IJBPAS, February, 2024, 13(2): 814-824

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

RELATIONSHIP BETWEEN CATALASE AND URIC ACID PROFILE IN DIABETIC AND SENILE CATARACT PATIENTS- A COMPARATIVE STUDY

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Received 6th Jan. 2023; Revised 27th March 2023; Accepted 26th June 2023; Available online 1st Feb. 2024

https://doi.org/10.31032/IJBPAS/2024/13.2.7786

ABSTRACT

Oxidative mechanisms play a major role in the aetiology and pathogenesis of cataract. The aim of present study is to investigate the activity of antioxidant enzyme catalase and the level of antioxidant compound uric acid in patients with diabetic and senile cataract and compare them. Total of 115 patients were included in this study out of which 40 diabetic cataract and 75 senile cataract patients attending by the Ophthalmology OPD of Shri Mahant Indresh hospital, Dehradun. Plasma/serum sample from diabetic and senile patients suffering from cataract were assessd for blood glucose, catalase, and uric acid levels. Whole blood for HbA1c levels was assessed. The activity of catalase (CAT) was significantly decreased in diabetic cataract patients in comparison to patients with senile cataracts ($p<0.001$). Concentration of serum uric acid is significantly increased in diabetic cataract patients as compared to senile catract patients (<0.001). Fasting and post-parandial blood glucose levels in patients of diabetic cataract were significantly higher ($p<0.001$) as compared to the patients of senile cataract. The current study revealed that oxidative stress indicated by decrease activity of antioxidant enzyme catalase and increased concentration of uric acid, might have a role in diabetes related complication specifically cataract formation.

Keywords: Oxidative stress, Antioxidant, Cataract, CAT, Diabetes

INTRODUCTION:

Oxidative stress implies that any alteration in which pro-oxidants predominate over the antioxidants. Pro-oxidants are the chemical compounds that are capable to trigger a cascade of oxdative reactions leading to protein unfolding and DNA damages. Oxidative stress may be due to either increased production of reactive oxygen species (ROS) or decreased levels of antioxidants **[1]**. The full reduction of four electrons of oxygen occurs within the mitochondria and the final product is water. A partial reduction produces superoxide and various reactive oxidative intermediated (free radicals and reactive oxygen species or ROS including hydroxyl radicals, singlet oxygen radicals and hydrogen peroxide). In addition to these endogenous oxidizers, other sources include food, air pollutants, tobacco smoke, exercise, ionising radiation. In the body cells reactive oxygen species oxygen species may initiate a surge of toxic biochemical reactions such as peroxidation of membrane-lipids and extensive damage to proteins causing intracellular protein aggregation and precipitation **[2]**. Oxidative insult probably originates at cell membranes **[3]** and the most important oxidants are free radicals. Free radicals are those molecules that have unpaired electrons in their outer shell. This unpaired state makes them highly unstable and responsive with other molecules to either gain or lose electrons. A major source of free radicals is the partial reduction of di oxygen by heme proteins and flavoproteins during mitochondrial electron transport. Oxidative stress has been reported to play a key role in both progression etiology and progression of ocular disorders, including cataract, glucoma, keratitis, uveitis, corneal inflammation. Previous studies have indicated that oxidative stress plays a vital role in pathogenesis of long term diabetic complications **[4]**. Cataract is a complete or a partial opacification of the human lens or capsule of eyes which impairs the vision. Long term exposure of high glucose is expected to increse metabolic influx including mitochondrial superoxide production which damages respiratory chain resulting in the accumulation of glycolytic intermediates **[5]**. Oxidative stress produced through the various pathways such as hexosamine and polyol pathway causes the production of advanced glycation end products (AGEs). Polyol pathway is involved in the pathophysiology of diabetic cataract. Enzymes of polyol pathway mainly aldose reductase and sorbitol dehydrogenase catalyze the conversion of glucose into sorbitol followed by convesion of fructose. Excessive production of fructose is considered as a major cause of reversible blindness in the world today **[6]**. In lens, reactive oxygen species may initiate

different toxic biochemical reactions leading to extensive changes in the lens protein **[7]**. When oxidative stress is induced by some external or internal factors, the homeostasis is disturbed and ROS will modify many intracellular molecules such as nucleic acids, proteins and lipids **[8]**. In diabetic cataract, osmotic stress resulting from the accumulation of sorbitol induces stress in the endoplasmic reticulum (ER), the main site of proteinsynthesis, resulting in the formation of free radicals **[9]**. Oxidative stress in the ER can also be caused by the fluctuation in the glucose levels that cause protein unfolding resulting in the production of reactive oxygen species and ultimately cause damage to the lens fibers. Furthermore increased glucose levels may involve in the glycation of lens protein and forms of advanced glycation end products **[10]**. Antioxidants inhibit both the production of free radicals and AGEs formation. Major protective antioxidants enzymes are superoxide dismutase (SOD), catalase (CAT), xanthine oxidase (XOD) and glutathione peroxidase (GSH Px). In addition, there are several non enzymatic antioxidants such as ascorbic acid, vitamins (A, E), glutathione, flavonoids, uric acid and minerals (Se, Mn, Cu, and Zn) are responsible to counteract the deleterious effect of reactive oxygen species **[11]**.

The occular lens and tissues are chronically exposed to environmental and intrinsic oxidants, such as superoxide anion radicals and hydrogen peroxide produced during mitochondrial respiration and photo-oxidation of endogenous UV filters, ascobate and structural proteins and crystallins. Hydrogen peroxide (H2O2) is a relatively stable reactive oxygen species until it reacts with transition metals, particularly ferrous iron and form highly reactive and toxic hydroxyl radicals, by Fenton chemistry and reacts with macromolecules causing tissue damage. Catalase (E.C. 1.11.1.6) is one of the most important antioxidant enzyme, present in all aerobic organisms. In most cells, it decomposes H_2O_2 to oxygen and water, thereby preventing radical formation catalyzed by transitional metals. Catalase plays an important role in preventing cellular oxidative stress mainly in peroxisomes.

Uric acid is the final product of the purine metabolism. Hypoxanthine is converted into xanthine and xanthine is converted into uric acid and this reaction is catalysed by xanthine oxidase. Uric acid is one of the important antioxidants of plasma **[12, 13]**, that contribute provide up to 65 % of total an antioxidant capacity **[14]**. It has been proven that one of the well reported functions of uric acid in human body fluids is to provide an efficient antioxidant capacity **[15, 16]**. Therefore, the activity of uric acid may also

be a compensatory mechanism to counteract oxidative damage related to degenerative diseases.

MATERIALS AND METHOD: This study was carried out in the department of Biochemistry in collaboration with department of Ophthalmology of Shri Guru Ram Rai University, Dehradun. A total of 115 patients (40 diabetic cataract and 75 senile cataract) attending the Ophthalmology OPD of Shri Mahant Indresh hospital, Dehradun were included in the present investigations. All patients were examined by well qualified and competent ophthalmologist of Shri Mahant Indresh hospital. Visual activity was tested and the opacity of the lens was examined using a slit lamp. Diabetic cataract patients (n=40) were aged 40 years or above and diagnosed as having diabetic cataract. Senile cataract (n=75) patients were aged 50 years or above and were diagnosed as senile cataract. The study protocol is approved by the Research Ethics Committee of Shri Guru Ram Rai University, Dehradun. After obtaining a written informed consent, venous blood sample was collected in Sodium fluoride-potassium oxalate anticoagulant vacutainers for blood glucose analysis. Serum separator tube (SST) was used to collect blood samples for catalase and uric acid analysis. For the measurement of HbA1c from diabetic cataract patients, blood was also collected in EDTA-coated vials. Plasma and serum sample were separated from whole blood by centrifugation at 3500 rpm for 15–20 min. Hemolysed and lipemic serum and plasma was excluded in this study. Prior to further investigation, the serum samples were stored at -80°C. The analysis of sample was performed in the department of Biochemistry of Shri Guru Ram Rai University, Dehradun. Patients who had hepatic disorders, gastrointertinal disorders, renal dysfunctions, anemia, osteoporosis, arthritis, cardiovascular diseases, thyrotoxicosis, traumatic cataract and toxic cataract were excluded from this study. Patients consumed alcohol and oral or tropical anti-oxidant preparation was also excluded from this study.

The following parameters were analyzed to assess the oxidative stress in diabetic cataract and senile cataract.

Serum Uric Acid: The measurement of serum uric acid level was done as per the method adopted by Trivedi and Kabasakalian, 1976 with a modified Trinder peroxidase method using the serum uric acid assay kit (Transasia Biomedical Ltd., ERBA Diagnostics Mannheim, Germany) (Trivedi *et al*., 1976) **[17]**.

Catalase: The activity of serum catalase was measured by using commercial kit based on sandwich ELISA method (Chongqing Biospes Co., Ltd., Chongqing, China). (Szychowski *et al*., 2021) **[18]**.

Blood Glucose: The fasting blood sugar level and post prandial blood sugar levels were estimated by enzymatic Glucose Oxidase- Peroxidase (GOD-POD) Trinder's method using commercial glucose assay kit (Transasia Biomedical Ltd., ERBA Diagnostics Mannheim, Germany) **[19]**.

Glycosylated Hemoglobin: The whole blood for glycosylated hemoglobin (HbA1c) was estimated by high performance liquid chromatography (HPLC) method by (Yis *et al*; 2019) using Bio- Rad D-10 Hemoglobin Testing System **[20, 21, 22]**.

Statistical Analysis: The results were expressed as mean ± standard deviation (SD). The SPSS software (SPSS® for Windows ® 10.0) was used to analyse the data. The post hoc student's t test was used to assess the statistical significance of the difference between the diabetic cataract group and the senile cataract group. Statistics were accepted to be significant at $p<0.001$.

RESULT: Oxidative stress markers such as catalase activity was significantly (<0.001) decreased in diabetic cataract (60.44±2.64 ng/ml) compared to senile cataract patients (85.65±2.18). Serum uric acid concentration significantly (<0.001) increased in diabetic cataract (8.38±1.18 mg/dl) when compared with senile cataract patients (4.67±1.29) **Table 1**. Basic demographical, clinical and biochemical characteristics of diabetic cataract and senile cataract patients are presented in **Table 2**. The mean age of the diabetic and senile cataract patients were 58±5.85 and 59±8.13 years respectively, fasting blood glucose in diabetic cataract was significantly higher (145±2.80 mg/dl) ($p<0.001$) whereas the senile cataractous patients had a normal fasting blood glucose concentration (85±2.60 mg/dl) and post parandial blood glucose in diabetic cataract was significantly higher (215±3.28 mg/dl) ($p<0.001$) as compared to that of senile cataractous patients (114±2.53 mg/dl). The HbA1c percentage in diabetic cataract patients is 7.9 ±0.7.

Table 1: The impact of oxidative stress markers on diabetic cataract and senile cataract

Stress markers	Diabetic cataract (n = 40)	Senile cataract (n= 75)	p- value
Catalase (ng/ml)	60.44±2.64	85.65±2.18	<0.001
Uric acid (mg/dl)	8.38±1.18	4.67±1.29	<0.001

Table 2: Demographic and biochemical characteristics of cataract patients

	Diabetic cataract (n = 40)	Senile cataract (n= 75)	p- value
Age (Years)	58±5.85	59±8.13	
Gender (male/female)	15/25	40/35	
Hypertension (yes/no)	12/28	25/50	
Current Smoker (yes/no)	8/32	13/62	
Duration of diabetes (years)	6±2.01	NA	
Fasting blood glucose (mg/dl)	145±2.80	85±2.60	<0.001
Post-prandial blood glucose (mg/dl)	215±3.28	114±2.53	<0.001
HbA1c (%)	7.9±0.7	NA	

DISCUSSION:

An imbalance between antioxidant defence systems and pro-oxidants results in the elevation of oxidative stress. This stress has been implicated in the progression of cataractogenesis **[23]**. Cataract is the major cause of blindness and visual impairment in the population around the world. Several studies have confirmed the presence of oxidative stress in ocular diseases, and ROS may also play an important role in the cataracts pathophysiology. A normal lens is well equipped with agents and systems of protection against oxidative stress. Over decades, chronic exposure to active forms of oxygen may lead to the gradual erosion of the antioxidant protective mechanisms present in the lens. This reduced activity is the main reason behind the susceptibility of the lens towards oxidative damages.

The reduction in the antioxidant properties is the main reason behind the lens susceptiblity towards oxidative damages. The cellular systems are composed of antioxidative enzymes such as XOD, SOD, CAT and glutathone peroxidase (GSH-Px), and antioxidant compounds such as vitamins (A, C, E) flavonoids, uric acid, bilirubin, etc. The present study investigated a relationship between an antioxidant enzyme catalase and an antioxidant compound uric acid in diabetic cataract as well as senile cataract. Catalase is an enzyme present in cellular compartment and catalyzes the decomposition of H_2O_2 into water and oxygen and act as 10^4 times faster than peroxidase **[24]**. The result of present study shows that significantly (<0.001) decreased activity of catalase in diabetic cataract patients as compared to senile cataract patients. Hasim *et al*; (2006) study reported that catalase activity significantly (<0.001) decreased in diabetic cataract patients as compared to senile cataract patients **[25]**. Considering the fact that during diabetes, the formation of reactive oxygen species and AGEs is greatly enhanced as compared to normal aging process. It is well-established that glycation not only affects long-lived proteins, but also influences shortlived molecules such as enzyme rendering them to be inactive. **Table 1** show the decrease in the activity is more evident in diabetic samples than that of senile samples. Antioxidant

enzymes are inactivated by glycation. In vitro studies have revealed that fructose inactivates CAT more quickly than glucose or its phosphate derivative **[26]**. Fructose is suggested to be ten times more potent glycating agent than glucose due to its acyclic form **[27]**. Considering the above facts, it may be postulated that high sugar level in diabetic subjects induces AGEs formation and hence inactivation of CAT. Ozmen *et al* (2002) also showed that catalase levels were significantly (<0.001) decreased in diabetic cataract patients as compared to senile cataract patients. Similarly, the decrease in Cu, Zn-SOD and catalase activities was more pronounced in diabetic cataract patients compared to senile cataract patients corresponding to glycation as a resut of hyperglycemia. Inactivation of these enzymes may result in the elevation of the H2O2 and O2 levels in the lens, and this may be responsible for the oxidative modification of lens protein. Sugars are reported to one of the well-known cataractogenic agents. Several reports suggested that the cataractogenic effect of the sugars in diabetes as well as in normal aging is initiated by the glycation of the proteins including the enzymes and subsequent formation of more complex and biologically inactive or harmful structures **[28]**. The study Chandrasena *et al*., (2006) demonstrated that the catalase activity is significantly decreased in senile cataract patients when compared with diabetic cataract.

Also, levels of uric acid is significantly (<0.001) higher in diabetic cataract patients as compated with senile cataract patients. Moreover, Hassan *et al*., (2001) also repoted that serum uric acid level was significantly increased in diabetic cataract patients compared with senile cataract ($p1<0.05$) synonomous to our study. This elevation of uric acid levels is due to the increased tissue protein catabolism conseuently causing hyperuricemia **[29]**. Furthermore, oxaloacetate was decreased in diabetic patients and therefore Kreb's cycle is inhibited. As a result, pyruvic acid was accumulated with subsequent increase in lactic acid. However, hyperlactacidemia leads to increase renal threshold level for uric acid clearance **[30]** (Yokogoshi & Saito, 1996). Madianov *et al*., (2000) reported that hyperuricemia in diabetic patients may be due to renal dysfunction and overproduction of uric acid **[31]**.

Although the occurrence of hyperuricemia in diabetic patients as a counteracting mechanism against oxidative damage **[32]** (Nieto *et al*., 2000), it has a lens capsular insult which is a possible pathophysiological explanation for cataract formation **[33]** (Beiran *et al*., 1994). The Hulya *et al*., showed that the levels of uric acid is significantly ($p<0.05$) lower in diabetic cataract patients as compared to senile

cataract patients. This is probably due to an increased fractional excretion of urate seen in diabetes patients **[34]**. Although usually recognised as a metabolic waste product, urate is a powerful antioxidant in vivo **[35]** and may be particularly important in diabetes where oxidative stress increases. Jitapunkul *et al* **[36]**, reported that the reduction of urate in patients with diabetes mellitus was associated with a significant elevation of protein glycation. This gives an assumption that urate might be involved in slowing the progress of protein glycation.

CONCLUSION:

Present study showed that there is decrease in the activity of catalase and elevation of uric acid levels in diabetic cataractous lenses patients than in senile catract patients. This may be due to increase in glycation resulting from hyperglycemia. Decrease activity of antioxidative enzymes causes oxidative stress, and therefore it can be suggested that antioxidants are helpful in combating oxidative stress. Therefore, antioxidant system augmentation can be a sound strategy to prevent or atleast delay the development of cataract.

Furthermore, it also proves that increase in blood glucose levels somewhat decreases the antioxidant capacity.

ACKNOWLEDGEMENT:

Authors pay gratitude to the Department of Biochemistry and Department of Opthalmology of Shri Guru Ram Rai Institute of Medical Health Sciences and Shri Mahant Indiresh Hospital (a tertiary care hospital), Dehradun, Uttarakhand.

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