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A STUDY OF GLYCOSYLATED HAEMOGLOBIN AND LIPID PEROXIDATION IN SENILE CATARACT PATIENTS WITH AND WITHOUT DIABETES MELLITUS IN A TERTIARY CARE HOSPITAL.

Biochemistry

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ABSTRACT

INTRODUCTION: Cataract associated blindness is a major cause of morbidity in developing countries. Oxidative stress leads to lipid peroxidation. One such product, malondialdehyde (MDA).Its role in cataractogenesis is due to its cross linking ability. Oxidative damage to the lens most likely arises as a consequence of an impaired antioxidant defence system, due to increased generation of ROS both by age and diabetes.

MATERIALS AND METHODS: The level of MDA & Glycosylated hemoglobin was measured in 30 randomly selected senile diabetic and 30 senile non diabetic cataract patients and matched against 30 suitable controls.

RESULTS: The study found statistically significant increased levels of MDA in senile diabetic cataract patients ($p<0.0001$) and increased Glycated hemoglobin in senile diabetic cataract patients ($p<0.0001$).

CONCLUSION: The present study concluded that oxidative stress plays role in formation of cataract in diabetics.

KEYWORDS

Malondialdehyde,cataractogenesis, oxidative stress.

INTRODUCTION

Cataract is characterized by partial or complete loss of transparency of the lens that leads to loss of vision. Globally, cataract associated blindness is a major cause of morbidity in developing countries. During the process of cataract formation, there is univalent reduction of oxygen which leads to free radical formation. Oxidative stress is a term used to describe any challenge in which pro-oxidants predominate over antioxidants; it may however be due to either increased production of reactive oxygen species (ROS) or decrease in the levels of antioxidants (enzymatic and non -enzymatic) or both.

Oxidative stress leads to lipid peroxidation. The end products of lipid peroxidation are both mutagenic and carcinogenic. One such product, malondialdehyde (MDA) reacts with deoxyadenosine and deoxyguanosine in DNA, to form DNA adducts. Its role in cataractogenesis is due to its cross linking ability. Changes in the oxidation of biomolecules can also be found in many human diseases, but cataract is one of the most common diseases, where oxidative modifications of proteins and lipids have a predominant metabolic substrate of pathological disorder. Oxidative modification of lens proteins, loss of protein function and the creation of protein aggregates of high molecular mass, are the main features of age-related cataract. Hyperglycemia, seen in diabetic cataract can stimulate oxidative stress by the process of auto oxidation of glucose in the presence of transition metals. Hence malondialdehyde (MDA) is measured to assess the level of oxidative stress in senile and diabetic cataracts.

AIMS AND OBJECTIVES

1) To estimate the levels of Glycosylated/ Glycated hemoglobin (HbA1c %) in the blood of cataract patients with and without diabetes mellitus and in controls.
2) Measurement of lipid peroxidation marker i.e. Malondialdehyde (MDA) in the serum samples of cataract patients with and without diabetes mellitus and in controls.
3) To find out the possible correlation of the results with routine parameters under study in cataract patients with and without diabetes.

Scheme of study

- The study was carried out in the Regional Institute of Ophthalmology, Gauhati Medical College & Hospital, during a period of one year (August, 2017 - July, 2018) after obtaining clearance from Institutional Ethical Committee.
- Prior informed consent was obtained from all the subjects under study.
- Cases both of senile diabetic and non- diabetic cataracts & equal number of age and sex matched subjects served as controls.
- Evaluation of malondialdehyde and HbA1c% done in subjects of both groups.
- Statistical evaluation will be done using GraphPad InStat Version 3.00 and one way ANOVA, Tukey- Kramer multiple comparisons test and Pearson's correlation co- efficient.

MATERIALS AND METHODS

In the present study, 3 groups of subjects of either sex in the age group of 51-80 years who attended the RIO, GMCH during the period from August 2017- July 2018 were analysed. The subjects were divided into 3 groups as follows:

I) 30 patients with Type 2 Diabetes with cataract in either eye or both the eyes constituted Group I
II) 30 non- diabetic patients with cataract in either eye or both eyes constituted Group II
III) 30 subjects of either sex in the age group of 51-80 years without diabetes and cataract were taken as control

Inclusion Criteria:

1) Patients diagnosed as a case of senile cataract.
2) Age: 51-80 years (both inclusive).
3) Sex: Both sexes were included.
4) Subjects without any history of cataract and diabetes who visited RIO, GMCH for other conditions like pterygium, ectropion, entropion etc; taken as controls.

In Group I where diabetic cataracts were included the following criteria were adopted along with the above mentioned inclusion criteria:

- HbA1c≥ 6.5%. This test was performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay OR
- FBS ≥ 126 mg/dl (7.0mmol/L). Fasting was defined as no caloric intake for at-least 8h OR
- 2 h plasma glucose ≥ 200 mg/ dl (11.1 mmol/ L) during an OGTT. The test was performed as described by the WHO, using a glucose load containing the equivalent of 75gm anhydrous glucose dissolved in water OR
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random blood glucose ≥ 200 mg/dl (11.1 mmol/L)

Exclusion criteria:

1) Not willing to participate in the study.
2) Cataract due to other secondary aetiology like trauma, radiation therapy etc.
3) Age: subjects below 51 years and above 80 years.

4) Patients with known history of cardiovascular disorders, hypertension, rheumatoid arthritis, ocular surgery, trauma, infection, inflammation of the eye, carcinomas and smokers where free radical damage has been incriminated were excluded from the study.
5) Acute or chronic diarrhoea, patients of acute or chronic renal failure.
6) Patients with history of intake of drugs like steroids, chemotherapeutic agents, antipsychotics and other antioxidants.

- **ESTIMATION OF MALONDIADEHYDE IN SERUM**: Malondialdehyde was assessed using QUANTICHROM™ TBARS Assay Kit (Bioassay, USA), and was done in Erba Mannheim Lisa Scan EM (ELISA microplate reader).
- **ESTIMATION OF GLYCOSYLATED HEMOGLOBIN IN PLASMA:** Glycosylated haemoglobin (HbA1c %) was measured in Autoanalyzer, VITROS 5600.
- **STATISTICAL ANALYSIS:** One way Analysis of Variance (ANOVA) test used to analyze differences in baseline characteristics between the studied groups. Tukey- Kramer Multiple Comparisons Test.
- Correlations were observed using Pearson's correlation co-efficient. The results were considered significant when the probability (p value) was less than 0.05. Statistical analysis was done using Graph Pad InStat Version 3.00. All the statistical graphs were prepared using Microsoft Excel.

RESULTS AND OBSERVATIONS

Table 1.1: Mean age of controls and cases.

	Controls (mean± SD)	Group I (mean ± SD)	Group II (mean± SD)	p value
Mean Age (years)	63.73± 6.97	59.76± 4.27	64.3± 6.65	0.0093**

** considered significant

Comparison of the mean ages in the three study groups. Values expressed as Mean± SD. One way ANOVA used for comparison of means between the groups

Table 1.2 Age wise frequency distribution in the study groups

Category	Age group in years	Frequency	Relative frequency
Controls	51-60	10	0.33
	61-70	15	0.50
	71-80	5	0.17
Group I	51-60	18	0.6
	61-70	11	0.37
	71-80	1	0.03
Group II	51-60	11	0.37
	61-70	13	0.43
	71-80	6	0.20

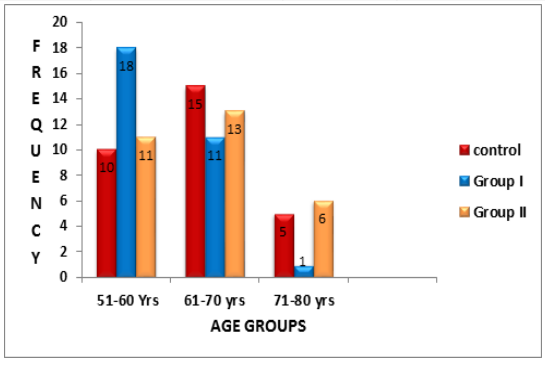

Figure 1.1: Age wise frequency distribution in the study groups.

Table 1.3 Means of MDA and HbA1c in study groups:

Parameter	Control (mean± SD)	Group I (mean± SD)	Group II (mean± SD)	p value
MDA	1.94±0.36	4.17±1.07	3.28±0.28	<0.0001***
HbA1c	5.19±0.45	8.14±1.9	5.14±0.67	<0.0001***
FBS	87.7±11.45	201.70±38.99	87.60±11.29	<0.0001***

*** considered highly significant

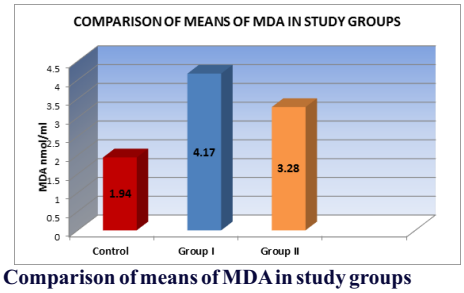

Fig 1.2 Comparison of means of MDA in study groups

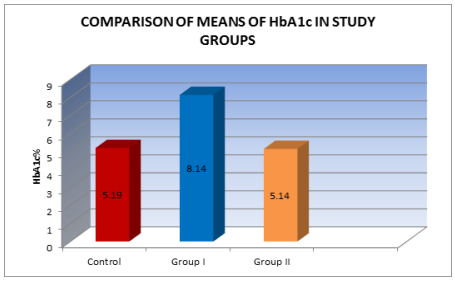

Fig 1.3 Comparison of the means of Glycated hemoglobin (HbA1c

TABLE 1.4: CORRELATION OF MALONDIALDEHYDE WITH OTHER BIOCHEMICAL PARAMETERS IN GROUP I.

Parameters	Malondialdehyde	
	r value	p value
Age	-0.1706	0.367 ns
BMI	-0.097	0.607 ns
W: H	0.023	0.901 ns
Duration of diabetes	0.544	0.0019**
FBS	0.612	0.0003**
HbA1c	0.719	<0.0001***

ns – not significant, ** considered highly significant, *** considered highly significant.

TABLE 1.5: CORRELATION OF MDA WITH OTHER PARAMETERS IN GROUP II

Parameter	Malondialdehyde	
	r value	p value
Age	-0.176	0.351ns
BMI	-0.042	0.821ns
W:H	-0.082	0.665ns
FBS	0.154	0.4176ns
HbA1c	0.28	0.123ns

ns -not significant.

DISCUSSION

The present study was conducted to assess oxidative stress parameters in senile cataract patients with and without diabetes in the age group 51-80 years in Gauhati Medical College & Hospital. In the present study it has been found that in cases of the patients presenting with cataract with diabetes(Group I) the mean age was 59.76±4.27 , while in Group II which included patients with only cataract the mean age found to be 64.3±6.65(Table 1.1). **Deepa. K, Manjunatha Goud B.K et al** (2011) in their study found the mean age of Group I was 60.36±7.46 and Group II to be 66.72±7.329 and found the difference of means between the Group I and Group II statistically significant ($p<0.01$).[6]This also corroborates with the study of **Bhatia G. et al** (2017) where they found the mean of Group I to be 60.36±7.46 while that in Group II was found to be 66.72±7.33 years.[7]

Malondialdehyde is a major end product of the free radical reaction on the membrane fatty acids.[8] In the present study the mean value of MDA in the patients with diabetic cataract (Group I) was found to be 4.17 nmol/ml (Table 1.3) and the value was found to be statistically significant when compared with Group II and control group ($p<0.0001$) (Table 1.4) and this corroborates with the study done by **Deepa K et al**. (2011)[6] where they compared the mean of MDA between two study groups; senile cataract and senile diabetic cataract on 25 patients in each group and found a highly significant p value (<0.0001). **Bhatia G et al.** (2017) in their study done on 135 subjects

which was divided into three groups aged between 50-75 years found a significant increase in serum MDA levels in diabetic ($p < 0.001$) and senile cataract patients.[7] Results of the present study indicated that raised MDA levels may be involved in the development of cataract associated with diabetes. This finding is supported by study done by **Fahad Alwadani et al** (2016)[9] where they found similar association.

In the present study, the serum Malondialdehyde values were positively correlated with FBG values in senile diabetic cataract patients i.e. Group I ($r=0.612$ and $p=0.0002$) (Table 1.5). In a population based longitudinal study done by **Tan AG et al.** (2008) they found that patients with baseline fasting glucose or with diabetes, had an increased risk of developing cataract.[10] The findings of our present study highlight the harmful effect of glucose on lens which has also been previously demonstrated by **Rowe N Et al.** (2000) in their studies.[11] The hyperglycaemic conditions in the presence of oxidative stress have seen to have more adverse effect on the lens.

Our correlation study revealed a significant positive association of serum MDA levels with HbA1c ($r=0.719$, $p<0.0001$) (Table 1.5) which tallies with the findings of **Desai V et al**. (2011)[12] where they found significant positive association between MDA levels and HbA1c ($r=0.277$, $p<0.001$).The increase in the lipid peroxides in the blood coupled with poor glycemic control in diabetics probably serves a background for the pathogenesis for cataract formation in diabetic cases.

Also there was significant positive correlation between the levels of MDA and the duration of diabetes. ($r=0.544$, $p=0.0019$). These corroborates with the findings of **Desai Vet al.**[13]

CONCLUSION

In this study an attempt was made to find the correlation between serum MDA levels FBS & HbA1c levels in senile diabetic cataract and senile cataract patients. There was a significant positive correlation of serum MDA levels with FBS and HbA1c. No correlation of MDA has been found with the parameters measured in case of senile cataracts.

The present study supported the hypothesis that hyperglycemia activates cellular and tissue injury by oxidative stress but the compensatory mechanisms for defense against the Reactive oxygen species to normalize oxidative stress is not achieved in diabetic patients. Moreover, this oxidative stress increased with poor glycemic control. As a result, patients with hyperglycemia and oxidative stress present a high risk for development of diabetic complications and thereby need early intervention.

In this study an attempt was made to find the correlation between serum Malondialdehyde (MDA) levels and serum Fasting blood glucose & HbA1c levels in senile diabetic cataract and senile cataract patients. There was a significant positive correlation of serum MDA levels with FBS and HbA1c. No correlation of MDA has been found with the parameters measured in case of senile cataracts.

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