Journal of Molecular Biomarkers & Diagnosis
ISSN: 2155-9929

El-Sayyad et al., J Mol Biomark Diagn 2017, 8:2
DOI: 10.4172/2155-9929.1000318

Research Article Open Access

Biochemical and Molecular Markers of Congenital and Senile Cataractous Lenses

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Abstract

Background: Cataractous lenses represent one of the main public health problem involved in impairing vision. Different multiactorial agents are involved in its development.

Material and methods: In this study, 15 congenital and 56 senile cataractous lenses obtained post-operative surgery from patients admitted to Ophthalmic Center, Mansoura University Hospital, Mansoura, Egypt were investigated. Non-opaque lenses were extracted from infants aging 8 M-2 y-old (n=5) and young adult 20-30 years (n=6) after accident death of 1-4 hours. Medical Research Ethics committee, Egypt, approved the study protocol. A written informed consent had been taken beforehand from all patients or parent of infants to use their extracted lens experimentally after operation. Biochemical analysis was carried out to outline the differences between congenital and senile cataractous lenses in comparison with the control. Different parameters were investigated including amino acids, protein carbonylation, glycation end products, phospholipids, caspases, endothelin, heat shock protein, sorbitol, fructose and comet assay for single DNA damaged and scanning electron microscopy of lens fibres were investigated.

Results: The findings revealed marked increase of protein carbonylation, glycation end products, phospholipids, caspases, endothelin, heat shock protein, sorbitol and fructose coincides with single DNA damage. Aspartic, alanine, cysteine, isoleucine, leucine, methionine, tyrosine, histidine, tryptophan, valine and phenylalanine were significantly decreased in senile cataractous lenses in comparison with control and congenital cataracts. Scanning electron microscopy revealed abnormal disorganized lens fibers in cataractous lens which become widely separated and possessed deformation of ball and sockets. Some lens fibers possessed apparent accumulation of calcium salts.

Conclusions: Different factors are involved in cataractous formation coincides with increased of glycation end products and caspases reflecting apoptosis of lens.

Keywords: Congenital and senile cataractous lenses; AGE; Lipid peroxidation; Sugar; Isoenzyme electrophoresis; DNA

Introduction

Cataract is a clouding of the crystalline lens of the eye which causes visual impairment. It is of a multifactorial origin with unknown cause. Cataractous lenses were observed in more than 17 million people and 2800 new cases are detected throughout the world daily [1]. There are different forms of cataractous lenses; congenital and senile type. Congenital cataract occurred through environmental and genetic factors and it is observed during intra-uterine growth [2,3]. The genetic incidence accounted for about 8.3 and 25% [4,5]. Merin and Crawford [6] divided congenital cataracts into total (complete), polar (anterior or posterior), zonular (nuclear, lamellar, or sutural), and capsular or membranous. Senile cataracts developed as a result of age-related changes in opacification of lens fibres. Nuclear black cataract or posterior subcapsular starts as immature then become hypermature [7,8]. The incidence reached to approximately 56% of the superimposed diseased epithelial cells [7].

Lipids such as sphingomyelins, phosphatidylcholines, and phosphatidylethanolamines are the main structural components of the lens membranes [9]. There was a marked increase of lipids conjugated proteins in the nuclear cataract more than in the cortical one [10]. Age-related changes could be a contributing factor for altered protein-lipid interaction leading to protein aggregation and cataract formation [11]. Lipid peroxidation (LPO) is a pathogenic factor in cataract. It includes diene conjugates, lipid hydroperoxides, oxy-derivatives of phospholipid fatty acids and lipid moieties of aqueous humour and lenses of senile patients [12]. Cataract may also result from accumulation of advanced glycation end product (AGE) [13], decreased of antioxidant defense [14] and increase liberation of free radicals [15].

The present study aimed to assess the overexpression of bioactive components related to the induction of congenital and senile cataractous lenses taking in consideration the origin of both types is different.

Materials and Methods

Patients

The study was approved by the Medical Research Ethics committee, Egypt. A written informed consent had been taken beforehand from all patients to use their extracted cataractous lens for studying assessments after operation. Fifteen congenital and 56 moderate and mature senile cataractous lenses were obtained from patients admitted to Ophthalmic Center, Mansoura University Hospital, and Mansoura Egypt. In cases of congenital, irrigation aspiration surgery was performed in infant

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Received October 25, 2016; **Accepted** November 28, 2016; **Published** November 30, 2016

Citation: El-Sayyad HI, Tag-Eldin YM, Khalifa SA, AbdEl-Wahab AA, El-Desoky TMG (2017) Biochemical and Molecular Markers of Congenital and Senile Cataractous Lenses. J Mol Biomark Diagn 8: 318. doi: 10.4172/2155-9929.1000318

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Type of disease	Number and %	Gender		Type of cataract
		♂	♀	
S1	6/56(10.7%)	4	2	4NC, 1PSC, 1CC
S2	9/56(16.1%)	5	4	4NC, 2PSC, 3CC
S3	8/56(14.3%)	5	3	3NC, 3PSC, 2CC
S4	6/56(10.7%)	3	3	2NC, 1PSC, 3CC
S5	6/56(10.7%)	3	3	3NC, 2PSC, 1CC
S6	5/56(8.9%)	2	3	1NC, 2PSC, 2CC
S7	8/56(14.3%)	3	5	3NC, 2PSC, 3CC
S8	8/56(14.3%)	4	4	4NC, 2PSC, 2PSC

CC, Cortical cataract; PSC, Posterior subcapsular cataract; NC, Nuclear cataract; PSC; S_1, Hypertensive; S_2, Diabetic; S_3, Hypertensive and diabetic; S_4, Hypertensive and renal failure; S_5, Hypertensive and cardiac; S_6, Cardiac; S_7, Hepatic and diabetic; S_8 Hypertensive, diabetic and cardiac of senile cataractous lens

Table 1: Etiology of cataract.

at 8M-2y-old and fragmented lenses were collected in 5 mL ringer solution and kept in refrigerator at -20°C. However, senile cataractous lenses were obtained by extracapsular cataract extraction leaving the elastic capsule and categorized according to their age-related diseases (60-80y-old) such as diabetic, hypertensive and diabetic, hypertensive and renal failure, hypertensive and cardiovascular, cardiovascular disease, hepatic and diabetic, hypertensive, diabetic and cardiovascular (n=7). Normal non-opaque lenses were collected from infants (n=5) at 8M-2 years and adult (n=6) of 20-30 years within 1-4 hours of accident death. The etiology of the disease, gender differences and kinds of cataracts are illustrated in Table 1. The Lenses (included its epithelium) of control, congenital and senile patients were kept stored in refrigerator at -20°C for 15 days' prior biochemical investigation. The following parameters were assayed follows:

Biochemical investigations

Carbonyl and Glycation end products: Protein carbonylation was assessed by the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine (DNPH) to form protein-bound 2, 4-dinitrophenylhydrazones. The amount of protein-hydrazone produced is quantified spectrophotometrically at an absorbance between 360 nm to 385 nm and the carbonyl content can be used as the standard protein concentration. AGE products contain CML (carboxymethyl lysine), pentosidines and other AGE structures. Advanced glycation end product (AGE) was determined using Cell Biolabs, Inc kit (catalogue no. STA-317). The quantity of AGE adduct in protein samples is determined by comparing its absorbance with that of a known advanced glycation end product and bovine serum albumin (AGE-BSA) standard curve.

Endothelin: Endothelin-1 was determined using enzyme linked immuno-assay kit (USCN Life Science Inc (catalogue No. CCA482Hu). The method is base on competitive inhibition reaction between biotin labelled Endothelin1 and unlabelled Endothelin 1 with the pre-coated antibody specific to Endothelin 1. Avidin conjugated with horseradish peroxidase is added to the samples, the amount of bounded HRP is proportional to the amount of EDN1 in the skin, the absorbance was measured at 450 nm (within 30 min to avoid fading) Standard curve was plotted using EDN1.

Determination of intercellular and vascular adhesion molecule adhesive molecules-1 (ICAM-1 and VCAM-1): Lens samples were homogenized and centrifuged at 3,000 g at 4°C for 10 min, and supernatant was frozen at -80°C until measurement of adhesion molecules using ELISA kit (R&D Systems; Minneapolis, MN). 100 µL of antibodies against recombinant human rat ICAM-1 and VCAM-1 conjugated to horseradish peroxidase were added to each well of both treated and control specimens. The plates were sealed and incubated at room temperature for 1.5 h. After washing, 100 µl of a stabilized substrate solution (tetra-methyl-benzidine) was added to each well and the plates were re-incubated at room temperature for 30 min. Color development was carried out and measurement was assayed at a wavelength of 450 nm with the wavelength corrected at 620 nm.

Caspases 3 and 7: It is determined colorimetrically by using a Stressgen Kit (USCN Life Science Inc., Wuhan, China; Cat. No. 907-013 for Caspase 3 and Cat. No.: E0449Ra for Caspase 7). Lens tissues were lysed to collect their intracellular contents, and the lysate was tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline. The cleavage of the peptide can be quantified spectrophotometrically at a wavelength of 405 nm for Caspase 3 and wavelength of 450 nm for Caspase 7.

Heat shock protein 70 (Hsp 70): Lens Hsp 70 antibody titers were measured by ELISA Kit (Nunc Immunoplate Maxisorp; Life Technologies, UK). A 96-well microtiter plate was coated with human recombinant Hsp-70 by adding 10 ng of recombinant Hsp in phosphate-buffered saline (PBS) and incubating overnight at 4°C. Plates were rewashed and bound with anti-Hsp70 antibodies by the addition of peroxidase-conjugated-goat anti-human immunoglobulin G, which were diluted at 1:100 with PBT (Sigma-Aldrich, Inc, USA). After washing with PBS/Tween-20, o-phenylenediamine in citrate/phosphate/hydrogen peroxide was added and incubated for 5 min. The reaction was terminated by the addition of 3 M hydrochloric acid and absorbance was measured at 492 nm by using a plate reader with Genesis 2 Software (Life Sciences, Basingstoke, and Hampshire, UK) [16].

Phospholipids: Lens phospholipids contents (phosphatidylcholine, phosphatidylethanolamine, phosphatidyl serine and sphingomyelin) were determined according to Bligh and Dyer [17]. Lipid extract was carried from a known weight of retina with a mixture of chloroform and methyl alcohol (2:1 respectively). After homogenization of retina in the previous mixture, the supernatant was removed and evaporated and the crude lipid materials were remixed with 0.4 mL of the mixture of chloroform and methyl alcohol (2:1 v/v). Fifty µL of the sample were injected into high phase liquid chromatography.

Sugars (Sorbitol and fructose): Sorbitol is one of the 6 carbon sugar alcohols. It is determined using BIOVISION KIT (Catalogue No. Catalog #K631-100; 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA). D-sorbitol is oxidized to fructose leading to intense color development at 560 nm. Sorbitol concentration in samples can be calculated as follows: C=Sa/Sv*D nmol/µl or mM. Where: Sa is the amount of sample (nmol) from standard curve. Sv is the volume of sample (µL) added into the reaction wells. D is the sample dilution factor if any. D-Sorbitol MW: 182.17 g/mol.

Amino Acids	Control infant	Congenital Patients	Control adult	Senile Patients							
				S1	S2	S3	S4	S5	S6	S7	58
Essential Amino Acids											
His	259.5 ± 4.4	250.0 ± 3.0-	282.4 ± 2.3	241.4 ± 4.2***	240.4 ± 3.3***	231.6 ± 1.6***	242.7 ± 3.3***	228.5 ± 2.7***	257.0 ± 1.9***	238.8 ± 2.7***	236.3 ± 0.3***
Ile	96.25 ± 1.5	92.2 ± 1.2*	113.3 ± 0.9	92.4 ± 0.5***	88.50 ± 0.4***	82.5 ± 0.7***	93.00 ± 0.456***	86.60 ± 0.2***	95.40 ± 0.5***	91.40± 0.4***	86.2 ± 1.1***
Leu	106.7 ± 1.4	101.9 ± 1.4*	127.0 ± 1.9	103.7 ± 0.6***	96.8 ± 0.6	95.2 ± 0.7***	101.4 ± 0.3***	94. 7 ± 0.3-	107.0 ± 2.6***	98.7 ± 1.4***	93.8 ± 1.2***
Lys	117.5 ± 2.6	113.4 ± 1.5*	136.9 ± 03	113.4 ± 2.4***	109.1 ± 2.5***	110.3 ± 1.8***	114.8 ± 2.2***	109.0 ± 0.6***	117.5 ± 0.3***	116.8 ± 1.1***	112.54 ± 2.0***
Met	188.5 ± 2.9	184.2 ± 12.9*	2265 ± 1.8	187.9 ± 0.7***	174.5 ± 2.5***	167.0 ± 4.9***	184. 8 ± 4.2***	174.0 ± 0.6***	193.4 ± 0.5***	178.1 ± 1.2**	178.9 ± 4.3***
Phe	92.3 ± 1.7	83.7 ± 0.5**	94.0 ± 1.4	84.2 ± 0.8***	80.6 ± 1.3***	75.8 ± 1.7**	84.2 ± 1.5**	81 ± 0.9**	87.4 ± 0.8**	83.2 ± 1.3**	79.9 ± 0.7**
Thr	85.9 ± 1.7	81.9 ±13*	92.4 ± 0.5	81.4 ± 0.6***	78.8 ± 1.6***	74.7 ± 1.3***	82.8 ± 1.4***	76.4 ± 1.4***	85.3 ± 1.5***	81.2 ± 1.1***	73.2 ± 1.6***
Trp	36.7 ± 1.7	23.9 ± 0.7**	39.5 ± 0.3	22.74 ± 2.7**	23.60.7***	21.7 ± 0.3***	24.5 ± 1.0 ***	24.1 ± 0.8***	26.7 ± 0.8***	33.0 ± 0.9***	30.6 ± 1.3***
Val	208.9 ± 2.8	200.9 ± 0.8**	225.3 ± 0.8	202.6 ± 3.6***	191.8 ± 4.4***	184.3 ± 1.3***	201.7 ± 3.5***	190.6 ± 2.3***	210.4 ± 3***	196.3 ± 2.9***	187.0 ± 3.4***
Non-Essential Amino Acids											
Ala	240.3 ± 7.9	214.7+2.9	216.9 ± 2.5	212.1 ± 3.9*	203.0 ± 1.4**	189.3 ± 5.5***	211.9 ± 4.7*	201.9 ± 3.8**	205.8 ± 3.8***	211.7 ± 0.4*	207.8 ± 1.7**
Asn	1.7 ± 0 .1	1.5 ± 0.1*	1.6 ± 0.1	1.36 ± 0.03***	1.24 ± 0.03***	1.34 ± 0.1*	1.50 ± 0.1*	1.20 ± 0.1**	1.4 ± 0.1*	1.3 ± 0.1**	1.2 ± 0.1**
Cys	2.15 ± 0.1	1.9 ± 0.2*	2.8 ± 0.1	1.9 ± 0.1***	1.7 ± 0.1***	1.6 ± 0.1***	1.8 ± 0.1***	1.6 ± 0.1***	1.9 ± 0.1***	1.90 ± 0.1***	1.9 ± 0.13***
Glu	24.8 ± 0.8	20.9 2.1*	26.0 ± 0.2	20.41 ± 0.3***	21.4 ± 20.4**	19.3 ± 0.2***	22.1 ± 0.4***	20.60 ± 0.4***	22.0 ± 0.2***	22.0 ± 0.4***	21.6 ± 1.3***
Gly	12.8 ± 0.4	11.1 ± 0.8*	13.6 ± 0.3	11.1 ± 0.5**	10.4 ± 0.6*	9.2 ± 1.2***	11.03 ± 1.2**	10.1 ± 1.3***	11.1 ± 0.6**	10.01 ± 4.5*	10.5 ± 0.5***
Ser	136.3 ± 2.6	131.2 ± 2.4*	159.0 ± 0.4	132.1 ± 2.3***	125.0 ± 2. 5***	123.2 ± 1.9***	132.2 ± 1.4***	124.9 ± 0.2 ***	134.7 ± 2.0***	130.5 ± 1.4***	124.5 ± 2.6***
Tyr	88. 1 ± 1.8	81.8 ± 0.8*	97.9 ± 0.5	81.8 ± 0.6***	76.8 ± 3.1***	71.40 ± 0.5***	82.2 ± 0.6***	74.2 ± 0.4***	81.8 ± 1.6***	78.9 ± 1.7***	72.8 ± 2.7***

Each result represents the mean ± SE (n=5). * means non-significant. Control Neonate non-opaque lens; CA, Control adult non-opaque lens**. Significant at P<0.05 and ***. Highly significant at P<0.001. Abbreviations; Asn, Aspartic acid; Ala, Alanine; Cys, Cysteine; Ile; Isoleucine, Leu; Leucine; Lys; Lysine, Thr; Threonine; Ser, Serine; Glu, Glutamic acid; Gly, Glycine; Met, Methionine; Tyr, Tyrosine; His, Histidine; Trp, Tryptophan; Val, Valine; Phe, Phenylalanine; S_1, Hypertesive;S_2, Diabetic;S_3;Hypertensive and diabetic; S_4, Hypertensive and renal failure; S_5, Hypertensive and cardiac; S_6, Cardiac; S_7, Hepatic and diabetic; S_8, Hypertensive, diabetic and cardiac of senile cataractous lens

Table 2: Amino acid contents of congenital and Senile cataracts (µg/g tissue).

Lens amino acids: Both normal, senile and congenital cataractous lenses were hydrolysed by 6 M hydrochloric acid. Sensitive amino acids (especially tryptophan and cysteine) will be partially destroyed. Any pulpy protein in the column was squeezed out and extracted several times with petroleum ether, followed by 95% ethyl alcohol and allowed to dry in a watch glass. The samples were dried under vacuum, redissolved in 10 to 100 µl 0.2 M sodium citrate buffer, pH 2.0, and loaded in the amino acid analyzer equipped with a cation exchange column (Amersham Pharmacia Biotech), equilibrated in 0.2 M sodium citrate buffer, pH 2.0 according the manufacturer. Detection of the modified amino acids was achieved calorimetrically at 440 nm for proline and hydroxy-proline and at 570 nm for all other amino acids [18].

Scanning electron microscopic investigation: Biopsies from lenses of senile cataractous patients were fixed in 2.5% glutaraldhyde in 0.1 M cacodylate buffer at pH 7.4 and dehydrated in ascending grades of ethyl alcohol. The specimens were allowed to dry in a carbon dioxide critical point apparatus, mounted in stubs, coated with a thin layer of gold by low voltage DC sputtering, and investigated under scanning electron microscope JOEL5300 JSM (Musashino 3-chome akishima Tokyo 196-8558, Japan).

Comet assay: Specimens of healthy control and senile and congenital lens of patients were collected and stored in refrigerator at -20°C. The specimens were homogenized in phosphate buffered solution pH 7.5. 6 µL of homogenate was suspended on 0.5% low melting agarose and put in between a layer of 0.6% normal-agarose and a top layer of 0.5% low melting agarose on fully frosted slides. Lysed cells were carried out of the slides followed by electrophoresis to allow unwinding of DNA. Electrophoresis was performed for 10 min at 300 mA and 1 V/cm. The slides were neutralized and stained with 20 mg/ml ethidium bromide. Each slide was analyzed using a Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope. One hundred cells were analyzed on each slide using the comet assay II automatic digital analysis system. Perspective tail length (mm) (DNA migration from the center of the body of the nuclear core) was used to determine DNA damage [19].

Statistical analysis: Results were recorded as mean ± SE. Data were analyzed using SPSS software (version 13) by one way analysis of variance between control and diseased groups and the lowest of $p<0.05$ was considered significant.

Results

Lens amino acids contents

Table 2 illustrates the marked variations of amino acids in congenital and senile cataractous lenses. The estimated amino acids appeared to be decreased in both types of cataractous lenses. Amino acids serine, glutamic and glycine showed the least affected amino acids. Aspartic, alanine, cysteine, isoleucine, leucine, methionine, tyrosine, histidine, tryptophan, valine and phenylalanine were significantly decreased in senile cataractous lenses in comparison with control and congenital ones.

Lens lipids and sugar contents

Table 3 exhibits the lipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidyl serine and sphingomyelin) and sugar (sorbitol and fructose) contents of both types of cataracts. The lipid ethanolamine content was apparently decreased in both congenital and senile cataract compared with significant depletion of phosphatidylcholine, phosphatidyl serine and sphingomylein. Highly Senile cataractous lenses showed the highest alterations compared with congenital cataracts. Congenital cataract revealed marked depletion of lens sorbitol content and marked increase of fructose level comparing with a considerable increase of both in senile cataractous lenses.

Lens contents of glycation end product, carbonylation, endothelin, adhesive molecules, caspases 3 and 7 and HSP70

Table 4 shows the levels of carbonylation, glycation end product, endothellin, adhesion molecules (ICAM, VCAM), caspase 3 and 7) and heat shock protein 70 in senile and congenital cataractous lenses.

		Lipids				Sugars	
		Phosphatidyl-choline (nmol/mg)	Phosphatidyl-ethanolamine (nmol/mg)	Phosphatidyl-serine (nrnol/mg)	Sphingomyelin (nmol/mg)	Sorbitol (nmol/gm)	Fructose (nmol/gm)
Congenital Patient	C	037 ± 0.01	1.23 ± 0.01	0.26 ± 0.01	050 ± 0.004	2.24 ± 0.10	2.16 ± 0.04
	Cg	0.32 ± 0.01***	0.91 ± 0.02**	0.22 ± 0.01**	0.43 ± 0.004***	1.16 ± 0.12**	3.91 ± 039**
Senile Patients	CA	0.42 ± 0.01	1.18 ± 0.02	0.29 ± 0.01	0.63 ± 0.02	1.90 ± 0.25	2.25 ± 0.18
	S I	035 ± 0.01**	0.97 ± 0.02	0.23 ± 0.01*	0.46 ± 0.01***	3.1 ± 0.24**	4.76 ± 0.29**
	S2	0.29 ± 0.01***	0.99 ± 0.1*	0.21 ± 0.01**	0.39 ± 0.01***	15.5 ± 0.54***	12.98 ± 0.51***
	S3	035 ± 0.02**	1.07 ± 0.03***	0.24 ± 0.01***	0.46 ± 0.02***	17.0 ± 0.23***	9.25 ± 0.45***
	S4	0.33 ± 0.01***	1.07 ± 0.02**	0.21 ± 0.01***	0.43 ± 0.01***	2.6 ± 0.07**	8.36 ± 0.40***
	S5	0.28 ± 0.01***	1.12 ± 0.01***	0.20 ± 0.01***	0.34 ± 0.01***	2.8 ± 0.12**	8.33 ± 0.61***
	S6	0.27 ± 0.01***	1.10 ± 0.01***	0.20 ± 0.04***	0.38 ± 0.03***	4.1 ± 0.38***	9.75 ± 0.28***
	S7	0.31 ± 0.03***	1.17 ± 0.1***	0.21 ± 0.01***	0.39 ± 0.02***	14.16 ± 0.5***	10.78 ± 0.77***
	S8	0.27 ± 0.01***	1.23 ± 0.1**	0.18 ± 0.01***	0.33 ± 0.01***	11.6 ± 0.68***	13.33 ± 0.32***

Each result represents the mean ± SE (n=5). Abbreviations; *, non-significant. C, Control infant; Cg, Congenital; CA, Control adult. *. Non-significant; **. Significant at P<0.05 and ***. Highly significant at P<0.001; S_1, Hypertensive; S_2, Diabetic; S_3, Hypertensive and diabetic; S_4, Hypertensive and renal failure; S_5, Hypertensive and cardiac; S_6, Cardiac; S_7, Hepatic and diabetic; S_8, Hypertensive, diabetic and cardiac of senile cataractous lens

Table 3: Lipid and sugar contents of congenital and senile cataracts.

		Carbonyl (ninolitng)	GEP (ng/100 in g)	Endothelin (pg/100 mg)	ICAM (ng/100 mg)	V CAM (ng/100 mg)	Casp3 (ng/100 mg)	Casp7 (ng/100 mg)	IISPI (ng1100)
Congenital Patient	C	3.4 ± 0.4	2.2 ± 0.1	4.7 ± 0.4	2.1 ± 0.2	3.3 ± 0.2	0.3 ± 0.02	4.3 ± 0.2	22.5 ± 2.2
	Cg	7.0 ± 0.7**	3.9 ± 0.2**	13.3 ± 0.5***	1.7 ± 0.1**	2.1 ± 0.1*	1.7 ± 0.4	6.3 ± 0.5**	30.6 ± 0.7***
Senile Patients	C	3.8 ± 0.4	3.8 ± 0.1	7.6 ± 0.2	1.9 ± 0.2	2.7 ± 0.3	0.4 ± 0.01	5.4 ± 0.4	23.3 ± 2.0
	S1	6.8 ± 0.6**	4.7 ± 0.1*	11.2 ± 0.3**	1.5 ± 0.1**	2.31 ± 0.1**	3.4 ± 0.5***	8.5 ± 0.4**	33.4 ± 0.6**
	S2	6.8 ± 0.2***	6.0 ± 0.2**	14.3 ± 0.4***	1.4 ± 0.1***	2.1 ± 0.1***	3.7 ± 0.3***	6.1 ± 0.1*	35.5 ± 0.6***
	S3	7.5 ± 0.5***	7.6 ± 0.2***	14.6±0.3***	1.3 ± 0.1***	2.1 ± 0.1***	4.8 ±0.5***	6.1 ± 0.1*	38.8 ± 1.4***
	S4	7.6 ± 0.2***	4.9 ± 0.4***	12.0 ± 0.2***	1.5 ± 0.1***	2.2 ± 0.1***	4.5 ± 0.5***	8.2 ± 0.4***	34.2 ± 1.0***
	S5	8.4 ± 0.5	4.6 ± 0.1**	11.4 ± 0.2**	1.4 ± 0.1*	2.3 ± 0.1	4.2 ± 0.4	9.3 ± 0.5	33.6 ± 1.1
	S6	8.0 ± 0.4***	4.4 ± 0.1*	10.0 ± 0.3**	1.6 ± 0.1***	2.3 ± 0.1***	2.9 ± 0.2***	7.6 ± 0.6***	37.6 ± 1.8***
	57	8.2 ± 0.3***	6.7 ± 0.2***	14.4 ± 0.3***	1.4 ± 0.1***	2.3 ± 0.1***	4.4 ± 0.5***	8.9 ± 0.6***	40.1 ± 1.4***
	S8	7.5 ± 0.3***	6.5 ± 0.2***	15.4 ± 0.4***	1.6 ± 0.1***	2.3 ± 0.1***	1.7 ± 0.3**	8.7 ± 0.7***	37.3 ± 0.9***

Each result represents the mean ± SE (n=5). * means non-significant; C, Control infant; Cg, Congenital; C, Control adult. *Non-significant; ** Significant at P<0.05 and *** Highly significant at P<0.001. Abbreviations; GEP: Glycation end product, ICAM: Intercellular adhesion molecule, VCAM: vascular cell adhesion molecule, Casp3: Caspase 3, Casp7: Caspase 7, HSP70: Heat shock protein 70, CA: Control lens, S1: Hypertensive, S2: Diabetic, S3: Hypertensive and diabetic, S4: Hypertensive and renal failure; S5: Hypertensive and cardiac, S6: Cardiac, S7: Hepatic and diabetic, S8: Hypertensive, diabetic and cardiac of senile cataractous lens

Table 4: Biochemical markers of congenital and senile cataracts.

Carbonylation, glycation end product and endothellin attained a significant increase in congenital and senile cataracts; however, adhesion molecules (ICAM, VCAM) were markedly decreased. There was a significant increase of caspases 3 and 7 and heat shock protein 70 in congenital and senile cataractous lenses.

Scanning electron microscopy

In normal infants, the lens fibers were arranged in concentric rings passing along the anterior posterior axis with minimal intercellular spaces and tightly attached with ball and socket (Figure 1C). However, congenital cataractous lens exhibited loosely attached lens fibers with apparent deformation of their ball and socket. Many of the lens fibers appeared degenerated (Figure 1Cg).

In normal adult lenses, the lens fibers are tightly interconnected by numerous ball and socket junctions on each planar surface. The regularity of the depressions and the existence of complementary projections and minimal intercellular spaces were recognized (Figure 1Ca). Senile cataractous lenses (S1-S5) showed loosely attached interfibrillar junctions of the ball and socket. Many of the lens fibers possessed deformation of the fiber structure and marked damage of their ball and sockets. A striking observation was detected in senile hypertensive patients (S1) characterized by massive accumulation of calcium salts on damaged lens fibers (Figure 1).

Comet assay

The genomic expression revealed increased detaching tail length in lenses of congenital and senile cataractous patients in comparison with the normal ones. There was a marked increase of tail length and DNA % of congenital and cataractous lenses in comparison with the control (Table 5 and Figure 2).

Discussion

The present findings revealed apparent depletion of the amino acids aspartic, alanine, cysteine, isoleucine, leucine, methionine, tyrosine, histidine, tryptophan, valine and phenylalanine especially in senile cataracts. Altered amino acids contents were detected in senile diabetic patients with either hepatic or cardiovascular and hypertension. On the other hand, apparent reduction of phenylalanine, alanine, tryptophan, cysteine and glutamic acid were detected in congenital cataract.

Cataractous lenses were found to be associated with oxidation of cyteine to either the disulfide form or putative cysteic acid [20].

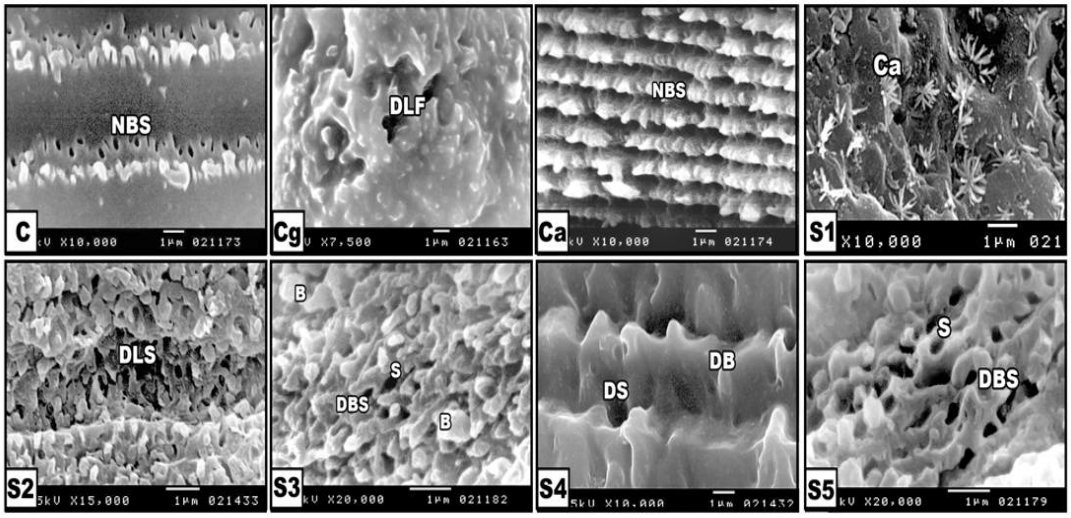

Figure 1: Scanning electron micrographs of congenital (Cg) and senile (S1-S5) cataractous lenses comparing with control (C and Ca). C. Control infant. C. Control adult. Note.

		Total	Tailed (%)	Normal (%)	Tail length (µm)	Tail DNA (%)
Control enfant		544 ± 46	4 ± 0.3	540 ± 52	0.87 ± 0.1	0.931-0.1
Congenital		666 ± 58	140 ± 23*	526 ± 64	2.70 ± 0.2*	2.05 ± 0.2*
Control adult		734 ± 78	61 ± 0.4	728 ± 85	0.92 ± 0.1	0.84 ± 0.1
Senile	hypertensive	654 ± 56	126 ± 28*	5281-48	2.56 ± 0.3*	2.83 ± 0.2*
	Diabetes	694 ± 83	184 ± 32*	5101-68	3.95 ± 0.4*	3.3 ± 0.2*
	Diabetes and Hypertension	694 ± 78	180 ± 17*	514 ± 39	3.45 ± 0.3*	3.69 ± 0.2*
	Hypertension and renal failure	656 ± 58	114 ± 13*	542 ± 47	2.66 ± 0.3*	3.29 ± 0.2*
	Hypertension and cardiovascular disease	740 ± 58	174 ± 27*	556 ± 68	2.32 ± 0.3*	2.86 ± 0.4*
	Cardiovascular disease	654 ± 40	114 ± 24*	540 ± 58	2.74 ± 0.3*	2.24 ± 0.5*
	Diabetic and liver disease	696 ± 47	172 ± 23*	524 ± 53	3.46 ± 0.6*	3.86 ± 0.5*
	Diabetic and hypertension and cardiovascular disease	690 ± 56	180 ± 10*	510 ± 32	3.61 ± 0.4*	3.42 ± 0.3*
* means significant at P<0.001						

Table 5: Morphometry of comet assay analysis of DNA damage of congenital and senile cataractous lenses.

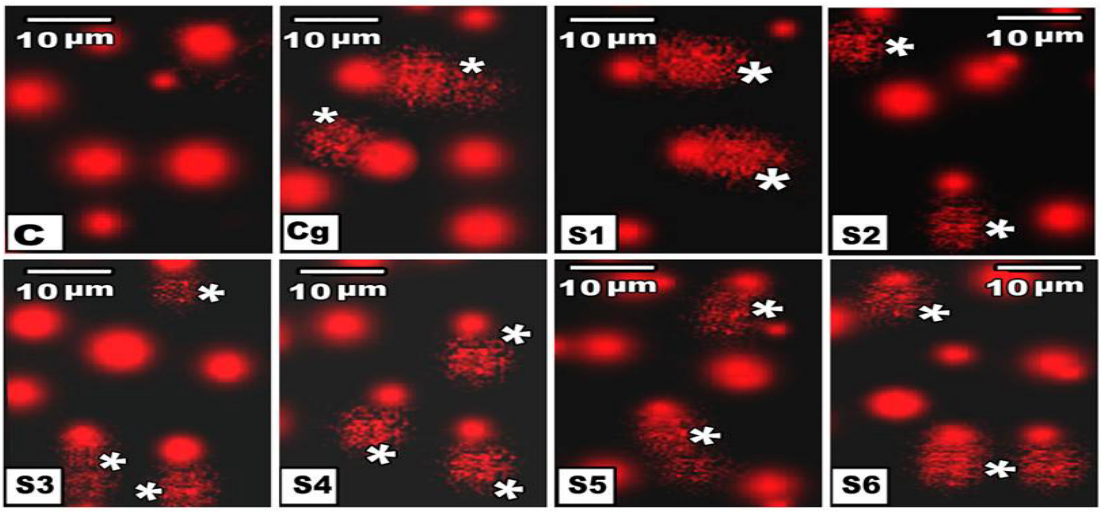

Figure 2: Comet assay of lens of congenital (Cg) and senile cataractous lenses (S1-6) asterisks showing stretched lens cells with DNA damage.

Rathore and Gupta [21] reported that increased concentration of each of the amino acids L-Tryptophan, L-tyrosine, L-phenylalanine, L-cysteine, L-glutamic acid protect against H_2O_2 induced cataractous lens *in vitro*.

Alterations of amino acids and subsequent protein conformation initiate the development of cataract. Senile cataractous lenses were found to possess aggregation of disulphide protein via oxidative changes

[20] and development of opacification [22]. Lenticular oxindolealanine (tryptophan oxidation product) and its byproduct kynurenines were observed in the lens nucleus of diabetic cataractous patients [23] and experimental diabetic rats [24]. Higher levels of dideoxyosones (DDOs); the intermediate product of advanced glycation end products (AGEs), such as pentosidine and glucosepane were detected in senile cataractous lenses [25]. Other findings mentioned deamidation of glutamine and asparagine residues in older lenses [26].

Protein represents the main structural components of lens. The present findings revealed that protein carbonylation and glycation end products were markedly increased in senile cataractous lenses comparing with that of congenital ones.

Experimental rats subjected to diabetes developed cataractous lenses by the end of 34th week as a result of increased protein carbonyls content and decrease of protein sulfhydryls as well as in age-related cataract [27].

Glycation and carbonylation of lens protein were apparently increased in diabetic senile patients. Mitochondrial represent the main target in glycation and carbonylation process of lens epithelium [28]. Lenticular diabetic cataract was found to increase the release of reactive oxygen species (ROS), which oxidized tryptophan (Trp) into kynurenines [29].

Also, the supplied data revealed depletion of assayed phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidyl serine and sphingomylein especially in senile cataractous lenses compared with congenital cataracts.

Similar findings of depleted phosphatidylcholine and two phosphatidylethanolamine-related phospholipids were reported in lenses of old people [30].

The lens transmitting light through thousands of cellular membranes are rich in phospholipids especially dihydrosphingomyelin. Most of the lipids are conjugated with proteins. Phospholipids constitute approximately 60% of human lens membranes and play the main role in its transparency [9]. The lipid contents especial dihydrosphingomyelin the main lens membrane component undergo dramatic alterations with age and cataractogenesis [31].

Scanning electron microscopy revealed that the normal lens showed regular arrangement of lens fibers with characteristic structure of ball and socket. However, Senile cataractous lenses possessed disorganized lens fibers attachments explained by loosely attached of the interfibrillar junctions and deformations of the ball and socket. Hypertensive patients with senile cataractous lenses showed abnormal accumulation of calcium salts in between the lens fibers.

Fleschner and Cenedella [32] showed apparent relative lipid compositions of the plasma membrane and fiber junction-enriched fractions of both human normal and cataractous lenses; however, cataracts involved deformational changes in the membrane lipid composition which influenced alterations of its membrane fluidity. Damage of the lens fiber cell membranes led to impairment of vision and interfered with light-scattering that cause the lens opacity. Age-related changes in lens lipid composition could be contributed to protein aggregation and cataract formation [11]. Lipid peroxidation (LPO) led to the formation of diene conjugates, lipid hydroperoxides, oxy-derivatives of phospholipid fatty acids which occurred in the lipid moieties of the aqueous humour and lenses of senile patients [12].

Also, senile cataractous lenses showed apparent increase of sorbitol and fructose sugars comparing with that of congenital ones. These may be attributed to the elevation of the activities of aldose reductase and sorbitol dehydrogenase, the promotor of polylol pathways [33,34]. Aldose Reductase is contributed to the reduction of development of glucose into sugar alcohol sorbitol, which is then metabolized to fructose by sorbitol dehydrogenase. Sorbitol was found to accumulate in lens epithelialcells causing osmolysis, and generation of free radicals which contributed to the formation of cataract [35,36]. Intracellular accumulation of sorbitol led to apparent increase of oxidative stress in the endoplasmic reticulum, the principal site of protein synthesis, and liberation of free radicals which cause disintegration of lens fibers [37].

In addition, endothellin attained a significant increase in congenital and senile cataracts. *In vitro* studies revealed that treating lens epithelial cells with endothelin-1 led to a marked increase of cytoplasmic calcium ion release and inhibition of sodium-calcium exchange [38]. Endothelin-1 is one of the principal potent vasoconstrictor peptide associated with increase ocular pressure [39]. The direct effect of its role in cataractous formation is not clear; however, pathophysiological changes of increased ocular pressure and its contribution of increased calcium release may be an influence in the development of cataract.

Also, the observed adhesion molecules in both congenital and senile cataractous lenses were markedly decreased.

It is well known that cell adhesion molecules (ICAM-1 and VCAM-1) are located in the epithelial cells, vascular endothelial cells and stroma cells. They play a main role in the cellular adhesion mechanisms characterized by cell-to-cell and cell-to-extracellular matrix (ECM) interactions for biological signal transducers in these interactions [40,41].

In vitro studies revealed that ICAM-1 was involved in lens epithelial cell attachment and growth on collagen and laminin as well as adhesion of lens epithelial cells to the extracellular matrix components of the lens capsule, which may facilitate inhibition of secondary cataract formation [42]. Fructose can directly increase the expression of ICAM-1 in endothelial cells [43] and renal tissues [44].

Expression of lens adhesion molecules N-cadherin, α-catenin, β-catenin and GR was significantly decreased in dexamethasone exposed lens cells with suspected to contribute to contribute to the pathogenesis of posterior subcapsular opacification [45].

On the other hand, Fan et al. reported increased expression of ICAM-1 in lens epithelial cells of type 2 diabetic patients suggesting its role in progress of cataractous formation [46].

The present work revealed increased incidence of apoptosis of lens cells which coincides with increased caspases 3 and 7, the markers of cell death and heat shock protein 70. Similar age-related reduction of Hsp-70 was reported by Bagchi et al. [47]. The authors attributed the increase of Hsp-70 to the structural deformation of lens and progress of cataract formation.

DNA damage of lens epithelium was markedly detected in cortical cataracts compared to the nuclear or posterior subcapsular cataracts and attributed mainly to the release of reactive oxygen species (ROS) [48]. Oxidative DNA damage of lens epithelial cells may be one of the etiology of senile cortical opacities [49, 50]. Increase sorbitol level was found to induce apoptosis of lens epithelium via elevated level of caspase 3 in diabetic rats [46].

Finally, it can be concluded that there are similarities between congenital and senile cataractous lens in the end product biochemical changes of amino acids, phospholipids, sorbitol and fructose levels

and glycation end products. Increased level of caspases and DNA damage assessed by comet assay reflected apoptosis of epithelial cells in aging lens.

There is no conflict interest.

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