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Original Research Article

Estimation of serum MDA (Malondialdehyde) in various morphological types and clinical stages of age related (senile cataract)

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ABSTRACT

Background: The aim was to estimate the Serum Malondialdehyde (MDA) in the various morphological types and clinical stages of Age Related (Senile) Cataract, a common cause of curable blindness, especially in India.
Methods: This was a study of estimation of serum Malondialdehyde (MDA) in 100 patients of Age Related (Senile) cataract with 80 cases of Senile Cortical type (40 cases of Posterior subcapsular and 40 cases of Mature) and 20 cases of Senile Nuclear type (10 cases of early Grades of I and II and 10 cases of Advanced Grades of III and IV) and in 20 cases of normal controls in the age group of 45 to 70 years.
Results: Serum MDA (nmol/ml) values of the Mean with SD were 3.34±0.93 (Range 2.02-4.65) in normal controls, 4.38±1.01 (Range 2.4 - 6.7) in senile cataract, 4.39±1.02 ((Range 2.4-6.7) in Senile Cortical Cataract with 3.82±0.73 (Range 2.4-5.1) in its Posterior Subcapsular stage and 4.96 ± 0.88 (Range 3.3-6.7) in its mature stage and 4.3±0.99 (Range 2.4-6.2) in Senile Nuclear cataract with 3.68±0.66 (Range 2.4-4.7) in its early Grades of I and II and 4.86±0.94 (Range 3.8-6.2) in its Advanced Grades of III and IV.
Conclusions: The present study showed a significant increased concentration of Serum MDA in Senile cataract especially in the advanced later clinical stages or grades of maturity than in the earlier clinical stages or grades of immaturity of senile cortical or nuclear compared to normal controls. And there was no statistically significant difference between the morphological types of Age related (Senile) cataract i.e. Cortical Cataract compared to Nuclear Cataract.

Keywords: Age related (senile) cataract, Posterior subcapsular (PSC), Serum malondialdehyde, Senile cortical, Senile nuclear

INTRODUCTION

Cataract is the leading cause of reversible curable blindness in the world today and the major cause of visual impairment leading eventually to blindness especially in developing countries like India. Age related (Senile) cataract is the most common type of acquired cataract and usually some degree of cataract is common after the age of 50 years. Cataract is an opacification of the human lens of sufficient severity to cause vision impairment.

Oxidative stress is a term used when oxidants predominate over antioxidants which results from an imbalance between the oxidants and the cellular antioxidant defense mechanisms due to either increased production of oxidants or decreased levels of antioxidants (enzymatic and nonenzymatic) or both.[1] Oxidative stress

is implicated to play a crucial role not only in the etio-pathogenesis of many diseases like Cancer, Diabetes mellitus but also in the development of age related cataract.[2,3] Oxidation initiates the sequence of events leading to cataract and the most important oxidants are free radicals like the reactive oxygen species (ROS) which initiate toxic biochemical reactions like peroxidation of cell membrane lipids with extensive damage to proteins causing intracellular protein precipitation.[4-14]

The main problems in studying the role of oxidants in human cataract formation are the inability to measure directly these oxidants in the lens in vivo and the difficulty in getting the normal human lenses to be used as controls.[15,16] So, many investigators used plasma or serum or erythrocyte to evaluate oxidant status in the patients of cataract and the normal controls.[15-20] One of the byproducts and index of lipid peroxidation is the toxic malondialdehyde (MDA), which is implicated in the development of the senile cataract manly due to its cross-linking ability.[21,22] Malondialdehyde (MDA) evidenced by the formation of thiobarbituric acid reactive substances (TBARS), was determined in serum.[23] The present study estimated the serum Malondialdehyde (MDA) in the form of thiobarbituric acid reactive substances (TBARS), in various morphological types and clinical stages, namely cortical, Posterior Subcapsualr (PSC) and nuclear cataracts of age related (senile) cataract and also in the age and sex matched normal controls.

The aim was to estimate the Serum Malondialdehyde (MDA) in the various morphological types and clinical stages of Age Related (Senile) Cataract, a common cause of curable blindness, especially in India.

METHODS

This study was conducted in the Depts. of Biochemistry and Ophthalmology, SV Medical College (Govt.) and SVRR govt. General Hospital, Tirupati, AP, India in 100 patients of age related (senile) Cataract and 20 normal controls in the age group of 45 to 70 years. Informed consent was taken from all the patients of senile cataract and normal controls. The study was approved by the institute ethical committee. Patients with a previous medical history of ocular conditions of surgery, trauma, infection and inflammation, metabolic or chronic systemic diseases like diabetes mellitus, cardiovascular disorders, rheumatoid arthritis and carcinomas were excluded from the study of the both the groups of normal controls and senile cataract cases.

A provisional clinical diagnosis of age related cataract, its morphological types and clinical stages was done by complete ocular examination (visual acuity, slit lamp examination, direct and indirect ophthalmoscopy). Patients, matched by sex and age, were divided in two groups: with lens opacities and without lens opacities. The Lens Opacities Classification System, Verso III (LOCS III) was used for grading lens opacities.[24] The basic types of age related cataract were mainly cortical and nuclear. Only pure cortical and pure nuclear cases were taken in the study excluding the mixed varieties of cataracts on the basis of the examination. Only those cases with a single type of opacity in both eyes were placed in the pure category.

There were 80 patients in pure cortical group (40 of Posterior Subcapsular stage and 40 of Advanced Mature stage) and 20 patients in pure nuclear group (10 of early Grades of I and II and 10 of Advanced Nuclear Grades of II and IV). Control group comprised of 20 persons with a visual acuity of 6/6 in both eyes without lens opacities in either eye.

The most frequently used technique to assess the oxidative stress is the estimation of the Lipid Per-Oxidation Product – Serum Malondialdehyde (MDA) in the form of Thiobarbituric acid reactive substances (TBARS) according to the method of YAGI.[25]

Principle

The lipid in the cell membranes, highly susceptible to oxidative damage is broken down into a number of units to form Malondialdehyde which reacts with Thiobarbituric acid (TBA) to form Thiobarburic acid reactive substances (TBARS), which has a pink color with absorption in Spectrophotometry at 535 nm wavelength. A molecule of Malondialdehyde reacts with two molecules of Thiobarbituric acid. So, the principle mainly depends on the measurement of the pink colour produced by the interaction of TBA with MDA in spectrophotometry at 532 nm wavelength.

Samples

10 ml of fasting blood sample of age related (senile) Cataract in the morning was drawn from a peripheral vein especially from the antecubital vein. For the separation of the serum, 5 ml of blood was taken into a plain vial first and then allowed to clot. Then this clotted blood was centrifuged at 3000 rpm for 5 minutes. This separated serum was used to estimate the serum MDA on the same day. 1 ml of blood was taken into a fluoride vial to estimate blood glucose to exclude diabetes mellitus.

Reagents

- 20% Trichloroacetic acid
- TBA Agent (200 mg of Thiobarbituric acid in 30 ml distilled water and 30 ml of Acetic acid
- N-Butanol
- Normal Saline (0.9% NaCl)

Standards

1,1,3,3 Tetramethoxy Propane

Stock standard

0.1 ml or 100 micro-liters of concentrated1,1,3,3 Tetramethoxypropane is taken to made up to 10.1 ml with Ethanol.

Working standard

0.1 or 100 microliters of stock standard solution is taken to made up to 10.1 ml with Ethanol. From that different solutions are made for different concentrations.

Technique

To each test tube 0.5 ml of serum, 0.5 ml normal saline, 1 ml of 20% Trichloroacetic Acid(TCA) and 0.25 ml of TBA reagent was added. The tubes were kept in boiling at 95 degrees centigrade for 1 hour. To the contents of the tube, 3 ml of N-Butanol was added and mixed well for 15 minutes.

After extraction, the tubes were centrifuged at 3000 rpm for 10 minutes and the separated Butanol layer was collected and measured against a reagent blank Spectrophotometry at the 535 nm wavelength. Lipid peroxide concentration is expressed in terms of nanomoles of Malondialdehyde (MDA) per ml (nmol/ml) of serum.

RESULTS

100 cases of Age Related(Senile) cataract with 80 cases of Senile Cortical type (40 Posterior Subcapsualr and 40 Advanced Mature) and 20 cases of Senile Nuclear type (10 cases of early Grades of I and II and 10 cases of advanced Grades of III and IV) and 20 cases of normal controls in the age group of 45-70 years were evaluated using the simple statistical methods. Serum Malondialdehyde (MDA) was measured in nmol/ml and all the results were expressed with Mean ±SD.

Table 1: Distribution of study groups with serum MDA values of range and mean with SD.

Study group	No.	Range	Mean±SD
Controls	20	2.02-4.65	3.34±0.93
Senile	100	2.4-6.7	4.38±1.01
Senile cortical	80	2.4-6.7	4.39±1.02
Posterior subcapsular	40	2.4-5.1	3.82±0.73
Mature	40	3.3-6.7	4.96±0.88
Senile nuclear	20	2.4-6.2	4.3±0.99
Early	10	2.4-4.7	3.68±0.66
Advanced	10	3.8-6.2	4.86±0.94

Table 1 shows the Serum MDA (nmol/ml) values of the Mean with SD, as 3.34±0.93 (range 2.024.65) in 20 cases of normal controls, 4.38±1.01 (range 2.4-6.7) in 100 cases of senile cataract, 4.39±1.02 (range 2.4-6.7) in 80 cases of senile cortical cataract with 3.82±0.73 (range 2.4-5.1) in its 40 cases of posterior subcapsular stage and 4.96±0.88 (range 3.3-6.7) in its 40 cases of mature stage and 4.3±0.99 (range 2.4-6.2) in 20 cases of senile nuclear cataract with 3.68±0.66 (range 2.4-4.7) in its 10 cases of early Grades of I and II and 4.86±0.94 (Range 3.8-6.2) in its 10 cases of advanced Grades of III and IV.

Table 2: Serum MDA (nmol/ml) in controls versus cases.

Parameter	Controls	Senile cataract
No.	20	100
Range	2.02-4.65	2.4-6.7
Mean with SD	3.34±0.93	4.38±1.01

Table 2 of the serum MDA (nmol/ml) values of the mean with SD in controls v/s cases shows 3.34±0.93 (range 2.02 - 4.65) in 20 cases of normal controls and 4.38±1.01 (range 2.4-6.7) in 100 cases of senile cataract.

Table 3: Serum MDA in controls versus morphological types of senile cataract.

Parameter	Controls	Senile Cortical	Senile Nuclear
No.	20	80	20
Range	2.02-4.65	2.4-6.7	2.4-6.2
Mean with SD	3.34±0.93	4.39±1.02	4.3±0.99

Table 3 of the serum MDA (nmol/ml) values of the mean with SD in controls v/s morphological types of Senile Cataract shows 3.34±0.93 (range 2.02-4.65) in 20 cases of normal controls, 4.39±1.02 (range 2.4-6.7) in 80 cases of senile cortical cataract and 4.3±0.99 (range 2.4-6.2) in 20 cases of senile nuclear cataract.

Table 4: Serum MDA in clinical stages of senile cortical v/s clinical grades of nuclear cataract.

Parameter	Senile cataract (n=100)			
	Cortical (n=80)		Nuclear (n=20)	
	Posterior subcapsular	Mature	Early	Advanced
No	40	40	10	10
Range	2.4-5.1	3.3-6.7	2.4-4.7	3.8-6.2
Mean with SD	3.82 ±0.73	4.96 ±0.88	3.68 ±0.66	4.86 ±0.94

Table 4 of the Serum MDA (nmol/ml) values of the mean with SD in clinical stages of senile cortical v/s clinical grades of nuclear cataract shows 3.82±0.73 (Range 2.4-5.1) in its 40 cases of posterior subcapsular stage and 4.96±0.88 (range 3.3-6.7) in its 40 cases of mature stage of cortical type and 3.68±0.66 (range 2.4-4.7) in its 10 cases of early Grades of I and II and 4.86±0.94 (range 3.8=6.2) in its 10 cases of advanced Grades of III and IV of nuclear type. Table 5 of the serum MDA (nmol/ml) values of the Mean with SD in morphological and clinical

types of senile cataract V/s controls shows 3.34±0.93 of normal controls, 4.38±1.01 of senile cataract, 4.39±1.02 of senile cortical cataract with 3.82±0.73 in its posterior Subcapsular stage and 4.96±0.88 in its mature stage and 4.3±0.99 in senile nuclear cataract with 3.68±0.66 in its early grades of I and II and 4.86±0.94 in its advanced Grades of III and IV.

Table 5: Serum MDA in morphological and clinical types of senile cataract v/s controls.

MDA	Controls	Cataract	Cortical	Cortical immature	Cortical mature	Nuclear	Nuclear early	Nuclear advanced
Mean	3.34	4.38	4.39	3.82	4.96	4.3	3.68	4.86
SD	0.93	1.01	1.02	0.73	0.88	0.99	0.66	0.94

Table 6: Values of t and P of serum MDA in senile cataract v/s controls.

MDA	Controls	Cataract	t value	P value	Remarks
Mean	3.34	4.38	4.3	<0.001	Significant
SD	0.93	1.01			

Table 6 of values of t and P of Serum MDA in Senile Cataract V/s Controls shows the t value of 4.3 and P value of <0.001.

Table 7 of Values of t and P of Serum MDA in clinical stages of Senile Cortical Cataract shows t value of 4.3 and P value of <0.001.

Table 7: Values of t and P of serum MDA in clinical stages of senile cortical cataract.

MDA	Cortical	Posterior subcapsular	Cortical mature	t value	P value	Remarks
Mean	4.39	3.82	4.96	4.3	<0.001	Significant
SD	1.02	0.73	0.88			

Table 8: Values of t and P of serum MDA in clinical grades of senile nuclear cataract.

MDA	Nuclear	Early nuclear	Advanced nuclear	t value	P value	Remarks
Mean	4.3	3.68	4.86	3.3	<0.001	Significant
SD	0.99	0.66	0.94			

Table 8 of Values of t and P of Serum MDA in Clinical grades of Senile Nuclear Cataract shows the t Value of 3.3 and P value of <0.001.

So, the Serum MDA (nmol/ml) values of the Mean with SD were

- Normal controls 3.34±0.93 (Range 2.02-4.65).
- Senile Cataract 4.38±1.01 (Range 2.4-6.7).
- Senile Cortical Cataract 4.39±1.02 ((Range 2.4-6.7) with 3.82±0.73(Range 2.4-5.1) in its Posterior Subcapsular stage and 4.96±0.88 (Range 3.3-6.7) in its mature stage.
- Senile Nuclear cataract 4.3 ±0.99 (Range 2.4-6.2) with 3.68±0.66 (Range 2.4 -4.7) in its early Grades I and II and 4.86±0.94 (Range 3.8-6.2) in its Advanced Grades III and IV.The serum MDA Values of Means with SD were compared statistically between
  - Normal Controls V/s Senile cataract as in Table 2.
  - Controls V/s Morphological Types of Senile Cataract i.e. Cortical and Nuclear Cataract as in Table 3.

Senile Cortical Posterior Subcapsular V/s Mature stages as in Table 4.

Senile Nuclear early Grades 1 and II V/s Advanced III and IV as in Table 4.

Controls V/s Morphological and Clinical Types of Senile Cataract as in Table 5.

Observations

There was a significant increase of Serum MDA in

- Senile cataract compared to Normal Controls as in Table 2.
- Senile Cortical Advanced Mature stage compared to early Posterior Subcapsular as in Table 4.
- Senile Nuclear Advanced Grades of III and IV compared to early Grades of 1 and II as in Table 4.
- Later Clinical Stages or Grades of Maturity than in Earlier Clinical Stages or Grades of Immaturity of Senile Cortical or Nuclear cataract as in Table 5.

There was no statistically significant difference of Serum MDA between the morphological types of Age related (Senile) cataract i.e. Cortical compared to Nuclear Cataract as in Table 3.

Values of t and P of Serum MDA were significant in Senile Cataract V/s Controls as in Table 6.

Clinical stages of Senile Cortical Cataract i.e. Early Posterior Subcapsualr and Advanced Mature as in Table 7. Clinical grades of Senile Nuclear Cataract i.e. Early Grades 1 and II and Advanced III and IV as in Table 8.

DISCUSSION

Age related (Senile) cataract is not only the most common type of acquired Cataract, but also the most common cause of curable blindness in the developing nations. A normal lens is well protected with agents and systems to overcome oxidative stress. But, continuous exposure over the decades to active forms of oxygen makes the lens susceptible to oxidative damage with depletion of the major antioxidant protective mechanisms of the lens (GSH and ascorbic acid), which results in accumulation of oxidized residues in the lens proteins and enzymes with a loss of normal metabolic function and derangement of the organization of the normal intracellular protein causing loss of transparency of the lens with cataract formation.[26-28] MDA is one of the by-products of lipid peroxidation and the lens MDA may be the result of lipid peroxidation of the lens cells membranes.

In present studyof 100 cases of age related (senile) cataract with 80 cases of senile cortical type (40 cases of posterior subcapsular and 40 cases of advanced mature) and 20 cases of senile nuclear type (10 cases of Grades I and II and 10 cases of Grades III and IV) and 20 cases of normal controls in the age group of 45 to 70 years, there was a statistically significant increased Serum MDA values of Mean with SD in senile cataract (4.38±1.01), especially in the later clinical stages or grades of maturity than in the earlier clinical stages or grades of immaturity when compared to normal controls (3.34±0.93) i.e. in Senile Cortical Cataract (4.39±1.02) with stage of Posterior Subcapsular (3.82±0.73) and advanced stage of maturity (4.96±0.88l) and in Senile Nuclear cataract (4.3±0.99) with early Grades of I and II (3.68±0.66)and advanced Grades of III and IV (4.86±0.94). There was no statistically significant difference between the morphological types of Age related (Senile) cataract i.e. Cortical Cataract (4.39±1.02) compared to Nuclear Cataract (4.3±0.99).

So, present study showed significantly increased levels of serum MDA in all the morphological and clinical stages of senile cataract especially in the later stages or grades of maturity than in the earlier clinical stages or grades of immaturity compared to the normal Controls, which may be due to its utilization by counteracting oxygen free radicals or due to its oxidation by O_2.

In the study of Pradhan AK et al, of 190 patients and 78 of normal controls in the age group of 50-80 years, Thiobarbituric acid reactive substances (TBARS), the indicator of lipid peroxidation (LPO) were significantly elevated in cortical, posterior subcapsular (PSC) and nuclear types of cataract when compared with control group i.e serum MDA (nmol/ml) values of Mean with SD was significantly higher in 190 patients of senile cataract with 40 of nuclear (2.81±0.638), 77 of PSC (2.94 ±0.685) and 73 of cortical (3.08±0.594) when compared to 78 normal controls (2.22±0.640).[29] No significant changes of parameters were seen among the different morphological types of age related cataract.

In the study of Cekic et al, the Serum MDA (umol/L) values of Mean with SD was significantly increased in 38 patients of senile cataract (20.24±8.12), with 16 patients of nuclear (17.39±2.32), 9 patients of posterior subcapsular (17.51±3.72) and 13 patients of cortical (17.22±2.27) compared to 38 normal controls (8.73±2.53), but, there was no statistical significance in the concentration of Serum MDA values of Mean with SD among patients of different types of age related cataract i.e. 16 patients of nuclear (17.39±2.32), 9 patients of posterior subcapsular (17.51±3.72) and 13 patients of cortical (17.22±2.27).[30]

In the study of Ates NA et al, the serum MDA (nmol/ml)) values of Mean with SD was significantly higher in 40 patients of senile cataract (4.47±0.35) as compared to 60 normal controls (2.94±0.26), but there was no statistical significant difference in the concentration of serum MDA between different subgroups of senile cataract.[31] In the study of Katta AV et al, serum MDA (nmol/ml) values of Mean with SD was significantly higher in 60 patients of senile cataract with 30 cases of nuclear (5.14±1.0) and 30 cases of cortical (3.95±0.71) when compared with 30 normal controls (2.92±0.50).[32]

Present study and the above studies showed that the increase in the level of serum MDA is directly related to the progression of the senile cataract.[7]

CONCLUSION

The present study showed a significant increased concentration of serum MDA in senile cataract compared to normal controls, in senile cortical advanced mature

stage compared to early Posterior Subcapsular and in Senile Nuclear Advanced Grades of III and IV compared to early Grades of 1 and II i.e. later clinical stages or grades of maturity showed increased serum MDA than in the earlier clinical stages or grades of immaturity of senile cortical or nuclear cataract and there was no statistically significant difference between the morphological types of age related (senile) cataract i.e. cortical cataract compared to nuclear cataract. The present study showed the statistically significant increased levels of serum MDA in the senile cataract with considerable interest to determine that if some measures are undertaken to slow and delay the development of the cataract by some years, it may enhance the quality of older aged people by reducing the prevalence of cataract with economic burden due to cataract disability and surgery.

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