Original Article

Assessment of changes in biochemical profile in primary open angle glaucoma patients presenting at a tertiary care hospital

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Abstract

Objective: To assess the changes in high sensitivity C Reactive protein (hs-CRP), serum cholesterol, uric acid, creatinine, bilirubin, ALT, Hb (hemoglobin) and TLC (Total leukocyte count) In patients of primary open-angle glaucoma (POAG).

Materials and Methods: Comparative cross-sectional study conducted between April 2021 and October 2021 at departments of Ophthalmology and Pathology, Fauji Foundation hospital Rawalpindi. 44 POAG patients and 54 healthy controls volunteered to participate in the study. POAG was diagnosed as per criteria. Patients were graded in to mild, moderate and severe groups by ophthalmologist according to established criteria. Venous blood was drawn for analysis of serum cholesterol, uric acid, creatinine, bilirubin, ALT and hs-CRP. hs-CRP was performed on ELISA plate reader Platos R496 while spectrophotometric analysis of serum uric acid, creatinine, and cholesterol was carried on Beckman Coulter AU-700.

Results: Mean Hb, TLC, serum urea, creatinine, uric acid, ALT, bilirubin and cholesterol were 12.80 ±1.50g, 8.88±1.92 mm3, 5.95±5.47 µmol/l, 92.19±21.81 µmol/l, 305.85±79.92 mmol/l, 34.31±18.26 IU/L, 9.26±3.11 µmol/l, 5.18±0.96 mmol/l respectively whereas the Mean IOP in POAG patients was 28mmHg, mean CCT was 516.6 µm, and mean vertical CDR was 0.6.

A High frequency of hs-CRP positivity (50 percent) was reported in our patients. Significantly lower uric acid levels were observed in primary open angle glaucoma patients versus controls i-e 305.85±79.92 mmol/l vs 344.36±37.24 mmol/l (p-value < 0.05). Serum creatinine was significantly different between mild, moderate and severe groups i.e. 82±9.0 vs 90.5±15.1 102.1±7.1. (p-value <0.001*)

Conclusion: High frequency of hs-CRP positivity and low uric acid levels suggest the presence of para inflammation in patients of POAG.

Keywords: Primary open-angle glaucoma (POAG), intraocular pressure (IOP), high sensitivity CRP (hs-CRP), uric acid (UA)

Introduction

Glaucoma is a devastating neurodegenerative disease that causes irreversible blindness.(1) It is estimated that by 2040 there would be a 45 percent increase in glaucoma cases from 76 million to 111 million worldwide. (2) As per Khan et al Pakistan has 8 million glaucoma sufferers. (3) According to a study by PS Mahar et al. amongst glaucoma cases 41.6% had primary open angle glaucoma (POAG)which makes it one of the most common type of glaucoma (4).

One of the most important risk factors involved in the pathogenesis of POAG is raised intraocular pressure, others being family history, ethnicity, old age, diabetes and hypertension. However, Insight in to the detailed mechanism of glaucoma especially POAG is important as less investigated risk factors pertaining to systemic inflammatory response especially biomarkers have been found to play a crucial role in several latest studies (5)(6). So there is a dire need of finding noninvasive biomarkers that may give clues to the presence of the disease early on and also aid in disease progression and assessment of its severity (7).

Neuro Inflammation and oxidant stress are the hall marks of POAG. This process is regarded as para-inflammation. The chemical mediators involved are similar to other inflammatory diseases. Similarly, In POAG cases rise in interleukins, hs-CRP, fibrinogen and transforming growth factor (TGF) beta is observed(6). Benoist et al 2016 carried out a meta-analysis of several studies that measured the total antioxidant status and oxidative stress markers in POAG patients. He found ascorbic acid and alpha-tocopherol which are part of the anti-oxidant system were significantly decreased in POAG patients(8).

Biomarkers like Interleukin 6, hs-CRP, serum cholesterol and neutrophil-lymphocyte ratio(NLR) ratio have been used as biomarkers for determining oxidative stress and disease progression in glaucoma(9)(10). hs-CRP is an acute phase protein well known for its role in inflammatory disorders. Al-Samak et al while investigating the relationship between vascular inflammation and glaucoma found significantly raised CRP levels in POAG(11). Uric acid and other routine biomarkers have been a subject of investigation in POAG and other types(12). Uric acid is routinely performed in the lab in the investigation of gout. Reduced GFR leads to under-excretion of uric acid and hence it's raised levels. Besides that, it is a known antioxidant and makes up about two third of the total anti-oxidant system(13).Similarly, many studies have found a positive correlation between glaucoma and raised blood cholesterol levels. Dube M et al. found significantly higher cholesterol, triglyceride and LDL levels in glaucoma patients when compared with controls(5)(10).

Our tertiary care hospital is catering to families of pensioners. Among other diseases one of the most common ocular diseases prevalent is glaucoma. By using simple biochemical tests, we tried to find the association between their derangements with POAG. This could yield evidence that would provide grounds for future recommendations pertaining to the testing of these indices for possible diagnosis of POAG, assessment of its severity thus reducing the sequelae associated with it.

The objective of the study was to measure changes in serum biomarker profile in POAG patients and to quantify the relationship between these markers including hs-CRP and Creatinine with glaucoma severity.

Materials and Methods

This case control study was conducted at the outpatient department of Ophthalmology and Pathology department Fauji Foundation hospital Rawalpindi after approval from institutional review board.

Forty-four POAG patients and fifty-four healthy controls were included in the study after taking written informed consent between April 2021 and October 2021.

A sample size of 94 was calculated by Rao soft calculator keeping confidence level 95 percent. POAG was diagnosed with at least two readings of IOP above 21 mm Hg by Goldmann tonometry, an open angle on gonioscopy or Visual field defects on Humphrey perimetry (14).

Exclusion criteria included Patients presenting with secondary causes of glaucoma, normal tension glaucoma and acute angle closure glaucoma. Patients with history of systemic disease or suffering from any cause of hyperuricemia. Patients taking lipid lowering drugs or patients suffering from renal failure.

Baselines demographic data including age, gender, family and drug history were recorded on a structured proforma. A thorough ophthalmic examination of POAG patients and controls was carried out by an experienced eye specialist. Glaucoma cases were graded as mild, moderate and severe based on visual field defects on Humphrey perimetry (14).

A 7 ml venous blood sample was taken from POAG patients for pathology lab tests. A 2ml was transferred to EDTA tube for Hb, TLC analysis while the rest 5ml was taken in clot activator tube after centrifugation at 3500 for 3 min, serum was extracted for chemical pathology tests i-e serum cholesterol, uric acid, creatinine, bilirubin and hs-CRP.

A Blood CP sample was taken in EDTA tube and run on a fully-automated hematology analyzer Sysmex XN-1000. The serum samples were analyzed on fully automated chemistry analyzer Beckman coulter AU-700 by spectrophotometry. Serum uric acid was analyzed by uricase, serum cholesterol by cholesterol oxidase CHOD-PAP, serum creatinine by Jaffe kinetic, serum ALT by IFCC method and serum bilirubin by the modified diazo method using Beckman Coulter reagents after verifying internal quality control. hs-CRP testing was carried out on ELISA 96 microwell plate reader Platos 496 using Amgenix international kits by the immunoturbidimetry method. Estimated GFR was calculated using the CKD-EPI equation for each participant(15).

All the results of tests and demographic data were entered in SPSS and were analyzed using SPSS version 25. Frequencies and percentages were calculated for qualitative variables and mean SD for quantitative variables. Means were compared using independent t-test between POAG patients and controls where as One way ANOVA was used for the comparison of means between different grades of glaucoma severity and ocular and biochemical characteristics of POAG and study participants.

Results

A total of 94 subjects i.e. 44 POAG patients and 54 age and gender-matched healthy volunteers participated in the study. The age range was between 40 and 75 years. Forty-two females and two males comprised POAG patients that participated in the study.

Mean age of POAG patients was 55 years. Mean Hb, TLC, serum urea, creatinine, uric acid, ALT, bilirubin, cholesterol and eGFR were 12.80 ±1.50 g, 8.88±1.92 mm^3, 5.95±5.47 µmol/l, 92.19±21 µmol/l, 305.85±79 mmol/l, 34.31±18 IU/L, 9.26±3.11 µmol/l, 5.18±0.96 mmol, and 67 ml/min respectively. (Table 1)

Table 1: Demographic and laboratory test parameters of the study population

Parameters	*Patients n=44*	*Controls N=54*	*P-value*
Hb	12.80 ±1.50	12.85±1.22	0.88
TLC	8.88±1.92	7.62±1.81	0.003**
Urea µmol/l	5.95±5.47	5.18±1.87	0.38
Creatinine µmol/l	92.19±21.81	91.18±14.35	0.50
Uric acid mmol/l	305.85±79.92	344.36±37.24	0.001*
ALT IU/L	34.31±18.26	35.56±7.01	0.67
Bilirubin µmol/l	9.26±3.11	9.80±1.55	0.06
Total cholesterol mmol	5.18±0.96	4.80±.71	0.02*
hs-CRP ng/ml	7082.43±3377.19	2022.0±1000.0	0.001

TLC (Total leukocyte count), ALT (Alanine aminotransferase), hs-CRP (high sensitivity C reactive protein)

P value <0.05 was considered significant

Independent t test was applied to compare means of the quantitative variables

Whereas mean IOP in POAG patients was 28mm Hg, mean CCT was 516.6 µm, and mean vertical CDR was 0.6. Among POAG patients, the rate of hs-CRP positivity was 50 percent. (Figure 1)

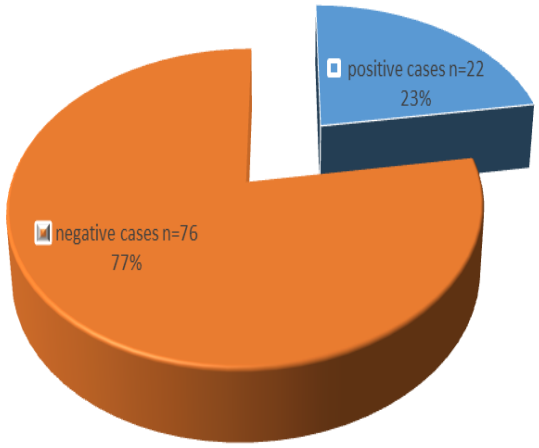

Figure 1: Frequency of hs-CRP Positivity in study population n=98

Means of quantitative variables i.e. Hb, TLC, urea, uric acid, creatinine and Hs-CRP were compared in POAG patients and controls using an independent sample t test. (Table 1) Uric acid and hs-CRP were significantly different in healthy controls from patients i.e. 305.85±79 mmol/l vs 344.36±37 mmol/land 7082.43±3377.19 ng/ml vs 2022.0±1000.0 ng/ml respectively. ($p<0.05$) where as other biochemical variables were not found to be significantly different among POAG group and control group. (Table 1)

Mild cases of glaucoma were the most frequent being 18 cases (41%) whereas moderate and severe POAG was noted in 17(40%) and 9 (19%) patients respectively.

Means of biochemical parameters were compared with different grades of glaucoma severity by applying One way ANOVA with post hoc LSD test. Among ocular parameters VCDR and CCT were significantly different among mild moderate and severe groups. ($p<0.05$) (Table 2)

Significant difference was observed among biochemical parameters for creatinine and estimated GFR between different grades of POAG severity. (Table 2) p value <0.05 was considered significant. Similarly, creatinine levels for these three groups were 82.1±9.0 vs 90.5±15.1µmol/l vs 102.1±7.1 µmol/l (p value <0.001). eGFR ranged between 47ml/min and 110 ml/min. Inverse relation was seen between POAG severity and eGFR. Lowest eGFR mean 55 ml/min was reported for severe POAG group whereas highest was reported for mild POAG group. (Table 2), (Figure 2)

Table 2: Biochemical and eye parameters among different grades of glaucoma severity

Parameters	*Mild n=18*	*Moderate n=17*	*Severe n=9*	*P value*
	Mean± SD	Mean ± SD	Mean± SD	
TLC mm	8.24±1.55	9.7±2.8	8.25±1.28	0.002*
Creatinine µmol/l	82.1±9.0	90.5±15.1	102.1±7.1	0.001*
eGFR ml/min	73±11	66±14	55±10	0.005*
VCDR	0.50±0.24	0.62±0.15	0.86±0.137	0.001*
CCT µm	526.4±42.3	513.7±44.4	501.4±40.5	0.13

TLC (Total leukocyte count), e GFR (Estimated GFR), VCDR (Vertical cup disk ratio), CCT (Central corneal thickness)

P value <0.05 was considered significant

One way ANNOVA with post hoc LSD was applied to compare means between three groups of glaucoma patients

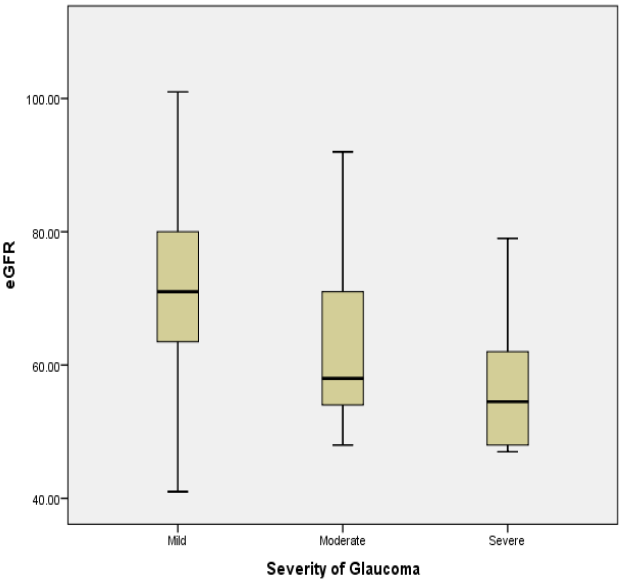

Figure 2 Distribution of e GFR among three grades of glaucoma severity

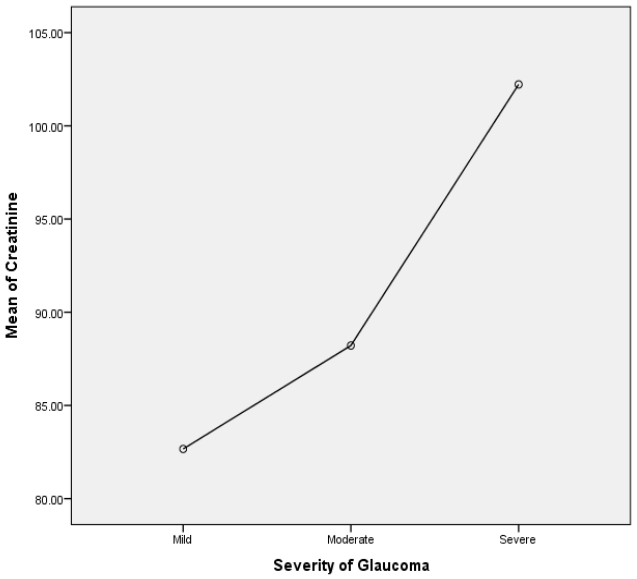

Figure 3: Serum Creatinine levels among POAG groups

Discussion

In this study, we sought to investigate the derangement in hs-CRP and other routine biochemical markers in POAG patients. The principal result of our study was the finding of a high rate of hs-CRP positivity, significantly raised cholesterol levels and reduced uric acid levels in POAG patients. This result points out other risk factors that may be involved in the pathogenesis of POAG besides the commonly assessed tool i.e. raised intraocular pressure.

Al-Samak et al found significantly raised CRP levels suggesting vascular inflammation in POAG patients. (11) Similarly Stefan C in a study on 14 glaucomatous subjects found positive association between raised CRP and glaucoma(9).The raised hs-CRP is in accordance with the hypothesis that glaucoma causes systemic inflammation. Other inflammatory markers like interleukins have also been found to be deranged in glaucoma in a similar background environment of oxidative stress(6). Hou P observed that treated cases of POAG had reduced CRP levels(16).On the contrary in the Beijing eye study raised CRP was found in diabetic retinopathy and no relation with glaucoma was established(17).Since the genetic makeup of our study population is different from Chinese this may have caused variation in results. Studies on gene polymorphism in POAG may give insight in to the detailed pathophysiology and candidate genes involved in individual study populations(18).

Limited numbers of studies have been done globally to check the association between POAG and uric acid. In a study by Li S et al serum uric acid level was about 12.7% lower than the healthy comparison group (12). This corroborates the results of our study; consolidating uric acid´s status as an antioxidant. In combating oxidant stress uric acid is depleted hence decreased levels in POAG patients in our study.

In a meta-analysis, it was deduced that serum cholesterol and triglycerides were significantly increased in patients with ocular pathologies associated with raised intraocular pressure(10). Another study by Dube M et al. found a positive correlation between dyslipidemia and POAG. Serum cholesterol was found to be elevated in POAG patients (5). One possible mechanism is the decreased anti-oxidant activity leading to increased oxidant stress, decreased removal of lipid peroxidation products and thus the acceleration of atherosclerosis. However, triglycerides and VLDL were not done in our POAG patients because of non-fasting samples so the derangement in triglyceride and LDL levels could not be assessed.

Inverse correlation was observed for serum creatinine and eGFR with different grades of glaucoma severity. eGFR less than 50ml/min was observed for two cases in the severe group. This explains the raised creatinine in the severe group relative to other groups. This is in accordance with the results of the meta-analysis which proves the bidirectional association of POAG with CKD (19). Ng Fung et al found that individuals with glaucoma had three-fold increased risk of CKD. In a study by Shim S, lower eGFR was found to be an independent risk factor for the development of POAG (20). eGFR reflects the rate of glomerular flow. Low eGFR translates to decreased removal of toxic substances including reactive oxygen species which are responsible for the para inflammation in the ocular trabecular meshwork and canal of schlem. Similarly, YC Tham et al. found in the Korean subjects association of CKD particularly at eGFR lower than 45 ml/min with POAG (21).

Since the study was conducted on a relatively small sample size and a predominant female population, a large sample size and heterogeneity in gender would further validate the results. More studies on advanced biomarkers involving interleukins, proteomics and molecular markers may better aid in the diagnosis, prognosis, severity checking and management in glaucoma(1)(22).

Conclusion

We conclude that there is evidence of systemic inflammation in our POAG patients demonstrated by positive CRP results and low uric acid. This could help us in devising a plan to minimize the potential harmful sequale associated with severe disease including development of dyslipidemia and CKD with timely intervention and management.

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