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Research Article

Expression of isoenzymes carbonic anhydrase, esterase, lactic dehydrogenae and superoxide dismutase in congenital and senile cataractous lenses of patients

Hassan IH El-Sayyad*, Tag-Eldin Y, Soad A Khalifa *, Amal A AbdEl-Wahab***, Taher M G E El-Desoky*****

*Biology Dept., Faculty of Science, Mansoura University, Egypt.
**Biology Dept., Delta University, Egypt.
***Ophthalmic Surgery, Ophthalmic Center, Mansoura University, Egypt
*Corresponding author: *elsayyad@mans.edu.eg*

Abstract

Assessment the biochemical and genetic changes of congenital and cataractous lenses of patients. In this study, 15 congenital and 56 senile cataractous lenses obtained from patients admitted to Ophthalmic Center, Mansoura University Hospital, Mansoura, Egypt were investigated. Non-opaque lenses were extracted from infants (n=5) and adult 20-30 years (n=6) after death of 1-4 hours. The study was approved by the Department of Evaluation and Quality Assurance of the Mansoura University of Human Research Ethics committee, Egypt. A written informed consent had been taken beforehand from all patients to use their extracted lens experimentally after operation. Isoenzyme electrophoresis was carried out for both control and experimental groups. isoenzyme electrophoresis of carbonic anhydrase, lactic dehydrogenase, esterase and superoxide dismutase was carried out for both control and experimental groups. The present findings revealed marked alterations of the assayed isoenzymes expression. Different factors are involved in cataractous formation and all contributed to the oxidative stress which altered the isoenzyme fractions contributed to the metabolism lens components.

Keywords: Congenital & senile cataractous lenses, isoenzyme electrophoresis.

Introduction

Cataract is a clouding of the eye lens 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 (Minassian et al., 2000). There are two 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 (Lambert et al., 1989). The genetic incidence accounted for about 8.3 and 25% (Francois, 1982; Semina et al., 1998). Merin and Crawford (1971) classified congenital abnormalities into total (mature or complete), polar (anterior or posterior), zonular (nuclear, lamellar, or sutural), and capsular or membranous. The epithelia of nuclear, posterior subcapsular, mature, mixed, hypermature, and black cataracts of male and female patients revealed that the 56% superimposed epithelial cells are probably the source of increased and altered cell activity (Vasavada et al., 1991). Senile cataract is classified into; immature or mature, nuclear cortical and posterior subscapular (Brazitikos et al., 1999).

The determination of serum enzyme activities has played an increasing role in clinical chemical diagnosis. In sera of patients with malignant tumor (retinoblastoma), there was a considerable increase of lactic dehydrogenase (LDH), isoenzymes specially LDH4 and LDH5 were higher and LDH1 and LDH2 were lower (Singh et al., 1991). Human cytoplasmic isoenzymes carbonic anhydrase (CA-) CA I and

CA II was found to detect in corneal endothelium, ciliary processes and pigmented and non-pigmented epithelium (Wisfrand et al., 1986).

Two isoenzyme fractions were detected in rat ocular tissues (Julia et al., 1986), meanwhile sclera and cornea expressed superoxide dismutase for protection against reactive oxygen species (Behndig et al., 1998).

Glaucoma and keratitis was found to exhibit increased level of Cyclooxygenase expression (Radi, 2009). Nitric oxide synthases (NOS) are involved in regulation of ocular vascular tone and blood flow endothelial NOS (eNOS) has recently been shown to mediate endothelium-dependent vasodilation in mouse retinal arterioles (Laspas et al.,2014).

The present study aimed to evaluate the expression of the isoenzymes lactic dehydrogenase, esterase, carbonic anhydrase and superoxide dismutase in normal, senile and congenital cataractous lenses of human subjects

Materials and Methods

Patients: Fifteen congenital and 56 senile cataractous lenses were obtained from patients admitted to Ophthalmic Center, Mansoura University Hospital, Mansoura Egypt. Non-opaque lenses were extracted from infants (n=5) and adult 20-30 years (n=6) after death of 1-4 hours. The study was approved by the Department of evaluation and quality assurance of the Mansoura University of Human Research Ethics committee, Egypt. A written informed consent had been taken beforehand from all patients to use their extracted lens experimentally after operation. However, senile patients were categorized according to their age-related diseases such as diabetic, hypertensive and diabetic, hypertensive and renal failure, hypertensive and cardiovascular, cardiovascular disease, hepatic and diabetic, hypertensive, diabetic and cardiovascular (n=7). The lenses were separated as fractionated parts in case of congenital or as a solid material in senile patients. Control lenses were collected from non-opaque infants (n=5) and adult (n=6) after 1-4 hours of death. Lenses of infants were selected for congenital (n=20) while the adult were used for comparing senile cataract. Immature cataract defined as possessed partial opacification of lens. Mature as defining of browning opaque of lens. Non-opaque and congenital and senile cataractous lenses were investigated for Isoenzyme electrophoresis of carbonic anhydrase (CAH), superoxide dismutase (SOD), lactic dehydrogenase (LDH) and esterase (EST). The lens samples were collected, cleaned and homogenized using 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose and their protein content was determined according to Lowry et al. (1951) and electrophoresis was carried out according to Laemmli (1970). The protein bands were stained with Coomassie blue R-250 (60 mg/L) in an acidic medium (Andrews, 1986). For visualization of the tested enzymes, electrophoresis of lens tissues were carried out in the selected incubated medium for each kind of the assessed enzyme as follows:

1.Carbonic anhydrase isoenzymes: It was carried out according to Topal and Gulcin (2014). Carbonic anhydrase (CA) catalyzes the reversible hydration and dehydration reactions of CO_2/H_2CO_3. Staining was carried out by mixing Tris-HCl (0.65 ml, 1 M, pH 6.8), SDS (3 ml, w/v: 10%), neat glycerol (1 ml), bromphenol blue (1 ml, w/v: 0.1%), B - mercaptoethanol (0.5 ml), and water (3.85 ml). and buffer solution (50 µl) and adjusting the pH to 7.0. Reaction was initiated by the addition of carbon dioxide as an aqueous solution.

2. Superoxide dismutase isoenzymes: SOD isoenzyme was determined according to Jevremovic et al. (2010). Proteins were separated by native polyacrylamide gel electrophoresis (PAGE) using 5% stacking and 10% running gels with a buffer consisting of 0.025M Tris and 0.192 M glycine (pH 8.3) at 100 V for 3.5 h. The total amount of protein applied per lane was 20 µg. After electrophoresis, the gels were incubated with 1 mM KCN and 30% H_2O_2 followed by incubation with a reaction mixture (0.1 M EDTA, 0.098 mM NBT, 0.030 mM riboflavin and 2 mM N,N,N,N-tetramethylethylendiamine in K phosphate buffer, pH 7.8) for 30 min in the dark. The gels were washed in distilled water and visualized with regular light.

3. Lactic dehydrogenase isoenzymes: LDH isoenzyme was determined according to Sarkar et al. (1978). After electrophoresis, the gels were incubated with H_2O 18.4 ml, 1 M Tris 4 ml, tetrazolium-blue 12 ml, Phenazine methosulphate 4 ml, Na-lactate 4 ml

and NAD 1.3 ml to develop color reaction for 20 min. In the color reaction, NAD and lactate serve as substrates, phenazine-methosulphate is the primary electron acceptor and tetrazolium-blue is the final electron acceptor.

4. Esterase: Esterase isoenzymes were estimated according to López-Soler et al. (2008). The gels were stained for esterase activity for 2-12 h at 4°C in 100 ml a-naphthyl acetate/fast blue solution in 0.2 M-phosphate buffer at pH 7.5. The resulting gels were measured and intensity of band staining was noted, the gels were also photographed. The esterase isoenzyme data for each isolate are based on at least two replicate extractions, each duplicated on a single gel.

Isoenzyme patterns were recorded on the basis of number and the rate of flow (Rf) values of the isoenzyme bands. The Rf value is the mobility of each isoenzyme band that traveled from the origin divided by the distance traveled by the front tracking dye. The presence or absence of a certain isoenzymatic band was considered as a differentiating feature.

Zymograms were drawn to scale and relative mobility values were calculated for each band.

Results

Isoenzyme electrophoresis of LDH, CAH, EST & SOD

Lens expressed two isoenzyme fractions of LDH in both control, congenital and senile immature and mature cataractous lenses. The intensities of isoenzyme fraction I was markedly increased in comparison with the control. However, senile cataractous lenses of hepatic and diabetic patients were apparently missing this fraction. Little changes were markedly detected, except moderate increase of band intensities (Fig.2).

In contrast, CAH expressed only two isoenzyme fraction of control infant compared with extra-middle isoenzyme fraction in congenital lens. In adult patient, the lens expressed three isoenzyme fractions I, II & III. However senile cataratous patients (S2-S4, S6 & SM) possessed the missing isoenzyme fraction II. Isoenzyme fraction III showed marked increased intensity in congenital & senile cataractous lenses of patients (Fig.1).

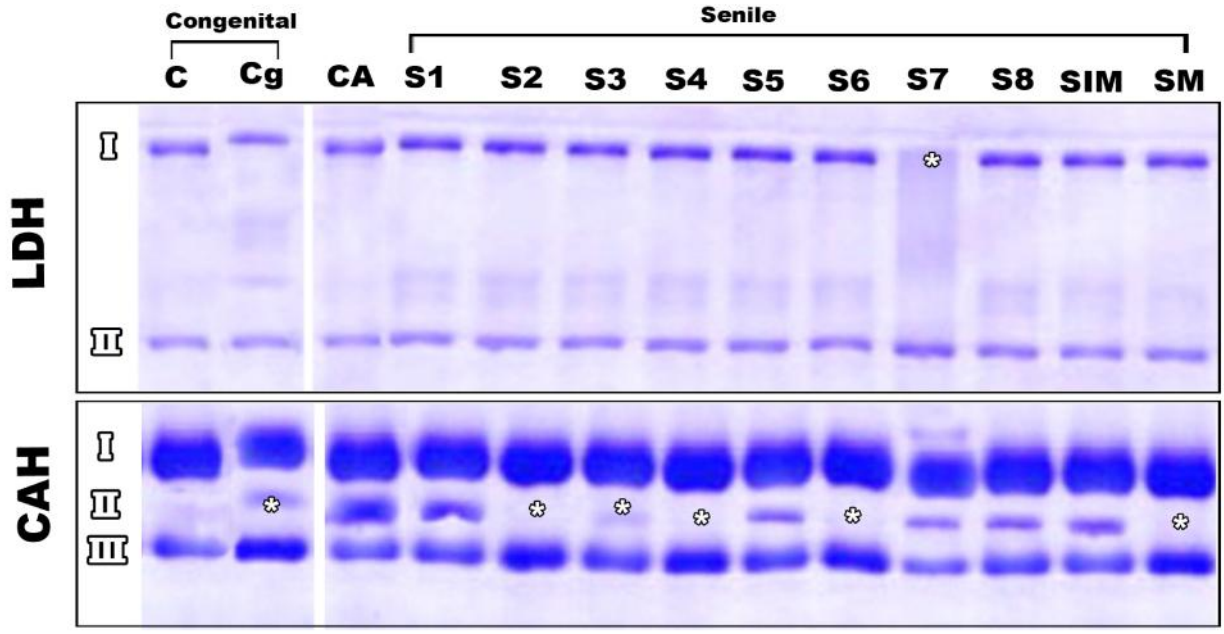

Fig.1. Isoenzyme electrophoresis of LDH and CAH of congenital (Cg) , senile (S1-S8) and immature and mature cataractous lenses of patients comparing with control (C). * indicating missing of fading isoenzyme expression. . S_1, Hypertesive;S_2, Diabetic;S_3;Hypertensive and diabetic; S_4, Hypertensive 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; SIM,senile immature cataract; SM, senile mature cataract.

Lens esterase expressed four isoenzyme fractions I, II, III & IV. There is increase of the isoenzyme fractions I & II especially in S5 and mature cataractous lenses.

There was a considerable change of the rate of flow of the isoenzyme fractions III & IV as well as missing of expression in S4, 5 and 7 and immature cataracts (Fig.2).

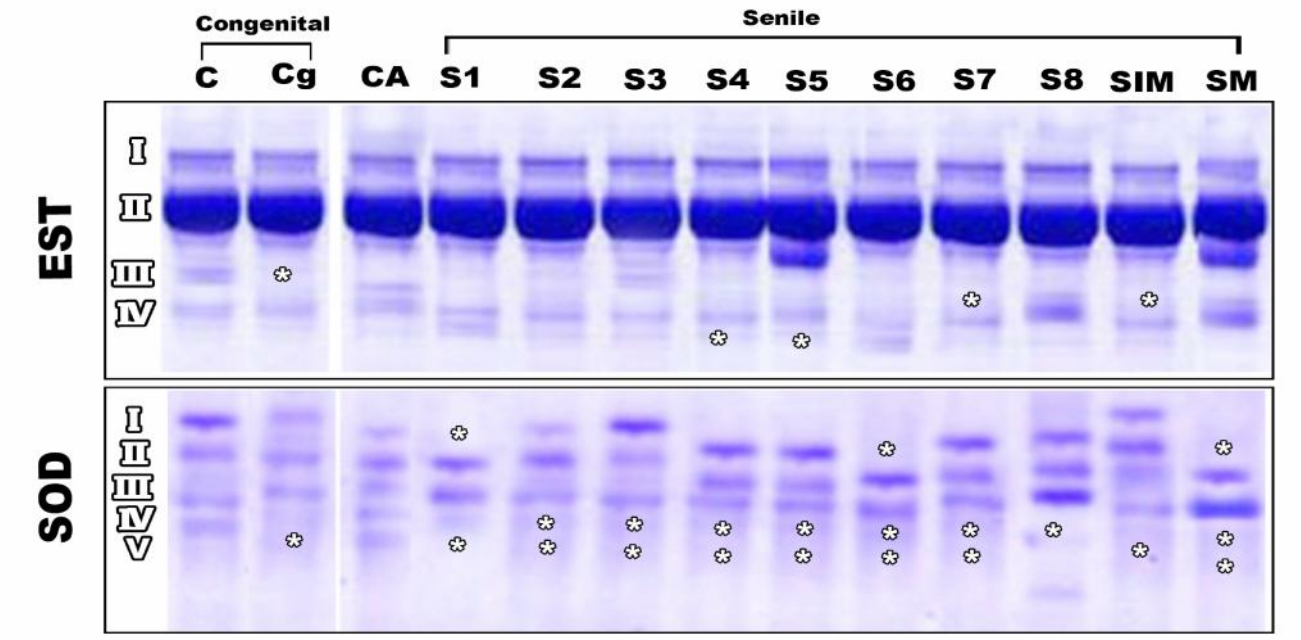

Fig.2. Isoenzyme electrophoresis of EST and SOD of congenital (Cg) , senile (S1-S8) and immature and mature cataractous lenses of patients comparing with control. * indicating missing or fading isoenzyme expression. S_1, Hypertesive;S_2, Diabetic;S_3;Hypertensive and diabetic; S_4, Hypertensive and renal failure;S_5, Hypertensive and cardiac; S_6, Cardiac; S_7, Hepatic and diabetic; S_8, Hypertensive, diabetic and cardiac of senile cataractous lens; SIM,senile immature cataract; SM, senile mature cataract.

Also, the normal non-opaque lenses expressed four isoenzyme fractions of superoxide dismutase. Comparing both congenital and senile cataractous lenses, the intensities of the isoenzyme fractions were either markedly decreased and or lacked expression in congenital and senile cataractous lenses (Fig.2).

Discussion

Congenital and senile cataractous lens exhibited marked alterations of LDH, CAH, EST & SOD isoenzyme fractions. Disruption of assayed isoenzymes may interfere with lens structure and function. CAH showed increased intensities of the isoenzyme fractions I & III as well as missing expression of isoenzyme II in senile cataractous lenses. Increased intensities may reflect increased CAH which led to increase of ocular pressure.

Carbonic anhydrase is a cytoplasmic enzyme presents in many mammalian and human lenses. It catalyses the hydration of metabolic CO_2 and subsequent production of carbonates, enhances the loss of CO_2 from the lens by ‘‘facilitated’’ diffusion (Wistrand & Knuuttila, 1980). As a result of tightly packed lens fibers which represent a functional syncytium, CAH may be involved in managing electrolytes exchange and volume of the whole lens (Wiederholt, 1980).

Increased isoenzyme fractions may disrupt lens functions through altering electrolyte levels and causing opacification of lenses.

Lactic dehydraogenase promotes the function of lens by catalysing the reversible reduction of pyruvic acid to lactic acid. A decrease in lactic dehydrogenase activity may contribute not only the lactic acid concentration but also indirectly the oxidation of glucose by the hexose-monophosphate shunt, which has been shown to play an important role in the oxidative metabolism of the lens (Kinoshita, 1955).

Thus, homogenates of total lenses show age-dependent reductions of enzyme activities, although enzyme activities remain at a physiological level in cortical lens fibers with recognizable cell nuclei. In lenses with immature supranuclear cortical and (particularly) or black nuclear cataracts, cortical fibers can still exhibit high LDH activities (Pau et al., 1997).

Lens esterase expressed four isoenzyme fractions; I, II, III & IV. There is increase of the isoenzyme fractions I & II especially in S5 and mature cataractous lenses. There was a considerable change of the rate of flow of the isoenzyme fractions III & IV as well as missing of expression in S4, 5 and 7 and immature cataracts.

The present findings supported the work of Hahn et al. (1976) who only reported that the esterase activity was increased in old mammalian lens. Parasympathetic nerves are known to affect the ciliary muscle which controls the lens to change its shape for vision accommodation. The targeted effect of the lens in response to the acetylcholine is terminated by the release of cholinesterase which yields inactive metabolites choline and acetic acid. Lens epithelium may contain the receptors of the acetylcholine which facilitated its action (Candia et al., 2002). Alterations in the enzyme activity may interfere with lens acetylcholine content (Massoulie, 2002), which may be involved in the formation of cataracts. Besides the direct excitatory effects of acetylcholine, the increase of intracellular calcium and decrease of magnesium (Agarwal et al., 2012) promotes the synthesis of nitric oxide (Nagai and Ito, 2007) and interferes with glucose metabolism, the promoter of cataract formation (Greiner et al., 1981). Increased acetylcholinesterase may activate muscarinic receptors and it is implicated in an increased risk of cataract (Collison et al., 2000).

Also, the findings revealed expression of four isoenzyme fractions of superoxide dismutase. The intensities of the isoenzyme fractions were either markedly decreased and or lacked expression in congenital and senile cataractous lenses.

The results of the present study indicate that the antioxidant capacity in the diabetic cataractous lenses was decreased and this result suggests a role of antioxidant enzymes in the genesis of diabetic cataracts. Similar findings of depleted SOD were reported in nuclear cataract of human, calf, rabbit and rat lenses (Fecondo and Augusteyn, 1983).

Ocular tissues were found to contain antioxidants that protect it from oxidative stress of free radicals such as redox antioxidant enzymes (catalase, superoxide dismutase, GSH peroxidase, glutathione S-transferase), ascorbic acid, glutathione, amino acids (cysteine and tyrosine) etc (Garland,1990; Kisic et al., 2012) that scavenge free radicals. The antioxidant redox system was detected in the epithelial layer and outer cortical regions. Cataract formation is believed to be attributed to reduction of the antioxidant defense and increase of oxidative stress.

Sorkhabi et al. (2011) observed increased expression in SOD activity in the lens capsule of patients with pseudoexfoliation (PEX) syndrome and cataract which suggests that oxidative mechanisms play a role in the etiopathogenesis of cataract.

Conclusion

Finally, it can be concluded that the alterations of the assayed isoenzyme fractions led to alteration of lens function and contributed to cataract formation.

References

Agarwal R, Iezhitsa I, Agarwal P, Spasov A (2012) Magnesium deficiency: does it have a role to play in cataractogenesis?. Exp Eye Res. 101, 82-89.

Andrews AT (1986) Electrophoresis: Theory, techniques and biochemical and clinical application. Oxford University Press, Oxford.

Behndig A, Svensson B, Marklund SL, Karlsson K (1998) Superoxide dismutase isoenzymes in the human eye. Invest Ophthalmol Vis Sci.; 39(3):471-5.

Brazitikos PD, Tsinopoulos IT, Papadopoulos NT, Fotiadis K, Stangos NT (1999) Ultrasonographic classification and phacoemulsification of white senile cataracts. Ophthalmology. 106(11):2178-2183.

Candia OA, Zamudio AC, Polikoff LA , Alvarez LJ (2002) Distribution of acetylcholine-sensitive currents around the rabbit crystalline lens. Exp Eye Res. 74(6):769-776.

Collison DJ, Coleman RA, James RS, Carey J , Duncan G (2000) Characterization of muscarinic receptors in human lens cells by pharmacologic and molecular techniques. Invest Ophthalmol Vis Sci.41(9):2633-41.

Fecondo JV , Augusteyn RC (1983) Superoxide dismutase, catalase and glutathione peroxidase in

the human cataractous lens. Exp Eye Res. 36(1):15-23.

Francois J (1982) Genetics of cataract. Ophthalmologica.18461- 71.

Garland D (1990) Role of site-specific, metal-catalyzed oxidation in lens aging and cataract: a hypothesis. Exp Eye Res. 50(6):677-682.

Greiner JV, Kopp SJ, Sanders DR, Glonek T (1981) Organophosphates of the crystalline lens: a nuclear magnetic resonance spectroscopic study. Invest Ophthalmol Vis Sci. 21(5):700-713.

Hahn U, Swanson AA, Hockwin O (1976)Age-related changes in the proteolytic enzymes of mammalian lens. Graefes Arch Clin Exp Ophthalmol 199(3): 197-206.

Jevremovic S, Petric M, Živkovic S, Trifunovic M , Subotic A (2010) Superoxide dismutase activity and isoenzyme profiles in bulbs of snakes's head fritillary in response to cold treatment. Arch. Biol. Sci., Belgrade, 62 (3): 553-558.

Julià P, Farrés J, Parés X (1986) Ocular alcohol dehydrogenase in the rat: regional distribution and kinetics of the ADH-1 isoenzyme with retinol and retinal. Exp Eye Res.;42(4):305-314.

Kinoshita JH (1955) carbohydrate metabolism of the lens.A.M.A. Arch.Ophthalmol.54:360-368.

Kisic B, Miric D, Zoric L, Ilic A ,Dragojevic I(2012) Antioxidant capacity of lenses with age-related cataract. Oxidative Med. Cellular Longevity, Article ID 467130, 8 pages doi:10.1155/2012/467130.

Laemmli UK(1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Lambert SR, Amaya L, Taylor D (1989) Detection and treatment of Infantile Cataracts. 1nt Ophthalmol Clinics. 29:5 1 -6.

Laspas P, Goloborodko E, Sniatecki JJ, Kordasz ML, Manicam C, Wojnowski L, Li H^2, Patzak A, Pfeiffer N, Gericke A.(2014) Role of nitric oxide synthase isoforms for ophthalmic artery reactivity in mice.Exp Eye Res. 127:1-8.

López-Soler N, Cervera A, Moores GD, Martínez-Pardo R, Garcerá MD (2008) Esterase isoenzymes and insecticide resistance in *Frankliniella occidentalis* populations from the south-east region of Spain. Pest Manag Sci. 64(12):1258-1266.

Lowry OH, Roseborough NJ, Farr AL, Randall R J (1951) Protein measurement with folin phenol reagent; J. Biol. Chem. 193**:** 265-275

Massoulie J (2002) The origin of the molecular diversity and functional anchoring of cholinesterases. Neurosignals 11:130-143.

Merin S , Crawford JS (1971)The etiology of congenital cataracts: a survey of 386 cases. Can J Ophthalmol. 6178- 182.

Nagai N, Ito Y (2007) Adverse effects of excessive nitric oxide on cytochrome C oxidase in lenses of hereditary cataract UPL rats. Toxicology.;242(1-3):7-15.

Radi ZA (2009) Pathophysiology of cyclooxygenase inhibition in animal models. Toxicol Pathol 37(1):1 34-146.

Sarkar S, Dubey AK, Banerji AP , Shah PN (1978) Patterns of lactate dehydrogenase isoenzymes during gonadogensis in the rat. J. Reprod. Fert. 53: 285-288.

Semina EV,Ferrell RE,Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC. (1998) A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. Nat Genet. 19(2):167-170.

Singh R, Kaurya OPS, Shukla PK, Ramputty R (1991) Lactate dehydrogenase (LDH) isoenzymes patterns in ocular tumours. 39(2):44-47.

Sorkhabi R, Ghorbanihaghjo A , Ahoor M (2011) Oxidative stress in pseudoexfoliation syndrome. Iranian J Ophthalmol 23(3):27-32.

Topal M , Gulcin I (2014) Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk J Chem 38: 894 - 902.

Vasavada AR, Cherian M, Yadav S, Rawal UM (1991) Lens epithelial cell density and histomorphological study in cataractous lenses. J Cataract Refract Surg. 17(6):798-804.

Wei et al. (2012) identified two phospholipase A (sPLA2) isoforms in lacrimal glands and epithelium of corneal and conjunctival of BALB/c and C57BL/6 mice.

Wiederholt M (1980) Physiology of epithelial transport in the human eye. Klin Wochenschr 58: 975–984..

Wisfrand PJ, Schenholm M, Lonnerholm G (1986) Carbonic anhydrase Isoenzymes CA I and CA II in the human eye. Invest Ophthalmol Vis Sci 27:419-428.

Wistrand PJ , Knuuttila KG(1980) Bovine lens carbonic anhydrases: purification and properties. Exp Eye Res. 30(3):277-290.