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Arab Academy for Science, Technology, and Maritime Transport, AASTMT Abu Kir Campus, Alexandria, EGYPT
P.O. Box: Miami 1029
Tel: [+203] 5622366/88 – EXT 1069 and [+203] 5611818
Fax: [+203] 5611818
Web Site:http://apc.aast.edu

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Study of Level of Malondialdehyde and Total Antioxidant Capacity (TAC) in Human Lens Epithelial Cells of Diabetic and Senile Cataract Patients

Kranti Santosh Sorte Gawali[1], Avinash Namdeo Jadhao[2]

[1,2] Seth GSMC & KEMH Parel Mumbai, India.

Email: krantisorte@kem.edu, avinashjadhao@kem.edu

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Abstract:

Background: *Oxidative stress is a condition where the balance between the production and elimination of reactive oxygen species (ROS) is disturbed. ROS can cause damage to various biomolecules, including DNA. DNA damage can impair the function and survival of cells, and may contribute to the development of cataracts. Several experimental studies have shown that oxidative stress is involved in cataract formation by inducing DNA damage in lens cells. ROS are created in diabetic tissues by glucose autoxidation as well as non-enzymatic protein glycation. ROS is thought to play a significant role in the development of microvascular problems in diabetic individuals.*

Objectives: *To measure the levels of total antioxidant capacity and Malondialdehyde (MDA) in diabetic and nondiabetic senile cataract patients and comparison between them.*

Patients and methods: *Thirty senile cataract cases and 30 diabetic cataract cases aged 50-80 years who were brought to the ophthalmology ward of Acharya Vinoba Bhave Rural Hospital for cataract surgery were used for TAC assay and malondialdehyde determination in lens epithelial cells of diabetic and senile cataract.*

Results: *TAC was decreased in human lens epithelial cells of diabetic and senile cataract group. TAC is more reduced in diabetic than senile cataract group, it is not statistically significant. higher levels of MDA in human lens epithelial of Diabetic cataract patients as compared to senile cataract patients.*

Conclusion: *In the present study, diabetes may cause ocular complications by affecting the lens of the eye. This is because diabetic patients have lower levels of TAC and higher levels of Malondialdehyde in their lens than senile cataract patients. These factors may contribute to pathogenesis of diabetic cataract.*

Key words: *Total antioxidant capacity, Malondialdehyde (MDA), Diabetic cataract.*

1. Introduction

Cataract is a major cause of blindness and vision impairment worldwide. It is caused by the clouding or opacification of the normally clear lens and its capsule [1]. In 2020, it was estimated that cataracts were responsible for 40% of all global cases of blindness, affecting over 17 million people [2]. Congenital cataract, senile or age-related cataract, and cataract linked to systemic disease are the three categories that can be made based on the aetiology of the condition. Human cataracts have been linked to a number of risk factors, including ageing, diabetes, malnutrition, poverty, sunlight exposure, smoking, hypertension, and renal failure [3]. Most experts believe that cataract formation is a multi-factorial disease, and that oxidative stress may be one of the main contributing factors [4].

Oxidative stress is a major factor in the development of senile cataract. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the endogenous antioxidant system to neutralize them. ROS can damage the lens proteins, lipids, and DNA, leading to structural and functional changes that impair lens transparency [5]. The main sources of ROS in the lens are UV radiation, glucose metabolism, and inflammation. The main antioxidants in the lens are glutathione, superoxide dismutase, and catalase [6]. Several studies have shown that oxidative stress markers, such as lipid peroxidation products and protein carbonyls, are increased in cataract patients compared to healthy controls. Moreover, antioxidant levels and activities are decreased in cataract patients, indicating a reduced defence against oxidative damage

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[7]. Therefore, oxidative stress plays a crucial role in the pathogenesis of senile cataract and may be a potential target for prevention and treatment strategies.

ROS are created in diabetic tissues by glucose autoxidation as well as non-enzymatic protein glycation. ROS is thought to play a significant role in the development of microvascular problems in diabetic individuals [8]. The current study aims to compare the levels of total antioxidant capacity and Malondialdehyde (MDA) in diabetic and nondiabetic senile cataract patients.

2. Materials and Methods

Patients and methods

After an in-depth description of the nature and possible consequences of the study, participants provided informed consent. The "Institutional Ethics Committee" granted the letter (Datta Meghe Institute of Higher Education & Research (DMIHER) /IEC/2008-09/151 dated 30th May 2008).

Subject Selection

Thirty senile cataract cases and 30 diabetic cataract cases aged 50-80 years who were brought to the ophthalmology ward of Acharya Vinoba Bhave Rural Hospital for cataract surgery were used for TAC assay and malondialdehyde determination in lens epithelial cells of diabetic and senile cataract.

Subjects with senile cataracts had normal fasting blood glucose levels and no history of diabetes. The average age of diabetic cataract patients was 66.6 $\pm$ 8.3 years. The average age of senile cataract patients was 62.4 $\pm$ 10.1 years. According to the requirements of the local ethical committee, all participants were notified.

The existence of a renal disease, anaemia, autoimmune disorders, infections, hypothyroidism, hyperthyroidism, cirrhosis, cerebrovascular disease, malignant tumour, alcohol usage, and traumatic and toxic cataract were all exclusion criteria.

After detailed evaluation of senile cataract and diabetic cataract patients, participants provided a detailed visual history. Professors from the researchers' Institute's Ophthalmology Department recorded and validated preoperative confirmation of cataracts and their type.

Details of the procedure to remove the lens anterior capsule from patients with senile cataract are as follows: Cataract patients were operated on under local anaesthesia, with a 2ml 2% lignocaine injection administered through a clear corneal incision (2.75 mm in length, made with a 2.2-mm double-blade corneal knife), and 5.5 mm continuous curvilinear capsulorhexis performed with the assistance of capsulorhexis forceps and a 25-gauge needle. In all cases, the anterior capsule was retrieved with Visco expression through a clean corneal incision and collected with forceps by a skilled surgeon. To avoid any direct injury to the human lens epithelial cells (HLECs) no further handling or irrigation was performed.

After removing the anterior capsule, the sample was immediately placed in an Essential Medium (containing 10% foetal bovine serum) and delivered to the Research laboratory. A single rhexis was preserved in a Minimal Essential Medium (MEM) containing 10% foetal bovine serum and cultured at 37 °C in an incubator containing 5% carbon dioxide (Gajjar et al., 2008). The maximum time between collecting the sample and starting the process was 1520 minutes.

Testing for HLEC viability: Before beginning the comet assay, the trypan blue exclusion test was utilised to determine whether or not the human lens epithelial cells were viable.

Total Antioxidant Capacity (TAC) Estimation in HLEC Aqueous Extract Principle: The reduction of ferric tripyridyl triazine (Fe3+-TPTZ) complex to ferrous form at low pH yields a strong blue colour which is proportional to the overall reducing power of the electron-donating antioxidant present in the reaction mixture. The intensity of this blue colour was determined to be 593 nm. Estimation of Malondialdehyde (MDA) by Thiobarbituric acid assay (TBA) method.

Statistical analysis

The data for biochemical analyses were expressed as mean ±SD. Student-t test was used to determine the significance of biochemical parameters among the patient groups. P value of <0.05 was considered as significant.

Results

A total of 60 rhexis samples were collected from diabetic (30) and senile cataracts (30) patients, having mean ages of 63.9±6.6 (Mean ±SD) years for senile cataract and 55.4±5.3 (Mean + SD) years for diabetic cataract.

Male/female ratio was 16/14 for senile cataract and male/female ratio was for 21/9 for diabetic cataract patients as shown in Table 1.

Table: 1 Demographic characteristics of patients

	Senile Cataract (30 Subjects)	Diabetic Cataract (30 Subjects)
Age in Years	63.9±6.6	55.4±5.3
Gender (Male/ Female)	16/14	21/9
Fasting Blood glucose in mg/dl	97 ± 4.5	198 ± 19.4
HbA1C in percentage	4.94 ± 0.89	8.33 ± 1.04

Table 2: Malondialdehyde levels in senile cataract and diabetic cataract patients

Sample	Senile Cataract (30 Subjects)	Diabetic Cataract (30 Subjects)	
Human Lens epithelial cells	**11.15 ± 2.22**	**14.59 ±2.09**	**(0.0204*)**

Table 3: TAC levels in senile cataract and diabetic cataract patients

Sample	Senile Cataract (30 Subjects)	Diabetic Cataract (30 Subjects)	P value
Human Lens epithelial cells	**1.57±0.11**	**1.45± 0.24**	**0.7317**

The levels of MDA were measured by TRAB method in human lens epithelial cells of senile cataract and diabetic cataract patients. Data are expressed in µmol/ml and represent mean ± standard deviation. Student *t* test analysis was used as shown in Table 2. The levels of TAC were measured by TRAB method in human lens epithelial cells of senile cataract and diabetic cataract patients. Data are expressed in µmol/ml and represent mean ± standard deviation. Student *t* test analysis was used as shown in Table 3.

3. Discussion

Total antioxidant capacity (TAC) is a measure of the ability of a substance to neutralize free radicals and prevent oxidative damage [9]. To study non-enzymatic antioxidant parameters like retinoic acid, tocopherols, ascorbic acid, glutathione, and an enzymatic-like catalase enzyme activity, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx), all above parameters, assessment is time-consuming. To avoid the cumbersome analysis of the various non-enzymatic antioxidant and enzymatic antioxidants with a single test, the total antioxidant capacity (TAC) had been assayed directly in human LECs. Lens epithelial cells (LECs) are the cells that line the anterior surface of the lens and play a crucial role in maintaining its transparency and function [10]. In this study, the researchers compared the TAC of LECs from patients with diabetic cataract (DC) and senile cataract (SC), two common types of cataracts that affect vision quality and cause blindness [11]. They hypothesized that DC and SC would have different values of the TAC of LECs, and that lower TAC would be associated with higher oxidative stress and more severe cataract formation.-

Oxidative stress is a condition in which the balance between the production and elimination of free radicals is disrupted. Free radicals are highly reactive molecules that can damage cellular components such as DNA, proteins, and lipids. Oxidative stress has been implicated in the pathogenesis of many chronic diseases, such as cancer, inflammation, and neurological disorders. The eye is constantly exposed to various sources of oxidative stress, such as UV light, oxygen, and blinking, which can generate reactive oxygen species (ROS) that damage the ocular tissues [12]. ROS are involved in the development of many eye diseases, such as age-related macular degeneration (AMD), cataract, and diabetic retinopathy. The eye has several mechanisms to protect itself from ROS, such as antioxidant enzymes and pigments.

However, these defences can be overwhelmed by excessive or chronic oxidative stress. Therefore, it is important to understand the role of oxidative stress in the eye and how to prevent or treat its deleterious effects [13].

Diabetes mellitus (DM) is a major cause of vision loss in many people. One of the complications of diabetes that can impair vision is cataract, which can be treated by surgery. Cataract can occur in any age group, but it is more common in people with diabetes. The main factors that increase the risk of cataract in diabetes are high blood sugar levels and long duration of DM. Oxidative stress, which damages the cells of the lens, may be present in both diabetic and age-related cataracts.

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The development of diabetic cataract involves various mechanisms, such as nonenzymatic glycation of lens proteins, oxidative stress, and polyol pathway activation in glucose metabolism. The polyol pathway converts excess glucose into sorbitol by the enzyme aldose reductase. Sorbitol accumulation in the lens may increase the osmotic pressure and water volume, leading to lens swelling and fiber rupture. These events, along with the impaired function of the lens fiber's cation pump, may cause osmotic changes and opacification in the lens [11].

The researchers discovered an amazing finding about the levels of TAC and uric acid in the aqueous humor of people with diabetic cataracts. They compared these levels with those of people who had senile cataract without diabetes. They found out that the diabetic group had much lower TAC levels than the senile group [14]. This means that diabetes affects the antioxidant capacity of the eye and may contribute to cataract formation. Another study by Gul et al. confirmed that TAC is reduced in both diabetic and non-diabetic people who have cataract compared to healthy people [15]. However, in the present study TAC was decreased in human lens epithelial cells of diabetic and senile cataract group. TAC value was lower in diabetic cataract than senile cataract group, it is not statistically significant. These studies show how important it is to monitor and maintain the balance of TAC in the body, especially for patients with diabetes.

Several studies have measured MDA levels in the lens and plasma of patients with different types of cataracts, such as age-related, myopic, diabetic, and traumatic cataracts [16]. The results have shown that MDA levels are significantly higher in cataract patients than in healthy controls, and that MDA levels increase with the severity of cataract. Moreover, MDA levels have been correlated with other indicators of oxidative stress, such as catalase activity and superoxide dismutase activity. These findings suggest that MDA plays a role in the pathogenesis of cataracts and that MDA may be a useful biomarker for assessing the oxidative status of the lens. Malondialdehyde (MDA) is a product of lipid peroxidation that interacts with deoxyadenosine and deoxyguanosine in DNA to create DNA adducts. Its contribution to the development of cataracts is attributed to its ability to cross-link lens proteins and cause oxidative damage [17]. According to Yildirim et al., diabetic cataract patients had significantly higher levels of MDA activities than nondiabetic senile cataract patients [18]. This finding corresponds with this study, the higher levels of MDA in human lens epithelial of

diabetic cataract patients as compared to senile cataract patients.

4. Conclusion

Cataract is a common condition associated with aging and it can impair the vision of affected individuals. Therefore, it is essential to explore strategies to slow down the progression of cataract formation, even though it cannot be completely prevented. Oxidative stress is a major factor that contributes to both senile and diabetic cataracts. This study demonstrated that the levels of MDA, a marker of oxidative damage, were increased and the levels of TAC, a measure of antioxidant capacity, were decreased lens epithelial cells of both senile and diabetic cataract patients. However, diabetic patients experienced more oxidative stress at an earlier age than senile patients. Thus, the researchers suggest that providing adequate antioxidant supplementation to cataract patients at an early stage may help delay the

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