



## Age-related changes of vitamin C levels in aqueous humour

### Promene vrednosti vitamina C u očnoj vodici u vezi sa starenjem

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#### Abstract

**Background/Aim.** Age-related cataract is a common disease among senior population. Vitamin C is the most effective reducing hydrosoluble antioxidant causing reduction in the levels of free radicals in crystalline lens. The aim of this study was to evaluate the age-related change of vitamin C (L-ascorbic acid) level in the aqueous humor of patients with senile cataract. **Methods.** This prospective study included 74 patients, divided into 3 age groups, with age-related cataract, underwent routine phaco cataract surgery with intraocular lens implantation. Aqueous humor was aspirated from 74 eyes at the beginning of phacoemulsification. The levels of vitamin C (L-ascorbic acid) in aqueous humor were determined with high-performance liquid chromatography (HPLC). **Results.** The average age of patients included in the study was  $65 \pm 9.85$  years (54–87 years). The level of vitamin C in aqueous humor was  $152.78 \pm 7.0125$   $\mu\text{g}/\text{mL}$  in the group A (50–59 years),  $134.15 \pm 5.1569$   $\mu\text{g}/\text{mL}$  in the group B (60–69 years) and  $106.51 \pm 5.44$   $\mu\text{g}/\text{mL}$  in the group C (over 70 years). **Conclusion.** The amount of vitamin C in aqueous humor of patients with age-related cataract is decreasing with age. There was a statistically significant change ( $p < 0.001$ ) of vitamin C aqueous concentration in the patients of different age. This decrease could play a role in susceptibility to cataract formation in older population.

#### Key words:

cataract; aqueous humor; aging; ascorbic acid; chromatography.

#### Apstrakt

**Uvod/Cilj.** Starosna katarakta je uobičajena bolest kod starijih osoba. Vitamin C je veoma efikasan redukujući hidrosolubilni antioksidant koji uzrokuje sniženje nivoa slobodnih radikala u očnoj sočivu. Cilj studije bio je da se utvrdi promena količine vitamina C (L-askorbinska kiselina) u očnoj vodici u zavisnosti od starosti bolesnika sa senilnom kataraktom. **Metode.** Ova prospektivna studija uključivala je 74 osobe sa senilnom kataraktom, podeljene u 3 starosne grupe, podvrgnuti rutinskoj hirurrgiji katarakte metodom fakoemulzifikacije sa ugradnjom intraokularnog veštačkog sočiva. Očna vodica aspirirana je iz 74 oka na početku operativnog zahvata. Nivo vitamina C (L-askorbinske kiseline) u očnoj vodici određen je metodom tečne hromatografije visokih performansi (HPLC). **Rezultati.** Prosečna starost bolesnika uključenih u studiju iznosila je  $65 \pm 9,85$  godina (54–87 godina). Nivo vitamina C u očnoj vodici iznosio je  $152,78 \pm 7,0125$   $\mu\text{g}/\text{mL}$  u grupi A (50–59 godina),  $134,15 \pm 5,1569$   $\mu\text{g}/\text{mL}$  u grupi B (60–69 godina) i  $106,51 \pm 5,44$   $\mu\text{g}/\text{mL}$  u grupi C (preko 70 godina). **Zaključak.** Količina vitamina C u očnoj vodici bolesnika sa senilnom kataraktom opada sa starenjem. Utvrđena je statistički značajna promena ( $p < 0,001$ ) količine vitamina C kod osoba različite starosti. Ovakav pad može imati ulogu u sklonosti ka nastanku senilne katarakte.

#### Ključne reči:

katarakta; očna vodica; starenje; askorbinska kiselina; hromatografija.

#### Introduction

Age-related cataract is a very common disease and continues to be one of the major health problems in developing countries, especially among senior population.

It is estimated that more than 18% of those older than 65 have cataracts, and that the cataract is the leading cause of reversible blindness.

Age-related cataract has a multifactor etiology<sup>1,2</sup>. Besides biochemical changes in the crystalline lens as a part of aging process, multitude of other factors are involved, as well.

The free radical theory of aging states that organisms age because cells accumulate damage caused by free radical over time<sup>3</sup>.

Ageing leads to morphological and biochemical changes in the crystalline lens. Contrary to other organs having epithelium,

the crystalline lens does not shed its old cells. Instead, they are being kept inside lens capsule throughout the entire lifetime.

The lens obtains nutrients necessary for metabolism and maintaining of its transparency, as well as antioxidative elements from aqueous humor. On the other hand, products of metabolism and oxidative processes in lens affect the composition of aqueous humor as well<sup>4</sup>. Production of aqueous humor decreases with ageing, affecting normal metabolism of the lens.

Human cataract in the older age groups seems to be caused by accumulation of various risk factors. There is an evident decrease in the antioxidative mechanism, levels of glutathione and vitamin C, as well as activity of catalase and superoxide dismutase, all of which can lead to cataract formation<sup>5</sup>.

Current hypothesis emphasises the role of free radicals and oxidative stress in the genesis of cataract<sup>6,7</sup>.

A certain number of metabolic reactions inside human organism require the presence of free radicals. However, on cellular level they can cause damage of important biomolecules leading to a wide range of pathological changes.

Within physiological conditions, the formation of free radicals is in the state of equilibrium with antioxidative systems in the organism. The disruption of this balance causes the increase of free radicals production or decrease in concentration of antioxidants, and it is termed oxidative stress<sup>8-10</sup>.

Antioxidative protection inside human body is based on the action of antioxidative substances that, depending on its concentration in substrate, can prevent or significantly reduce oxidation<sup>11</sup>.

Vitamin C (L-ascorbic acid) is the most effective reducing hydrosoluble antioxidant and one of essential non-enzymatic components of antioxidative protection<sup>12</sup>.

Ascorbic acid is especially active in oxidoreductive processes causing reduction in levels of free radicals in the crystalline lens<sup>13,14</sup>.

The aim of this study was to evaluate the level of vitamin C (L-ascorbic acid) in the aqueous humor of patients with age-related, senile cataract.

## Methods

This prospective study included 74 patients with age-related cataract who were undergoing routine phaco cataract surgery with intraocular lens implantation at the University Eye Clinic, Clinical Center Vojvodina in Novi Sad.

According to age, the patients with cataract were divided into 3 groups: the group A with 24 patients of 50–59 years; the group B with 26 patients of 60–69 years; the group C of 24 patients, 70 and more years.

Complete ophthalmological examination included: distance visual acuity testing using the Snellen method, near visual acuity using Jaeger's tables, intraocular pressure measurement with applanation tonometry, slit lamp examination of anterior and posterior segment of the eye with artificial mydriasis.

Patients with other ophthalmic (glaucoma, uveitis, retinal diseases etc.) and systemic diseases (diabetes, hyperlipemia etc.) that might have influence on oxidative stress and the level of ascorbic acid in patients with cataract were excluded.

Aqueous humor samples were obtained before creation of the scleral tunnel. A small amount (0.2 mL) of aqueous humor was aspirated through *ab externa* (outside- in) limbal paracentesis with a 27 gauge needle on a tuberculin syringe.

The extracted quantity of aqueous humor was replaced with the same amount of isotonic Ringer lactate solution, and the operating procedure was resumed in regular fashion.

This method of aqueous sampling did not disrupt the regular course of cataract surgery in any way.

The levels of vitamin C in aqueous humor were determined with high-performance liquid chromatography (HPLC). Vitamin C produced by J. T. Baker (The Netherlands) was used as a standard, and it was dissolved in 3% solution of metaphosphoric acid (Riedel-de Haën, Germany) and 8% acetic acid solution (Zorka, Šabac, Serbia).

Standard vitamin C solution was prepared in the following manner: 0.003 g of the standard was dissolved in 100 mL of 3% metaphosphoric acid. Such a solution contained 30 µg/mL of vitamin C.

All solutions were prepared in bidistilled water meeting HPLC quality guidelines.

All analysis used high-performance liquid chromatography (HPLC system „Agilent 1100“, USA), with 20 µL sample injection loop, C-18 column, particle size of 5 µm and UV-DAD detector. The flow of mobile phase was 0.4 mL/min, and column temperature was 37°C. Analysis duration was 5 minutes with three repetitions. Standard vitamin C solution, as well as native samples of aqueous humor filtrated with a membrane filter, were passed through the system.

Twenty microlitres of aqueous humor stabilised with a buffer system in the presence of metaphosphoric acid was injected. The amount of vitamin C was calculated by comparing the area under the peak of vitamin C standard solution, and the area under the peak of vitamin C from aqueous of patients with age-related cataract.

## Results

A total of 74 patients, average age of  $65 \pm 9.85$  years (54–87 years), were included in this study, of who 56% were men and 44% women. There was no statistically significant difference in gender distribution in the patients with senile cataract ( $p = 0.662$ ).

The level of vitamin C in aqueous humor was  $152.78 \pm 7.0125$  µg/mL in the group A,  $134.15 \pm 5.1569$  µg/mL in the group B and  $106.51 \pm 5.4416$  µg/mL in the group C ( $p < 0.01$ ) (Table 1).

Table 1

Average vitamin C content in aqueous humor	
Patients	Vitamin C (µg/mL)
	$\bar{x} \pm SD$ (min-max)
Group A (n = 24) 50–59 years	$152.78 \pm 7.01$ (142–168)
Group B (n = 26) 60–69 years	$134.15 \pm 5.15$ (120–149)
Group C (n = 24) > 70 years	$106.51 \pm 5.44$ (99–115)

The variation interval and average vitamin C content ( $\mu\text{g/mL}$ ) in aqueous humor in relation to age ( $n = 74$ ) was statistically significant ( $p < 0.01$ ).

## Discussion

The aqueous humor has low protein concentration. It maintains the intraocular pressure, provides nutrition (e.g. glucose and amino acids) for the avascular ocular lens and serves to transport ascorbate in the anterior segment which acts as an antioxidant agent.

Nutrients diffuse in and waste diffuses out through the constant flow of fluid from the anterior/posterior poles of the lens and out of the equatorial regions.

Numerous risk factors for the different types of age related cataracts have been identified. These include environmental factors, sunlight exposure, systemic diseases, cigarette smoking, indices of nutrition, socioeconomic factors etc.<sup>15</sup>.

A discovery made by Bietti in 1940, that the concentration of vitamin C in aqueous humour is much higher than the one in plasma, played a major role in our understanding of the important function of this vitamin. It is known to be the most important non-enzymatic antioxidant, taking part in numerous complex biochemical reactions, and protecting the eye from oxidative stress.

The lack of vitamin C causes the multitude of degenerative and pathological disorders<sup>16,17</sup>.

Our research showed that the amount of vitamin C in aqueous humour of the patients with age-related cataract is decreasing with age. There was a statistically significant variation ( $p < 0.001$ ) of vitamin C aqueous concentration in patients of different age and we, therefore, concluded this decrease may be the cause of susceptibility to cataract formation in older population.

In the same time, during the process of cataract maturation, vitamin C aqueous concentration in the patients included in this study decreased. The reason for this is thought to be its oxidation, due to accumulation of free radicals and hydrogen peroxide<sup>18</sup>.

The degree of vitamin C aqueous concentration fall in our study is consistent with previous results of other authors<sup>19</sup>.

Being hydrosoluble, vitamin C is always present in aqueous fraction of the cell in two of its forms: reduced (ascorbic acid), and oxidized form (dehydroascorbic acid). Vitamins C primary function is electron donor, i.e. reducing agent, preventing oxidation of other compounds.

Vitamin C serum concentration ranges from 0.6 to 2 mg/mL and maximal aqueous humor concentration is up to 20 times higher<sup>20,21</sup>.

Jacques and Chylack<sup>22</sup> have found out that there is a decrease of 75% in cataract formation in people taking large amounts of vitamin C ( $\geq 490$  mg/day). In people with high

vitamin C serum concentrations ( $> 90$   $\mu\text{mol/L}$ ) incipient cataract is one third rarer than in people with lower serum concentration ( $< 40$   $\mu\text{mol/L}$ ). The Roche European American Cataract Trial (REACT) reported small deceleration of cataract progression in persons with high daily dose supplements (vitamin C 750 mg, vitamin E 268 mg and  $\beta$ -karoten 15 mg) combined for 3 years<sup>23</sup>. Supplement with low doses of vitamin C (180 mg/day) and vitamin E (40 mg/day) decreased risk of nuclear cataracts among participants aged  $\geq 65$  years in the Linxian cataract studies<sup>24</sup>. Decreased risk of cataract was also reported in a Beaver Dam Eye Study<sup>25</sup>.

Other observational studies found that blood levels of antioxidants are inversely associated with age related cataract in Asian population but not in Western population<sup>26</sup>.

However, the hypothesis that high doses vitamin C supplement use may decrease the risk of age-related cataract has not received support in several studies.

Antioxidants in free radical rich environment may function as prooxidants and high doses of vitamin C may contribute to a disturbance in redox homeostasis promoting cataractogenesis<sup>27</sup>. A harmful effect from dehydroascorbate (oxidized vitamin C) has been reported. It contributes glycation of lens proteins, generation of superoxide anions and modification of lens proteins increasing risk of cataract formation<sup>28-30</sup>.

Rautiainen et al.<sup>31</sup> and Selin et al.<sup>32</sup> observed statistically significantly increased risk of age related cataract extraction among high doses of vitamin C supplement users but not among multivitamins with recommended daily allowances of vitamin C users. The positive association between high doses vitamin C use and risk of age-related cataract was stronger among patients with higher oxidative stress (reactive oxygen species-generating factors): older patients, corticosteroid users, long-term users.

Jacques et al.<sup>33</sup> and Rock<sup>34</sup> determined that steep increase in serum and aqueous humor vitamin C concentrations appear with daily intake of 150–250 mg of vitamin C, while higher intake ( $> 250$  mg/day) fails to further increase its concentration, neither in serum, nor in aqueous humor. It was also found that increase in serum and aqueous humor concentrations of vitamin C raise its concentration within crystalline lens in linear fashion, while increase in serum concentration of vitamin C induces its increase in aqueous humor, but in non-linear way, indicating the existence of saturating concentration.

## Conclusion

The amount of vitamin C in aqueous humor of patients with age-related cataract is decreasing with age. There was a statistically significant change ( $p < 0.001$ ) of vitamin C aqueous concentration in the patients of different age. This decrease could play a role in susceptibility to cataract formation in older population.

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