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### RESEARCH ARTICLE

#### EVALUATION OF URIC ACID LEVELS IN TEARS OF PATIENTS OF VARIOUS GRADES OF MYOPIA

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#### Abstract

Myopia is major health problem in India. Several studies showed that glaucoma risk increased with more severe myopia. Oxidative damage associated with hypoxia and oxidative stress alter regulatory pathways in myopia. Oxidative stress is caused by an imbalance between the production of reactive oxygen species and ability of the biological systems defense mechanisms necessary to eliminate the stress. Oxidative damage has also been reported to play a role in several ocular diseases including age-related macular degeneration, cataract, uveitis, retinopathy of prematurity, corneal inflammation and keratitis. The antioxidant status of tear fluid is of interest because tears are the first barrier protecting the cornea against light induced oxidative damage and other toxic chemicals. So Uric Acid was measured in tears of patients of myopia (20-40) years and age and sex matched healthy control.

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#### Introduction:-

Myopia is the most common form of the refractive error and a major cause of moderate to severe visual impairment worldwide. The myopic eye focuses images in front of the retina, resulting in blurred distant vision. Others symptoms may include discomfort after near work, sensitivity to light, floating black spots and sometimes flashes of light. Severe near sightedness increases the risk of retinal breaks, retinal detachment and glaucoma.<sup>1,2</sup>

Myopia which is measured in diopters (D) by the strength or optical power of a corrective lens that focuses distant images on retina has also been classified by degree or severity. Low myopia usually describes myopia of  $-3.00$  D or less. Moderate myopia usually describes myopia between  $-3.00$  and  $-6.00$ D. Those with moderate myopia are more likely to have pigment dispersion syndrome or pigmentary glaucoma. High myopia usually describes myopia of  $-6.00$ D or more.<sup>3,4</sup>

People with high myopia are more likely to have retinal detachment and primary open angle glaucoma. They are also more likely to experience floaters or shadow like shapes which appear singly or in clusters in the field of vision.<sup>5,6</sup>

Oxidative stress is caused by an imbalance between the production of reactive oxygen species and the ability of the biological defense mechanism to eliminate it. Oxidative damage has also been reported to play a role in several

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ocular diseases including age-related macular degeneration, cataract, uveitis, retinopathy of prematurity, corneal inflammation and keratitis.<sup>7-11</sup>

Recent awareness of association of oxidative stress damage with ocular surface diseases have incited researchers to establish the possible mechanisms. Cells produce reactive oxygen species (ROS) both as a metabolic by-product and for the signaling of certain cellular processes. Excessive ROS are scavenged by endogenous antioxidants that protect the tissue from oxidative damage. The balance between ROS production and antioxidant capacity is delicate, and disruption of it contributes to the patho-physiology of numerous diseases. The antioxidant status of tear fluid is of interest as ocular surface is the most environmentally exposed mucosal surface of the body, encountering challenges such as wind, extremes of temperature, ultraviolet radiations, irritants, pollutants and tobacco smoke. Such factors somehow, can influence the severity of ROS potential damage. A great number of different compounds have been demonstrated in tears like proteins, enzymes, lipids, metabolites, electrolytes and drugs which are secreted during topical treatment.<sup>12</sup>

#### **Uric acid in myopia:**

The uric acid in tears and in the blood plays a key role as a diagnostic indicator for antioxidant status in the eye and gouty arthritis respectively.<sup>13</sup>

The reference range of uric acid level in blood is 3.5 – 7.0 mg/dL and in human tears is 68 ±46 µmol/L.<sup>14</sup>

Camus Kar Man Choy et al. evaluated a novel method (FRASC) for total ferric reducing (antioxidant) activity and ascorbic acid concentration applied to human tears, to investigate the stability of ascorbic acid, and to determine the antioxidant status of human reflex tears. Total antioxidant activity and ascorbic acid and uric acid concentrations (mean ± SD) in reflex tears from 47 healthy subjects were 409 ± 162, 23 ± 9.6, and 68 ± 46 µM, respectively. Ascorbic acid and uric acid constituted around half the total antioxidant activity measured. There was a significant correlation between uric acid and total antioxidant activity ( $r = 0.754$ ;  $P < 0.0001$ ). Men had significantly ( $P = 0.0045$ ) higher tear ascorbic acid concentrations than women. They found FRASC is suitable for measuring total antioxidant activity and ascorbic acid in human tears.<sup>15</sup>

D Koracevic et al. conducted a study to develop a new, simple, and cheap method for estimating antioxidant activity in human fluids. They measured the capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. A solution of 1 mM/L uric acid was used as standard. The following mean (SD) anti oxidative activities were found (as uric acid) in the various biological fluids: serum, 2.04 ± 0.20 mM/L, urine 176.5 ± 25.6 µM/L, cerebrospinal fluid 95.0 ± 26.9 µM/L, aqueous humour oculi 61.25 ± 9.9 µM/L, saliva 838.5 ± 48.2 µM/L, tears 247.0 ± 17.0 µM/L, ascites fluid 270.0 ± 63.3 µM/L, kidney cyst fluid, 387.1 ± 28.1 µM/L.<sup>16</sup>

Moonseong Park et al. developed a plasmonic Schirmer strip for on-demand, rapid, and simple identification of biomarkers in human tears. The diagnostic strip features gold nanoislands directly and evenly formed on the top surface of cellulose fibers, which maintain a hygroscopic nature for an efficient collection of tear production as well as provide plasmonic enhancement in surface-enhanced Raman scattering (SERS) signals for identification of tear molecules. The uric acid in human tears was quantitatively detected at physiological levels (25–150 µM) by using SERS. They also found a strong linear correlation between uric acid level in both human tears and blood for gouty arthritis diagnosis. This functional paper strip enables noninvasive diagnosis of disease-related biomarkers and healthcare monitoring using human tears.<sup>17</sup>

Yuri Y Sautin et al. found that Uric acid, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension, and cardiovascular disease conditions associated with oxidative stress. While one explanation for this paradox could be that a rise in uric acid represents an attempted protective response by the host, they reviewed the evidence that uric acid may function either as an antioxidant (primarily in plasma) or pro-oxidant (primarily within the cell). They suggested that it is the pro-oxidative effects of uric acid that occur in cardiovascular disease and may have a contributory role in the pathogenesis of these conditions.<sup>18</sup>

Michael G. Simic et al. found that one-electron oxidation of uric acid generates the urate radical, which was studied in aqueous solution by pulse radiolysis and oxygen-uptake measurements. Acid-base properties of the uric acid radical were determined, i.e.  $pK_{a1} = 3.1 \pm 0.1$  and  $pK_{a2} = 9.5 \pm 0.1$ . The reaction of the radical with oxygen was too slow to be measured,  $k < 10^{-2} \text{ dm}^3 \text{ Mol}^{-1} \text{ s}^{-1}$ . Rapid reactions of uric acid with oxidizing species and peroxy radicals

were indicative of uric acid as a possible water-soluble physiological antioxidant. Rapid reaction of uric acid with the guanyl radical indicates that uric acid may also act as a repair agent of oxidative damage to DNA bases.<sup>19</sup>

The association of oxidative stress with myopia has not been clearly established, hence the present study was planned to establish the levels of Uric Acid as antioxidants in the tears of various degree of myopia.

### **Materials And Method:-**

The present study was hospital based case control study conducted on seventy five newly diagnosed cases of various degree of myopia and twenty five controls.

This study was conducted in the Department of Biochemistry in collaboration with Regional Institute of Ophthalmology (Glaucoma unit), at a tertiary care centre, Pt B.D. Sharma Post Graduate Institute of Medical Sciences , Rohtak. Patients of different degree of myopia in the age group of 20-40 years attending the ophthalmology OPD were enrolled. Diagnosis was established with help of detailed history, clinical examination and ocular examination.

#### **Sample size:**

Twenty five patients each with mild myopia (Group 1), moderate myopia (Group 2), high myopia (Group 3), age and sex matched healthy controls (Group 4).

#### **Inclusion Criteria:**

Patients between 20- 40 years of age and newly diagnosed clinically as myopia.

#### **Exclusion Criteria:**

Patient having glaucoma, any systemic disease like diabetes, hypertension, thyroid disorders, any surgery or trauma to eye with in 1 month of presentation, any major illness like liver disease, cancer, in intensive care unit, any form of local or systemic steroids and or sex hormones, any antioxidant supplementation.

#### **Sample collection and storage:**

##### **Tear collection:<sup>20</sup>**

After explaining the procedure and making the person comfortable and informed consent, the tear sample was collected from one eye of each subject.

1. One drop of 0.5% proparacaine was instilled on the cornea and conjunctiva and the patient was made to wait for five minutes.
2. Tear collection was accomplished by using graduated 100µL glass capillary rods which was procured from Scientific Drummond company Pennsylvania, USA.
3. The tip of the glass capillary rod was placed in contact intermittently with the tear fluid at the lower cul-de-sac for a minimum time to obtain fluid without irritating the subject's eye (30 to 60 seconds).
4. The collected sample was placed gently in to a 1.5-ml Eppendorf tube.
5. The sample was immediately transferred to the laboratory in an insulated container on cold packs for analysis of various parameters.
6. The sample was stored at -70°C, if it could not be analyzed immediately.



**Fig 1:-** Glass graduated 100µL capillary rods.

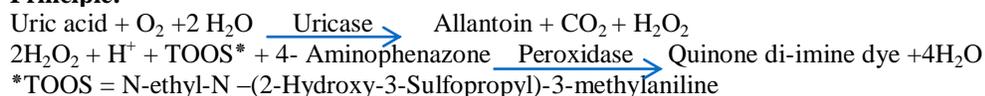
#### Sample processing:

Tear sample was analyzed for Uric Acid levels within thirty minutes of collection. Separate blood sample was collected for routine investigation in red capped vacutainer and Serum was separated by centrifugation at 2000 rpm for 10 minutes. Separated serum was stored at -20°C if not analysed immediately.

#### Estimation of Uric acid:<sup>21</sup>

Estimated by enzymatic method on autoanalyser

#### Principle:



#### Observations And Results:-

The following were the observations of the present study.

#### Comparison of baseline data:

In this study 68 % of the subjects were males and 32% were females in the control and myopia group.

**Table 1:-** Gender and age distribution in cases and controls.

		CONTROL N=25	DEGREE OF MYOPIA		
			MILD N=25	MODERATE N=25	SEVERE N=25
Gender	Male	17	19	18	14
	Female	8	6	7	11
Age (yrs)	Mean ±s.d.	21.68 ± 3.95	24.56 ± 6.35	24.12 ± 5.45	23.24 ± 5.17
	Range	20 – 37	20 – 37	20 -38	20 -36
	Median	20.40	20.86	21.50	20.58

Table 1. shows that the mean ( $\pm$ SD) age was  $21.69 \pm 3.95$ ,  $24.56 \pm 6.35$ ,  $24.12 \pm 5.45$ , and  $23.24 \pm 5.17$  in the control group, group with mild, moderate and severe myopia respectively. Age was comparable in all the four groups.

**Table 2:-** Mean uric acid levels in tears of myopia and controls.

URIC ACID ( $\mu$ MOL/L)		Control	Myopia	P value
	Mean $\pm$ s.d.	22.36 $\pm$ 16.04	15.78 $\pm$ 12.03	
	Range	.00 – 59.48	.00 – 59.48	
	Median	21.24	13.43	

Table 2. shows that the mean uric acid levels in tears is lower in myopia patients as compared to controls. The decrease was statistically significant ( $p < 0.05$ ).

**Table 3:-** Mean uric acid levels in tears of various degrees of myopia and controls.

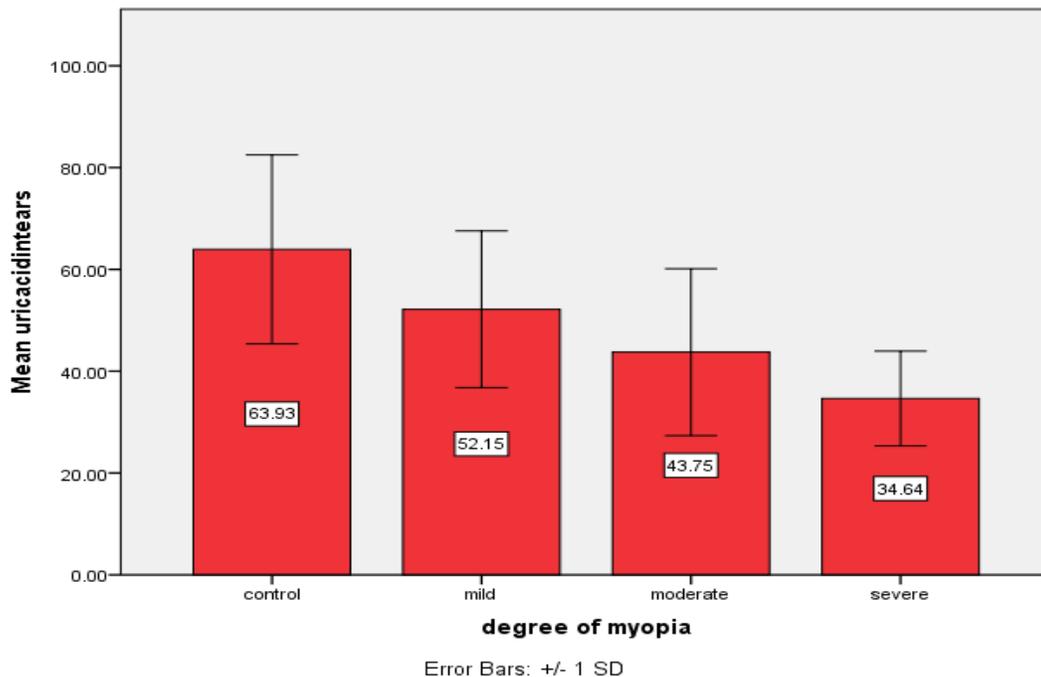
URIC ACID ( $\mu$ MOL/L)		Control	Degree of myopia		
			Mild	Moderate	Severe
	Mean $\pm$ s.d.	63.92 $\pm$ 18.5	52.15 $\pm$ 15.39	43.75 $\pm$ 16.40	34.64 $\pm$ 9.30
	Range	30.33-104.09	23.79-77.72	24.39-73.16	23.79 - 60.67
	Median	50.55	36.87	32.11	65.4
p value ( in relation to control)		.008	.000	.000	

Table 3. shows the level of uric acid in tears was decreased in the various degree of myopia patients as compared to the controls. The decrease in the level of uric acid in tears was inversely proportional to the degree of myopia. The decrease was statistically significant ( $p < 0.05$ ).

**Table 4:-** Intra group comparison of uric acid levels in tears of various degrees of myopia.

URIC ACID ( $\mu$ mol/L)	Degree of myopia intra group comparison		Mean difference	Student Unpaired t test (p value)
	Mild	Moderate	8.398	0.055
		Severe	17.510	0.000
	Moderate	Severe	9.112	0.038

Table 4. shows there was statistically significant difference in the mean uric acid values in mild versus moderate and moderate versus severe degree of myopia ( $p < 0.05$ ).



**Figure 1:-** Mean uric acid levels in tears of various degree of myopic cases and controls.

Figure 1. shows that the mean ( $\pm$ SD) of uric acid in tears was  $63.93 \pm 18.57$ ,  $52.15 \pm 15.39$ ,  $43.75 \pm 16.40$  and  $34.64 \pm 9.30$  in the control group, group with mild, moderate and severe myopia respectively. p value  $<0.05$  of mild, moderate and severe cases was found to be highly significant in relation to control.

**Table 5:-** Comparison of findings of current study with other studies in literature.

Authors	Finding	Conclusion
Camus Kar Man Choy et al.	Ascorbic acid and uric acid constituted around half the total antioxidant activity measured in human reflex tears. There was a significant correlation between uric acid and total antioxidant activity ( $r = 0.754$ ; $P < 0.0001$ ).	Uric Acid can be used to evaluate status of antioxidant activity.
D Koracevic et al.	They measured the antioxidant activity in human fluids and capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction	Anti oxidative activities were found (as uric acid) in tears
Moonseong Park et al.	The uric acid in human tears was quantitatively detected at physiological levels ( $25-150 \mu\text{M}$ ) by using SERS in newly developed Plasmonic Schirmer strips featuring gold nanoislands.	They found a strong linear correlation between uric acid level in both human tears and blood for gouty arthritis diagnosis.
Michael G. Simic et al.	Found rapid reactions of uric acid with oxidizing species and peroxy radicals were indicative of uric acid as a possible water-soluble physiological antioxidant.	Uric acid may also act as a repair agent of oxidative damage to DNA bases.
Present study	Uric Acid levels in tears of patients of various grade of myopia were estimated and found decremental effect as compared to the controls. The decrease was statistically significant ( $p < 0.05$ ), indicating depleting antioxidant status in with severity of myopia.	Anti oxidative activities of uric acid were found in tears. Seems to be significant role of oxidative stress in development of myopia and other degenerative disorders.

### Discussion:-

This work was done in the department of biochemistry in collaboration with the department of ophthalmology at Pt. B.D. Sharma institute of medical sciences. The principle aim was to evaluate uric acid levels in tears of patients of various degree of myopia. A total of 75 patients of different degree of myopia as cases and 25 age and sex matched healthy controls were enrolled in this observational case-control study. The diagnosis of myopia was confirmed by retinoscopy and refraction. Uric Acid was measured in tears. Routine biochemical investigations were also performed in all the cases and controls.

### Age and Sex:

In the present study mean ( $\pm$ SD) age was  $21.69 \pm 3.95$  (20-40 years) in the controls, in mild cases  $24.56 \pm 6.35$  (20 – 40 years), in moderate cases  $24.12 \pm 5.45$  (20-40 years) and  $23.24 \pm 5.17$  (20 - 40 years) in severe cases. The age and sex were comparable in both i.e, cases and control.

### Mean Uric Acid levels with in tears of various degree of myopic cases and controls:

Mean ( $\pm$ SD) of uric acid in tears was  $63.93 \pm 18.57$ ,  $52.15 \pm 15.39$ ,  $43.75 \pm 16.40$  and  $34.64 \pm 9.30$  in the control, mild, moderate and severe myopia groups respectively. The level of Uric Acid in tears were decremental in the various degree of myopia patients as compared to the controls. The decrease was statistically significant ( $p < 0.05$ ), indicating depleting antioxidant status in with severity of myopia.

There was also statistically significant difference in the mean uric acid values in mild versus moderate and moderate versus severe degree of myopia ( $p < 0.05$ ). It depicts there is a linear correlation with various degree of increasing myopia.

Thus there seems to be significant role of oxidative stress in development of myopia and other degenerative disorders. Tears can be used as a non invasive method to study various oxidative and other markers for this purpose. Further studies with more oxidative markers in tears can also be compared with blood values in larger groups of patients with long term follow up to validate the finding.

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