/dl	umol/l
Immature	0.2 -1.1	11.36 - 62.5	0.58	32.96	± 0.24	±13.64
Mature	0.3 -0.9	17.05 -51.14	0.52	29.55	±0.20	±11.36

**Table 7: Serum vitamin C in senile nuclear type-20 (Grade I & II) + 20 (Grade III & IV) = 40.**

Grades	Range		Mean		SD	
	mg/dl	umol/l	mg/dl	umol/l	mg/dl	umol/l
I & II	0.2 -1.0	11.36 - 56.82	0.56	31.82	± 0.29	± 16.48
III & IV	0.2 -0.9	11.36 -51.14	0.52	29.55	± 0.22	± 12.5

**Table 8: Comparison of serum vitamin C mg/dl in senile cortical and senile nuclear types.**

Type	Range		Mean		SD	
	mg/dl	umol/l	mg/dl	umol/l	mg/dl	umol/l
Cortical	0.2 -1.1	11.36 - 62.5	0.55	31.25	± 0.23	13.07
Nuclear	0.2 - 0.9	11.36- 51.14	0.54	30.68	± 0.23	13.07

In our study of 100 cases of senile (age related) cataract with 60 cases of senile cortical type (30 case of immature and 30 cases of mature) and 40 cases of senile nuclear type (20 cases of Grades I and II and 20 cases of Grades III and IV) in the age group of 45 to 70 years, vitamin C mean plasma level was significantly decreased in senile cataract ( $0.54 \pm 0.23$  mg/dl or  $30.68 \pm 13.07$  umol) and especially in the later stages or grades of maturity when compared with the earlier stages or grades of immaturity i.e. The mean level of Serum Ascorbic acid was  $0.55 \pm 0.23$  mg/dl ( $31.25 \pm 13.07$  umol) in senile cortical cataract with  $0.58 \pm 0.24$  mg/dl ( $32.96 \pm 13.64$  umol) in its Immature stage and  $0.52 \pm 0.20$  mg/dl ( $29.55 \pm 11.36$  umol) in its mature stage and the mean level in senile nuclear cataract was  $0.54 \pm 0.23$  mg/dl ( $30.68 \pm 13.7$  umol) with  $0.56 \pm 0.29$  mg/dl ( $31.82 \pm 16.48$  umol) in its Grades I and II and  $0.52 \pm 0.22$  mg/dl ( $29.55 \pm 12.5$  umol) in its Grades III and IV.

So, our study showed significantly decreased levels of serum vitamin C in all the morphological types and clinical stages of senile cataract especially in the later stages or grades of maturity compared to the earlier clinical stages or grades of immaturity, which may be due to its utilization by counteracting oxygen free radicals or due to its oxidation by  $O_2$ .

In the study of Ravilla et al, an Indian study of vitamin C was inversely associated with cataract with the serum

ascorbic acid level of 0.61 (0.51-0.74) and similar results were with vitamin C by type of cataract: nuclear cataract 0.66 (0.54-0.80), cortical cataract 0.70 (0.54-0.90), and posterior subcapsular cataract (PSC) 0.58 (0.45-0.74).<sup>30</sup>

In the study of Kamath et al, an Indian study of 131 participants in each group, the patients with denser cataracts had a significantly lower level of serum vitamin C.  $0.91 \pm 0.40$  mg/dl in Group 1 with cataract compared to  $1.16 \pm 0.50$  in Group 2 without minimal cataract and the low serum vitamin C levels were strongly associated with nuclear and cortical cataracts, but not with posterior subcapsular cataracts.<sup>31</sup>

In the study of Dherani M et al, an Indian study of 821 patients in the age group of  $>$  or  $=$  50 years, serum Vitamin C level of 0.64 (Range 0.48-0.85) was inversely associated with cataract the highest ( $>$  or  $=$  15 micromol/L) compared with the lowest ( $<$  or  $=$  6.3 micromol/L).<sup>11</sup>

In the study of Leske et al, US study of 945 patients in the age group of 40-79, the serum Ascorbate was 0.72 (range 0.46-1.12) with 0.80 (range 0.50-1.29) in its cortical type, 0.73 (range 0.32-1.73) in posterior subcapsular (PSC) type and 0.48 (0.24-0.99) in nuclear type.<sup>32</sup>

In the study of vital et al, a US study of 431 patients in the age group of  $\geq 40$  years, the serum ascorbate was 1.01



(range 0.45-2.26) in cortical type and 1.31 (0.65-2.6) in Nuclear cataract.<sup>33</sup>

In the study of Jacques et al, a US study of 163 patients in the age group of 53-73 years, serum ascorbate was 0.54 (range 0.28-1.02) in Nuclear type.<sup>34</sup> In the study of Taylor et al in, a US study of 332 patients in the age group of 53-73 years, serum ascorbate was 1.4 (range 0.86-2.52) in Cortical type and 0.74 (range 0.32- 1.68) in Nuclear cataract.<sup>35</sup>

In the study of Lin Wei et al, serum ascorbate level was 0.704 (range 0.564-0.879) and the subgroup analysis by cataract type showed the significant associations in nuclear type 0.587 (Range 0.347-0.995) and PSC 0.478 (range 0.348-0.655), but not in cortical type.<sup>36</sup>

Our study and the above studies showed that the decrease in the level of serum vitamin C level is directly related to the progression of the senile cataract. In summary, inverse association of serum ascorbate with the progression of the senile cataract suggested that serum ascorbate might reduce the risk of cataract.

## CONCLUSION

The present study with the low levels of serum Vitamin C in the senile cataract and in its morphological types of cortical and nuclear, especially in the later clinical stages or grades of maturity when compared to immature stages, it may be justified to use antioxidants to raise serum level to prevent the onset and progression of the senile cataract. If some measures are undertaken to slow and delay the development of the cataract by some years, it may reduce the prevalence of cataract with economic burden due to cataract disability and surgery and will enhance the quality of old people.

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