Biochemical and Molecular Markers of Congenital and Senile Cataractous Lenses

Hassan IH El-Sayyad1*, Tag Eldin YM2, Soaad A Khalifa1, Amal A AbdeEl Wahab1, and Taher MG El Desoky2

1Biology Department, Faculty of Science, Mansoura University, Saudi Arabia
2Biology Department, Delta Universities, of Science and Technology, Saudi Arabia

Abstract

Background: Cataractous lenses represent one of the main public health problem involved in impairing vision. Different multifactorial 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 or posterior 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

*Corresponding author: Hassan IH El-Sayyad, Faculty of Science, Mansoura University, Saudi Arabia, Tel: 0020502254850; E-mail: elsayyad@mans.edu.eg

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treated and control specimens. The plates were sealed and incubated of antibodies against recombinant human rat ICAM-1 and VCAM-1 molecules using ELISA kit (R&D Systems; Minneapolis, MN). 100 μL homogenized and centrifuged at 3,000 g at 4°C for 10 min, and adhesive molecules-1 (ICAM-1 and VCAM-1):

Lens samples were was plotted using EDN1.

The method is base on competitive inhibition reaction between biotin immuno-assay kit (USCN Life Science Inc (catalogue No. CCA482Hu). Advanced glycation end product (AGE) was determined using Cell (carboxymethyl lysine), pentosidines and other AGE structures.

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 washed and bound with anti-Hsp70 antibodies by the addition of peroxide-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.

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Number and %</th>
<th>Gender</th>
<th>Type of cataract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>♂</td>
<td>♀</td>
</tr>
<tr>
<td>S1</td>
<td>6/56(10.7%)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>S2</td>
<td>9/56(16.1%)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>S3</td>
<td>8/56(14.3%)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>S4</td>
<td>6/56(10.7%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>S5</td>
<td>6/56(10.7%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>S6</td>
<td>5/56(8.9%)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>S7</td>
<td>6/56(14.3%)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>S8</td>
<td>8/56(14.3%)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Type of disease: CC, Cortical cataract; PSC, Posterior subcapsular cataract; NC, Nuclear cataract; PSC; 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 cataracts lens
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 acids (especially tryptophan and cysteine) will be partially destroyed. Any pulpy protein in the column was squeezed out and extracted.

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, phenylalanine, S hypertensive and diabetic, S hypertensive and renal failure, S Hypertensive and cardiac, S Cardiac, S Hepatic and diabetic, S Hypertensive, diabetic and cardiac of senile cataractous lens

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

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Control infant</th>
<th>Congenital Patients</th>
<th>Control adult</th>
<th>Senile Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
</tr>
<tr>
<td></td>
<td>Non-Essential Amino Acids</td>
<td></td>
<td>Essential Amino Acids</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>259.5 ± 4.4</td>
<td>250.0 ± 3.0</td>
<td>282.4 ± 2.3</td>
<td>241.4 ± 4.2***</td>
</tr>
<tr>
<td>Ile</td>
<td>96.25 ± 1.5</td>
<td>92.2 ± 1.2</td>
<td>113.3 ± 0.9</td>
<td>92.4 ± 0.5***</td>
</tr>
<tr>
<td>Leu</td>
<td>106.7 ± 1.4</td>
<td>101.9 ± 1.4</td>
<td>127.0 ± 1.9</td>
<td>103.7 ± 0.6***</td>
</tr>
<tr>
<td>Lys</td>
<td>117.5 ± 2.6</td>
<td>113.4 ± 1.5</td>
<td>136.0 ± 0.9</td>
<td>113.4 ± 2.4**</td>
</tr>
<tr>
<td>Met</td>
<td>185.8 ± 2.9</td>
<td>184.2 ± 12.9</td>
<td>2265 ± 1.8</td>
<td>1879 ± 0.7***</td>
</tr>
<tr>
<td>Phe</td>
<td>92.3 ± 1.7</td>
<td>83.7 ± 0.5**</td>
<td>94.0 ± 1.4</td>
<td>84.2 ± 0.8***</td>
</tr>
<tr>
<td>Thr</td>
<td>89.5 ± 1.7</td>
<td>81.9 ± 13</td>
<td>92.4 ± 0.5</td>
<td>81.4 ± 0.8***</td>
</tr>
<tr>
<td>Trp</td>
<td>36.7 ± 1.7</td>
<td>23.9 ± 0.7**</td>
<td>39.5 ± 0.3</td>
<td>22.7 ± 2.7**</td>
</tr>
<tr>
<td>Val</td>
<td>208.9 ± 2.8</td>
<td>200.9 ± 0.8*</td>
<td>225.3 ± 0.8</td>
<td>202.6 ± 3.6***</td>
</tr>
</tbody>
</table>

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. Abbreviation: Asn, Aspartic acid; Ala, Alanine; Cys, Cysteine; Ile, Isoleucine; Leu, Leucine; Lys, Lysine; Thr, Threonine; Ser, Serine; Glu, Gluamic acid; Gly, Glycine; Met, Methionine; Tyr, Tyrosine; His, Histidine, Trp, Tryptophan; Val, Valine; Phe, Phenylalanine, S Hypertensive, S Hyperglycemic and diabetic, S Hypertensive and renal failure, S Hypertensive and cardiac, S Cardiac, S Hepatic and diabetic, S Hypertensive, diabetic and cardiac of senile cataractous lens

Comet assay: Specimens of healthy control and senile and congenital lens 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 in between a layer of 0.6% normal-agarose and a top layer of 0.5% low melting agarose on fully frieded 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. Perspectival tail length (mm) (DNA migration from the center of the body of the nuclear core) was used to determine DNA damage [19].

Lens lipids and sugar contents

Table 3 exhibits the marked variations of lipid contents of both types of cataractous lenses. The lipid ethanol-nolamine, phosphatidyl serine and sphingomyelin) and sugar (sorbitol and fructose) contents of both types of cataracts. The lipid ethanol-nolamine content was apparently decreased in both congenital and senile cataract compared with significant depletion of phosphatidylcholine, phosphatidyl serine and sphingomyelin. 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, carboxylation, endothelin, adhesive molecules, caspases 3 and 7 and HSP70

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

In normal adult lenses, the lens fibers are tightly interconnected passing along the anterior posterior axis with minimal intercellular spaces and tightly attached with ball and socket (Figure 1C). However, in normal infants, the lens fibers were arranged in concentric rings (Figure 1Cg).

In normal adult lenses, the lens fibers possess deformation of the fiber structure and marked damage of their ball and sockets. A striking observation was detected in senile diabetic patients characterized by massive accumulation of calcium salts on damaged lens fibers (Figure 1).

**Scanning electron microscopy**

In normal adults, the lens fibers are tightly interconnected by numerous ball and socket junctions on each planar surface. The regularity of the deformations 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.

**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.

Other hand, apparent reduction of phenylalanine, alanine, tryptophan, cysteine and glutamic acid were detected in congenital cataract.

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

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine (nmol/mg)</td>
<td>Phosphatidylserine (nmol/mg)</td>
</tr>
<tr>
<td><strong>Conventional Patient</strong></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Cg</td>
<td>0.32 ± 0.01**</td>
</tr>
<tr>
<td>S1</td>
<td>0.42 ± 0.01</td>
</tr>
<tr>
<td>S2</td>
<td>0.29 ± 0.01**</td>
</tr>
<tr>
<td>S3</td>
<td>0.35 ± 0.02**</td>
</tr>
<tr>
<td>S4</td>
<td>0.33 ± 0.01**</td>
</tr>
<tr>
<td>S5</td>
<td>0.28 ± 0.01**</td>
</tr>
<tr>
<td>S6</td>
<td>0.27 ± 0.01**</td>
</tr>
<tr>
<td>S7</td>
<td>0.31 ± 0.03**</td>
</tr>
<tr>
<td>S8</td>
<td>0.27 ± 0.01**</td>
</tr>
</tbody>
</table>

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. 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.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid and sugar contents of congenital and senile cataracts.</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylcholine (nmol/mg)</td>
<td>Phosphatidylserine (nmol/mg)</td>
</tr>
<tr>
<td><strong>Conventional Patient</strong></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Cg</td>
<td>7.0 ± 0.7**</td>
</tr>
<tr>
<td>S1</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>S2</td>
<td>6.8 ± 0.6**</td>
</tr>
<tr>
<td>S3</td>
<td>6.8 ± 0.2***</td>
</tr>
<tr>
<td>S4</td>
<td>7.5 ± 0.5**</td>
</tr>
<tr>
<td>S5</td>
<td>7.6 ± 0.2**</td>
</tr>
<tr>
<td>S6</td>
<td>8.4 ± 0.5</td>
</tr>
<tr>
<td>S7</td>
<td>8.0 ± 0.4***</td>
</tr>
<tr>
<td>S8</td>
<td>7.2 ± 0.3***</td>
</tr>
<tr>
<td>S9</td>
<td>7.5 ± 0.3***</td>
</tr>
</tbody>
</table>

Each result represents the mean ± SE (n=5). * means non-significant; C, Control infant; Cg, Congenital; CA, 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.
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$_2$O$_2$ 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 deoxysteroyxosines (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 deamination 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 sulphydrys 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 carbylonylation 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 sphingomyelin 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 dihydrosphingomyelins. 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 special dihydrosphingomyelins in 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.

Fleschnerg 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 promoter of polyol 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 epithelial cells causing osmosis, 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, endothelin 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 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.

References


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