Original Research Paper

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Ophthalmology

ANALYSIS OF AQUEOUS HUMOR PROTEINS IN MYOPIC PATIENTS

Dr Tojum Gongo Assistant Professor, Department of Ophthalmology, TRIHMS, Naharalgun-791110, Arunachal Pradesh

ABSTRACT **Introduction:** myopia is the most common eye disease in the world in with substantial social, educational and economic impact. Some studies have shown changes in aqueous humour proteins in myopic patients. **Aim:** To estimate total protein concentration, types of proteins in aqueous humour and the correlation with myopic patients. **Material and Methods:**This is a Prospective study conducted on 36 eyes of 36 patients attending Department of Ophthalmology, TRIHMS, Naharlagun, who were undergoing cataract operation. Group A served as a control group with 22 eyes of non myopic patients. Group B consisted of 14 eyes of 14 myopic patients having axial length more than 26mm.Aqueous humour collected from both the groups during cataract surgery was sent for Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). **Result:** Significant difference in relative bands width (%) in the gel patterns in myopic and non myopic patient was seen.Myopic patients had higher magnitude of protein expressions /bands in molecular weights being 55kDa (Transthyretin), 69kDa (Albumin) and 110kDa (Vitamin-D binding protein) than non-myopic patients. In non myopic patients ,70-90 kDa (Heat shock Protein) were very highly expressed than myopic patients. **Conclusions:** Aqueous humour proteins were estimated to be different between myopic and non myopic patients significantly. These proteins can be candidates for broadening of our existing knowledge of the pathophysiological characteristics of myopia. They may help in early diagnosis and monitoring of the myopic patients can be done. They may also help in deeper understanding of mechanism which cause axial elongation in myopia.

KEYWORDS : aqueous humor proteins, myopia, SDS-PAGE

INTRODUCTION:

Myopia is the most common ocular abnormality. Its prevalence varies among different ethnic groups, being least in Blacks and greatest in Asians [1,2]. It has been reported that 30% to 70% of high myopes display at least some lesions of retina and choroid [3,4].

It has been demonstrated that various tissues of the eye are involved in the pathogenesis of high myopia which is associated with active scleral elongation and tissue remodelling[5].An excess of GO/GROW signals or a defect of STOP signals is thought to underlie the excessive longitudinal growth of the ocular globe observed in myopic subjects[6].Aqueous humor is an important intraocular fluid responsible for the supply of nutrients to and removal of metabolic wastes from the avascular tissue of the eye[7]. Most of the recent insights regarding high myopia have come from the studies on animal models. It is known that protein levels in aqueous humor are changed in various eye diseases. Not only had such changes in aqueous humor proteins observed in anterior segmentdisordersbut also in posterior chamber disorders [8,9,10,11,12]. In addition an increasing number of studies have demonstrated that some proteins that change in age correlate with mechanisms or prognosis of many eye disorders[13,14,15]. Duan *et al*,2008, reported that the total protein concentration in the aqueous humor of high myopia was significantly greater than that of non-myopia.[16]

Frost and Norton (2007) undertook a more neutral proteomic analysis using two-dimensional gel electrophoresis and mass spectrometry to identify scleral proteins that are differentially expressed during development of and recovery from lens induced myopia in tree shrew [17]. They found that in the recovering eyes 78 kDa glucose regulating protein (GRP) , a member of Heat Shock Protein70 families, was slightly up regulated 1.3 fold. This means that there must be some factors or regulators which down regulates GRP 78 in myopia. GRP 78 is a chaperone , facilitating multimeric protein assembly in the endoplasmic reticulum that recognises and binds to malfolded or denatured proteins such as type-1 procollagen [18,19].

AIMS AND OBJECTIVES:

1. To estimate protein total protein concentration in aqueous humor in myopic patients.
2. To identify various types of proteins in aqueous humor of myopic patients.
3. To find any correlation between proteins in aqueous humor and axial length of the eyeball.

Materials and methods

The present study was conducted on 36 eyes of 36 patients attending in OPDs of Department of Ophthalmology in TomoRiba Institute of Health and Medical Sciences (TRIHMS), Naharlagun, Arunachal Pradesh.

Group A served as control group consisting of 22 eyes of 22 nonmyopic patients undergoing cataract surgery and having axial length less than 25mm.

GroupB served as a Study group consisting of 14 eyes of 14 myopic patients undergoing cataract surgery and having axial length more than 26mm.

All the patients were evaluated clinically and details were documented on specially designed Performa(Appendix-1). A thorough clinical history was taken from all the patients. A baseline ocular examination for measurement of visual acuity, refractive status, slit lamp examination, direct and indirect ophthalmoscopy, and applanation tonometry was done in all patients. Special emphasis was given to keratometry and A-scan Ultrasonography (for axial length, anterior chamber depth, lens thickness and posterior segment length.

Aqueous humor samples were collected from all the patients recruited in the study before ocular incision during cataract surgery. Approximately 100-200 µl (microliter) of aqueous humor was collected by 26 gauge cannula fitted on tuberculin syringe under binocular microscope. The aqueous humor was immediately stored in the microtubules at -18 degree Celsius until further analysis. Total protein was estimated in all the aqueous humor samples by Bradford method. Different proteins were identified by SDS-PAGE and silver staining.

Statistical analysis was done using IBM SPSS 27 -2019 for windows software. Unpaired t-test was applied to analyse statistical significance of change in aqueous humor protein concentration between two groups. Pearson Correlation Coefficient was used to find out correlation between various variables in the study. P value <0.05 was taken as significant.

Results

Age and gender wise distribution of patients in various groups:

AGE RANGE (years)	GROUP A		GROUP B		TOTAL
	MALES No. (%)	FEMALES No. (%)	MALES No. (%)	FEMALES No. (%)	
0-1	1(4.55)	_		_	1(2.78)
11-20	_	_	1(7.14)	_	1(2.78)
21-30	_	_	_	1(7.14)	1(2.78)
31-40	_	2(9.09)	_	1(7.14)	3(8.33)
41-50	1(4.55)	5(22.73)	2(14.29)	1(7.14)	9(25.00)
51-60	4(18.18)	4(18.18)	1(7.14)	2 (14.29)	11(30.56)
61-70	3(13.63)	1(4.55)	_	3 (21.43)	7(19.44)

71-89	_	_	1(7.14)	1(7.14)	2(5.56)
81-90	_	1(4.55)	_	_	1(2.78)
TOTAL	9(40.91)	13(59.09)	5(35.71)	9(64.29)	36(100)

In age Group A, age of patients ranged from 9 years to 85 years, with the mean age of 55.45+/- 15.14 years. The male to female ratio in this group was 0.7:1. The maximum number of patients were in the age group of 51-60 years i.e., 8 (36.36%) cases. In Group B, age of patients ranged from 19 years to 75 years, with the mean age of 51.79 ± - 16.43 years. The male female ratio in this group was 0.5:1. The maximum number of patients were in the age groups pf 41-50 years and 51-60 years i.e., 3 (21.42%) cases each.

Visual acuity in various groups:
The baseline visual acuity in Group A ranged from Hand Movement to 6/18 with maximum patients having a visual acuity of hand movement i.e., 8 (36.36%) cases. In group B, the baseline visual acuity ranged from Hand movement to 6/18 with maximum patients having a visual acuity of 1/60 i.e., 7 (50.00%) cases.

Baseline intraocular pressure in various groups:
The mean intraocular pressure in group A was 15.34 ± 2.108 mm of Hg, with a minimum and maximum intraocular pressure of 10.20 mm of Hg and 17.30 mm of Hg respectively. Whereas the mean intraocular pressure of group B was 16.74 ± 1.833 mm of Hg with a minimum and maximum intraocular pressure of 12.20 of Hg and 20.20 mm of Hg respectively. The difference between the intraocular pressures between the two groups was statistically insignificant with a p-Value of 0.5.

Mean of biometric variables in various groups:
The mean axial length in Group A, was 22.89±0.84 mm, whereas, in Group B the mean axial length was 27.77±1.83 mm. On the statistical analysis, the difference between the two groups was found to be statistically significant (t=9.39, p=<0.001)

The keratometric mean in Group A was 44.41 ±1.76 D, whereas, in Group B the keratometric mean was 44.40±2.35 D. On statistical analysis, the difference between two groups was found to be statistically insignificant (t=0.978, p=-0.028).

The mean of anterior chamber depth in Group A was 2.76±0.44 mm, whereas, in Group B the mean anterior chamber depth was 2.90 ±0.57 mm. On statistical analysis, the difference between the two groups was found to be statistically significant (t=0.792, p=0.156).

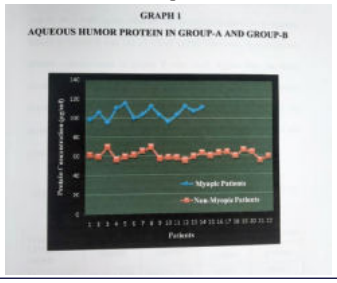
The mean of lens thickness in Group A was 3.816 ±0.767mm , whereas , in Group B, the mean of anterior chamber depth was 3.99± 0.57 mm. On statistical analysis, the difference between the two groups was found to be statistically insignificant (t=0.133,p=0.552).

The mean of posterior segment length in Group A was 16.25 ±1.17 mm, whereas, in Group B the mean posterior segment length was 19.54 ±16.43 mm. On statistical analysis , the difference between the two groups was found to be statistically significant (t=4.868,p < 0.001).

Aqueous humor protein levels in various groups:
In group A, 12 (54.55%) patients had aqueous humor protein concentration in the range of 61-70 µg/ml.

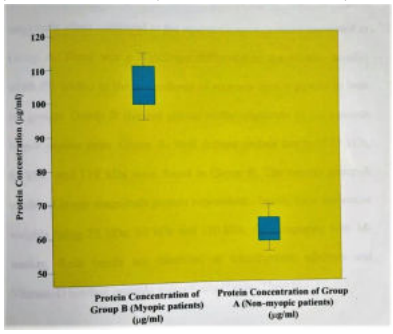
In group B, 5 (35.71%) patients each had aqueous humor protein concentration in the range of 101-110 µg/ml and 110-129 µg/ml respectively, whereas, 4 (28.58%) patients had aqueous humor protein concentration in the range of 91-100 µg/ml .

The aqueous humor protein concentration in Group B was higher than the aqueous concentration in Group A.



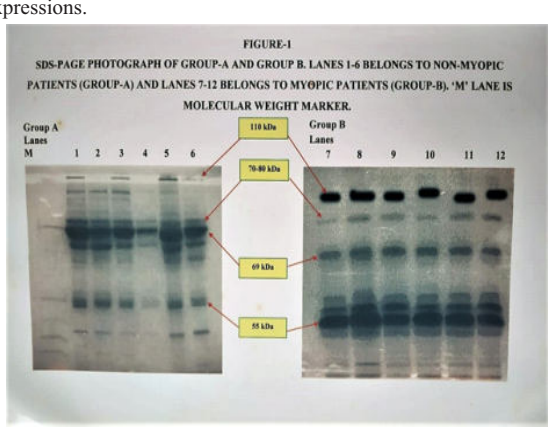
Mean Aqueous humor protein levels in various groups:
The mean protein concentration in aqueous humor in Group A was 61.06 -=4.06 µg/ml, whereas in Group B it was 105.27=-6.41 µg/ml. The mean aqueous humor protein concentration in Group B was much higher than the mean aqueous humor protein concentration in Group A. The difference between the mean aqueous humor protein concentration of the aforementioned groups was statistically significant (t value=23.02 and p value =.001).

GRAPH-2
THE BOX CHART SHOWING THE MEAN STANDARD DEVIATION AND THE 9% CONFIDENCE INTERVAL OF AQEOUS HUMOR PROTEINS IN GROUP A(NON MYOPIC PATIENTS) AND GROUP B(MYOPIC PATIENTS)



Analysis of electrophoretic pattern of aqueous humor proteins in various groups:

On Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the aqueous humor of the two groups, remarkable differences were noted in their electrophoretic patterns. In Group B, the expression of aqueous humor proteins/bands was of higher magnitude when compared to aqueous humor proteins expressed in Group A. There is a significant difference in the band(s) width (%) in the gel patterns of aqueous humor protein in both the groups. Group B showed greater width/magnitude of the aqueous humor protein than Group A. Well defined protein bands of 55kDa, 68 kDa and 110kDa were found in Group B. The control Group A exhibited lower magnitude protein expressions /bands, their molecular weights being 55kDa,69 kDa and 110 kDa. On comparing with M-marker, these bands are identified as transthyretin, albumin and Vitamin D binding protein respectively. Interestingly, band (s) of 70-90kDa were highly expressed in Group A. On the contrary, it was poorly expressed in Group B. Further studies are warranted by employing monoclonal antibodies against heat shock proteins in order to confirm their expressions.



Correlation between concentration of aqueous humor protein and biometric variables of all proteins:

There was a positive correlation between the axial length of the eye under study and protein concentration in the aqueous humor of all the patients. On statistical analysis, this positive correlation was found to be highly significant (r=0.884 and p <0.001).

There was a weak positive correlation between the aqueous humor protein concentration and the anterior chamber depth of the eye under study, but this positive correlation was statistically insignificant (r=0.178 and p=0.308).

There was a weak positive correlation between the aqueous humor protein concentration and the lens thickness of the eye under study, but this positive correlation was statistically insignificant (r=0.028 and p=0.873).

There was apositive correlation between aqueous humor protein concentration and posterior segment length of theeye under study. On statistical analysis, this positive correlation was found to be highly significant. (r=0.638 and p <0.001).

CONCLUSIONS

On the basis of observations made in the present study, following conclusions were drawn:

1. Posterior segment length was directly proportional to axial length. Myopic patients with greater axial lengths had larger posterior segment length.

2. Total protein levels in aqueous humor were higher in myopic patients than non myopic patients.

3. On sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the aqueous humor of myopic and non myopic patients , a significant difference in the relative band(s) width (%) in gel patterns of aqueous humor protein was observed between the groups. The myopic patients showed greater width /magnitude of the aqueous humor protein than non-myopic patients.

4. On SDS-PAGE, in myopic patients, there were higher magnitude of protein expressions/bands, their regular weights being 55kDa (Transthyretin),69 kDa(albumin) and 110kDa (Vitamin –D binding protein) then non myopic patients.

5. On SDS-PAGE, in nonmyopic patients ,70-90 kDa(Heat shock Protein) were very highly expressed than myopic patients.

6. There was statistically significant positive correlation between the axial length and protein concentration in the aqueous humor of all the proteins.

7.There was statistically significant positive correlation between posterior segment length and aqueous humor protein concentration of all the patients.

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