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PERCENTAGES OF SOLUBLE AND INSOLUBLE PROTEINS IN DIFFERENT TYPES OF CATARACTOUS LENSES COMPARED TO TOTAL PROTEIN WITH PHOTOGARPHIC APPERANCE

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ABSTRACT

Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours. Cataract is a multifactorial process in which many intrinsic and extrinsic factors act cumulatively. The percentages of soluble protein (SP) and Insoluble protein (ISP) with respect to total protein in different types of cataractous lenses. The lowest (14.05%) and highest (85.94%) value of SP and ISP respectively is found in Brown cataract. The highest (44.76%) and lowest (55.23%) value of SP and ISP respectively is found in CS-NS=PP type of cataract. A "heavy molecular weight aggregate" (HMW protein) with an apparent molecular weight in excess of $15 * 10^6$ can be isolated from the water soluble proteins by gel filtration

KEYWORLDS: proteins, NS-Nuclear sclerosis, PSC- Posterior sub capsular, CS- Cortical spoke, ISP- Insoluble proteins, SP- Soluble proteins.

INTRODUCTION

The word cataract is derived from the Latin word "Cataracta" meaning, cloudiness of the water fall' refers to the presence of an opacity in the normally clear and transparent ocular lens. The adjective "senile" has become attached to the most common of all forms of cataract, about the precise etiology of which nothing is known because cataract is a multifactorial process in which many intrinsic and extrinsic factors act cumulatively.

Most of the 4,00,000 cataracts extracted each year were discarded after a gross pathological examination, this was possible only because there was so little demand for human lenses for

study. The magnitude of this overburden of cataract in the developing countries is indicated by a survey in Punjab in India (Chatterjee et al., 1982).

Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours (Hiller et al., 1977, Zigman et al., 1979). Content wise, lens has a higher protein concentration than any other tissues and the concentration in the nucleus is greater than that in the cortex. The lens protein contributes 35% of the wet weight of the lens, and crystalline account for 80-90% of the soluble proteins of the lens.

The crystalline are generally referred to as structural proteins of the lens indicating that they are possibly responsible for the structural properties, consistency and transparency of the lens (De Jong, 1981). There major structural lens proteins are α, β & Γ crystalline. In cataractogenesis the level of Glutathione decline rapidly in lenses with increase in age (Ajit et.al, 2010). Since Glutathione is a substrate for ϒ- GTP, its decrease would inhibit the feedback mechanism thus lowering the activity.

MATERIALS AND METHODS

The soluble, insoluble, total proteins of the lens and total proteins of the AQH were determined by the standard method of Lowry et al., (1951).

Reagents and Chemicals

1. 0.3 N Sodium hydroxide solution
2. 2% Potassium-Sodium tartrate solution
3. 1% Copper Sulfate solution
4. 2% Sodium carbonate solution in 0.1 N Sodium hydroxide
5. Folin-ciocaulet Phenol reagent diluted with distilled water (1:2 ratio)
6. Alkaline copper sulfate solution (Fresh)

100 ml solution number 4

+1 ml solution number 2

+1 ml solution number 3

Procedure: The weighed lenses were homogenized in cold distilled water and centrifuged at 4,000 x g for 30 mins. The supernatant was used for determination of the soluble protein levels. The pellet was dissolved in known volume of 0.3 N NaOH and aliquote from this was taken for estimation of water insoluble proteins.

0.1ml of both the soluble and the insoluble protein sample solutions, 0.9 l of distilled water and 5.0 ml of alkaline copper sulfate solution were added. The contents of the test tube were mixed thoroughly and incubated at room temperature for 10 minutes. Then, to each tube 0.5 ml of Folin-phenol reagent was added, shaken immediately and incubated for 30 mins. at room temperature. The absorbance of the blue color sample, 0.03 M NaOH was used.

The protein concetration was calculated using the formula.

$$= \frac{530 \text{ X Dilution X Abs.}}{\text{Aliquote x Tissue wt.}}$$

The unit for protein concentration is ug/mg lens weight.

Fractionation of lens proteins

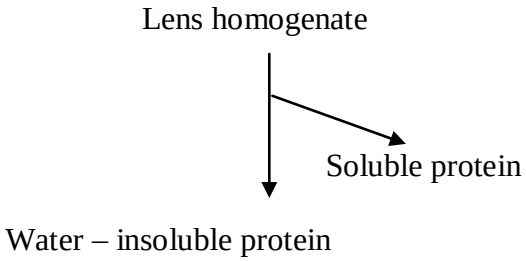
The water soluble, insoluble fractionation was done by the standard method of Coghland and Augusteyn (1977). Determination of protein was done by the method of Lowry et al., (1951).

Reagents and Chemicals

1. 8 M Urea
2. 8 M Urea containing 0.10 mM dithiothretol (DTT)
3. All regents used for protein estimation as mentioned earlier.

Procedure: The weighed lenses were homogenized in cold distilled water and centrifuged at 4,000 g for 30 mins. and the supernatant was used for determination of the water soluble proteins. The residues were dissolved in 8 M urea and centrifuged again. The supernatant obtained was used again dissolved in 8 M urea containing 0.1 nm DTT and centrifuged again.

The supernatant obtained was used for the estimation of yellow fraction of lens proteins. The residues were dissolved in 0.3 N NaOH and estimated as brown fractions of lens proteins. The procedure is summarized in figure.



RESULTS

Table-1 shows the level of TP, SP and ISP in different types of cataractous human lens. The amount of TP shows a variation within the range of 327.1 to 617.7 ug/mg/ The highest value

is found in Brown cataractous lenses and is 617.7 ± 44 ug/mg (mean ± s.e.). Whereas lowest value is found in Mature cataractous lenses and is 327.1 ± 32 ug/mg (mean ± s.e.). The level of TP in NS, PSC and NS-PSC-CS is found to be more or less the same. In rest of the cataractous lenses TP content shows a small variation. The same table also show a variation in the amount of Sp within the range of 86.8 – 177.9 ug/mg. The lowest value of SP is found in Brown cataractous lenses and is 86.8 ± 10 ug/mg (mean ± s.e.) Whereas highest value of SP is found in CS-NS-PP types of cataractous lenses and is 177.9 ± 12 ug/mg (mean ± s.e.). The level of SP in NS-PSC, PSC-CS and NS-CS types of cataractous lenses were found to be consistent. The table also shows variation in the amount of ISP within the range of 188.4 – 530.8 ug/mg. The highest value is found in Brown cataractous lenses and is 530.8 ± 32 ug/mg (mean ± s.e.).Whereas the lowest value is in PSC-CS types of cataractous lens and is 184.4 ± 20 ug/mg (mean ± s.e.).

Table 2 shows the percentages of Soluble protein (SP) and Insoluble protein (ISP) with respect to total protein indifferent types of cataractous lenses. The lowest (14.05%) and highest (85.94%) value of SP and ISP respectively is found in Brown cataract. The highest (44.76%) and lowest (55.23%) value of SP and ISP respectively is found in CS-NS=PP type of cataract.

The values for SP and ISP are similar in NS and NS-CS, also in SPC and NS-PSC. In other types of cataracts these values show slight variations compared to these cataracts.

The average values for total protein (TP), soluble protein (SP) and insoluble protein(ISP) in normal and cataractous human lenses are shown in this studies. It shows significant differences between normal and cataractous lens. There is significant increase in the level of ISP in cataractous condition where as significant decrease in the level of SP in cataractous lens compared to normal lens.

The change in amount of TP is negligible. With reference to TP there is insignificant difference between normal and cataractous lenses. The percentage of SP in normal and cataractous lenses ate 79.19% and 35.56% respectively, whereas that of ISP is 20.8 and 64.30 respectively compared to total protein. If shows increase in the level of ISP during cataractous condition. Significant difference exists between these two parameters in normal and cataractous lenses. All values are expressed as mean ± s.e. and p-value is less than 0.01. Age groups matched for these parameters.

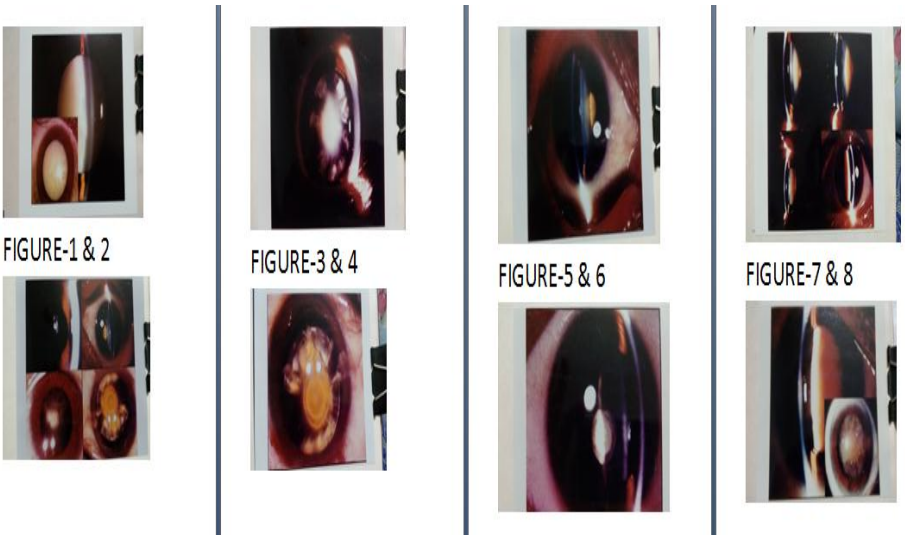
Table – 1: Total Protein, Souble Protein And Insoluble Protein Contents Of Cataractous Human Lenses

Type of Cataracts	TP (ug/ mg)	SP (ug / mg)	ISP (ug /mg)
NS (4)	415.9 ± 28	167.8 ± 15	248.1 ± 20
PSC (4)	423.2 ± 39	159.0 ± 13	264.2 ± 21
CS (5)	348.1 ± 37	151.9 ± 18	196.2 ± 21
NS, PSC (5)	384.5 ± 22	142.9 ± 11	241.6 ± 21
PSC, CS (6)	331.8 ± 31	147.4 ± 11	184.4 ± 20
NS, CS (6)	360.1 ± 31	144.6 ± 16	210.4 ± 21
NS, PSC, CS (6)	415.7 ± 30	134.8 ± 12	280.8 ± 25
CS, NS, PP (3)	397.4 ± 41	177.9 ± 12	219.5 ± 22
MATURE (5)	327.1 ± 32	117.2 ± 13	209.9 ± 17
BROWN (5)	617.7 ± 44	86.8 ± 10	530.8 ± 32

-------► All values are expressed as mean ± S.E.
-------► Number in the parenthesis is sample sizes.
-------► p-value ∗ < 0.01

Table – 2: Percentages Of Soluble Protein And Insoluble Protein In Different Types Of Cataractous Lenses Compared To Total Protein.

Type of Cataracts	S P %	I S P %
NS (4)	40.346	59.653
PSC (4)	37.570	62.429
CS (5)	43.636	56.363
NS, PSC (5)	37.165	62.834
PSC, CS (6)	44.424	55.575
NS, CS (6)	40.155	59.844
NS, PSC, CS (6)	32.427	67.572
CS, NS, PP (3)	44.765	55.234
MATURE (5)	35.830	64.169
BROWN (5)	14.052	85.947



DISCUSSION

The yellow and brown protein fractions are uniquely associated with different types of cataract especially nuclear cataract and increases with the progression of the cataract (Truscott and Augusteyn, 1977, Pandya A V and Rawal U M,2006). The brown color of lens in brown cataract is due to very high amount of brown fraction of protein. It contain about 637 times higher amount of brown fraction compared to yellow fractions of protein. The color of les is due to glycation and aggregation of lens proteins.

A "heavy molecular weight aggregate" (HMW protein) with an apparent molecular weight in excess of $15 * 10^6$ can be isolated from the water soluble proteins by gel filtration (Jedziniak et al., 1973) or by differential centrifugation (Roy and Specter, 1976). It has been suggested that this protein is an intermediate in the Insolubilization of lens proteins and that calcium play a key role in this process (Specter and Rothschild, 1973).

The cataractous protein appears to be stabilized by disulphide bonds whereas the protein from normal lenses is not (Dilley, 1975). The unsterilized protein appears and remain in aqueous humor and due to same reason the aqueous humor (AQH) protein content increases during cataractous conditions. It seems possible that ascorbic acid, GSH, NAD (P) H could form part of a protective system due to anti oxidative action in lens and AQH (Ajit Pandya, 2014). Evidence for the existence of such a system must await further experimental studies. It has been reported that the cataractous protein has a much lower tyrosine and much higher leucine content than normal protein (Jedziniak et al., 1973).

The slit lamp photography was carried out before cataract operation shows following figure 1 to 8.

The first photograph shows MATURE cataract, second NS-PSC-CS, third NS-CS, fourth PSC-CS, fifth NS-PSC, sixth PSC, seventh NS and eighth photograph shows BROWN cataract. The two types were deleted from record by mistakes and are not attached in this photographs.

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