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Original Research Article

Association between platelet aggregation parameters and primary open angle glaucoma

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ABSTRACT

Background: The aim of the research was to study association between platelet aggregation parameters and primary open angle glaucoma.
Methods: An analytical cross sectional study with comparison group was conducted on 120 patients (60 cases, 60 controls). All enrolled patients underwent a comprehensive ophthalmological examination which included visual acuity assessment and refractive error testing, slit lamp examination, intraocular pressure (IOP) measurement with Goldmann's applanation tonometer, Gonioscopy using Sussman 4 mirror gonioscope, optic nerve head examination by slit lamp and biomicroscopy using +90D lens, visual field analysis and mean deviation assessment using automated perimeter in both eyes, retinal nerve fiber layer (RNFL) analysis by optical coherence tomography (OCT), complete blood count (CBC) analysis for the study of platelet aggregation parameters, platelet count (PLT), platelet distribution width (PDW), plateletcrit (PCT), mean platelet volume (MPV), and platelet large cell ratio (P-LCR).
Results: Based on the direction of assessment, PCT was evaluated for prediction of glaucoma, a cut off of PCT <0.203 was found to be 83.3% sensitive and 53.3% specific. Severity of POAG did not show any significant association with any of the platelet aggregation parameters.
Conclusions: In the present study, we did not find a significant association of different platelet parameters with severity of POAG either.

Keywords: Primary open angle glaucoma, Platelet parameter, Vascular risk factors

INTRODUCTION

Glaucoma is a multifaceted disorder that impacts the optic nerve, leading to the gradual death of retinal ganglion cells (RGCs) and resulting in the excavation of the optic nerve head. If left untreated, this condition progresses to visual field loss and can ultimately cause irreversible blindness. It is the second most common cause of blindness globally, responsible for 12.3% of blindness cases.[1] Glaucoma is primarily understood through two key theories regarding its causative factors.

Mechanical theory

This theory argues that increased intraocular pressure (IOP) causes the laminar beams to stretch, damaging the axons of retinal ganglion cells.

Vascular theory

This theory suggests that glaucomatous optic atrophy is due to an insufficient blood supply to the optic nerve.[2]

The vascular theory of glaucoma proposes that vascular dysregulation may play a role in the pathogenesis of glaucomatous optic neuropathy. However, the precise mechanisms by which vascular factors contribute to the disease process remain areas of active research.

This study suggests a potential link between platelet parameters and glaucoma risk. Larger platelets, known for their increased activity in hemostasis, may indicate a higher risk for conditions such as coronary thrombosis. Incorporating platelet parameters into routine hematological assessments could potentially aid in identifying individuals at risk for glaucoma-related complications. Understanding these diverse factors is essential for developing effective strategies in glaucoma prevention, early detection, and personalized treatment approaches.

Platelet function and their interactions with endothelial cells are critically important in the microcirculation of the eye, as evidenced by both clinical observations and experimental studies. Research indicates that platelet coagulation and inflammation directly affect the endothelium of Schlemm's canal in primary open-angle glaucoma (POAG).[3]

Platelet distribution width (PDW) parameter indicates the variation in the size of platelets in the bloodstream. A higher PDW value suggests a greater variation in platelet size, which can be indicative of platelet activation and changes in their morphology.[4] Plateletcrit (PCT) parameter represents the proportion or fraction of platelets in the total blood volume. It is a measure of the concentration of platelets circulating in the blood.

In summary, this research aims to provide critical insights into the underlying mechanisms of glaucoma, particularly regarding platelet-related factors, and emphasizes their clinical relevance in managing this debilitating eye condition.

METHODS

Type of study

It was a hospital based analytical cross-sectional study with comparison group.

Selection of cases

Cases were selected from glaucoma clinic of department of ophthalmology, RAMA Medical College, Kanpur. The study was done over a period of 1 year (January 2023 to December 2023).

Inclusion criteria (cases)

Patients of either gender between the ages of 40-60 years, patients detected to have glaucoma based on IOP more than 21 mmHg, vertical cup and disc ratio above 0.7, inter-eye asymmetry of above 0.2 with notching, neuroretinal rim thinning, retinal nerve fiber layer defect (detected on OCT), and patients with visual field defects (typical of glaucoma) were included as cases.

Exclusion criteria (cases)

Patients on antiplatelet or anticoagulant medication in the past 6 months such as aspirin, clopidogrel, and warfarin; patients with haematological disorders such as aplastic anaemia and primary thrombocytosis; patients with abnormal coagulation functions; patients with severe cardiovascular (CHF), renal or hepatic disorders; patients with acute infections, autoimmune diseases, cancers or thyroid dysfunction; patients not willing to participate in the study (non-consenting); patients having isolated raised IOP i.e. ocular hypertension, secondary causes of glaucoma (angle closure glaucoma), ocular abnormalities preventing good measurement of IOP, and any previous eye surgery or laser therapy were excluded as cases.

Selection of controls

Healthy controls were selected from ophthalmology department, RAMA Medical College, Kanpur.

Inclusion criteria (controls)

Patients of either gender between the ages of 40-60 years, patients with IOP lesser than 21 mmHg with normal optic disc (VCDR<0.5) and no clinical evidence of glaucoma were included as controls.

Exclusion criteria (controls)

Family history or personal history of glaucoma; complaints of eye discomfort; prior ocular trauma or surgery; severe systemic diseases, receiving antiplatelet/anticoagulant medications, eye diseases, IOP ≥21 mmHg, VCDR >0.5, thyroid dysfunction, cardiovascular diseases, hepatic diseases, renal diseases, acute infectious diseases, autoimmune diseases, recent surgery, and cancer were excluded as controls.

Methodology

Proposed sample size was 120 patients (60 case, 60 controls). All the enrolled patients underwent a comprehensive ophthalmological examination which included visual acuity assessment and refractive error testing; slit lamp examination (Labomed EVO300 model); IOP measurement with Goldmann's applanation tonometer (Haag-streit); gonioscopy using Sussman 4 mirror gonioscope; optic nerve head examination by slit lamp and biomicroscopy using +90D lens; visual field analysis and mean deviation assessment using automated perimeter (Oculus centrefield, Zeiss) in both eyes; RNFL analysis by OCT (NIDEK); and complete blood count (CBC) analysis for the study of platelet aggregation parameters. The parameters to be tested were: platelet count (PLT), platelet

distribution width (PDW), plateletcrit (PCT), mean platelet volume (MPV), and platelet large cell ratio (P-LCR). Blood samples for platelet parameters were taken in laboratory tubes with: a minimum of 1 ml of whole blood was taken as sample, following standard venipuncture of the veins in the antecubital fossae; ethylenediamine-tetraacetic acid (EDTA) raised bottom microtainer was filled above the 250 ul line; sample was sent to pathology lab within 6 hours of collection; sample was analyzed using an automated hematology analyzer (ERVA H360 3 part analyser).

Statistical tools employed

The outcomes evaluated in the present study were continuous/parametric in nature. Hence, a parametric evaluation plan was adopted. Data has been represented as numbers and percentages for qualitative data and mean±standard deviation for continuous data. Data analysis was performed using IBM statistical package for the social sciences (SPSS) stats 25.0 version software. Chi-square test, independent samples 't'-test and analysis of variance (ANOVA) were used to compare the data. Receiver operator characteristic curve analysis was used to evaluate the discriminant value of platelet aggregate parameters. A brief description and formulas used for different statistical evaluations is as follows.

Mean

To obtain the mean, the individual observations were first added together and then divided by the number of observations. The operation of adding together or summation is denoted by the sign Σ. An individual observation is denoted by the sign X, number of observations denoted by n, and the mean by $\bar{X}$.

$$\bar{X}=\frac{\sum X}{\text{No. of observations (n)}}$$

Standard deviation

It is denoted by the Greek letter σ.

$$\sigma=\sqrt{\frac{\sum(X-\bar{X})^2}{n}}$$

Chi square test

$$x^2=\sum\frac{(O-E)^2}{E}$$

Where O=observed frequency, E=expected frequency.

Independent samples 't' test

To test the significance of two means the student 't' test was used.

$$t=\frac{\bar{X}_1-\bar{X}_2}{S\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$$

Where $S^2=\frac{(N_1-1)SD_1^2+(N_2-1)SD_2^2}{N_1+N_2-2}$

Where $\bar{X}_1$and $\bar{X}_2$ are means of group 1 and group 2; N_1, N_2 are number of observation group 1 and group 2 SD_1, SD_2 are standard deviation in group 1 and group 2.

Analysis of variance

The ANOVA test was used to compare the within group and between group variances amongst the study groups. Analysis of variance of different study groups at a particular time interval revealed the differences amongst them. ANOVA provided "F" ratio, where a higher "F" value depicted a higher inter-group difference.

$$F=\frac{Mean\ of\ sum\ of\ between\ group\ differences}{Mean\ of\ sum\ of\ within\ group\ differences}$$

Table 1: Mean of sum of between and within groups differences.

Differences	Sum of squares	df	Mean square	F
Between groups	A	N_1	$X=A/N_1$	X/Y
Within groups	B	N_2	$Y=B/N_2$	

ROC curve

To draw a ROC curve, only the true positive rate (TPR) and false positive rate (FPR) are needed. TPR determines a classifier or a diagnostic test performance on classifying positive instances correctly among all positive samples available during the test. FPR, on the other hand, defines how many incorrect positive results occur among all negative samples available during the test.

In signal detection theory (detection theory, or signal detection theory, is a means to quantify the ability to discern between signal and noise; much of the early work in detection theory was done by radar researchers), a receiver operating characteristic (ROC), or simply ROC curve, is a graphical plot of the sensitivity (sensitivity, or recall rate, is a statistical measure of how well a binary classification test correctly identifies a condition, whether this be medical screening tests picking up on a disease, or quality control in factories deciding if a new product is good enough to be sold. The results of the screening test are compared to some absolute (gold standard); for example, for a medical test to determine if a person has the disease, the test will be positive) versus (1 – specificity) (the specificity is a statistical measure of how well a binary classification test correctly identifies the negative cases, or

those cases that do not meet the condition under study. For example, given a medical test that determines if a person has a certain disease, the specificity of the test to the disease is the probability that the test indicates `negative' if the person does not have disease) for a binary classifier (binary classification is the task of classifying the members of a given set of objects into two groups on the basis of whether they have some property or not. Some typical binary classification tasks are medical testing to determine if a patient has certain disease or not (the classification property is the disease) as its discrimination threshold is varied. The ROC can also be represented equivalently by plotting the fraction of true positives (type I errors (or α error, or false positive) and type II errors (β error, or a false negative) are two terms used to describe statistical errors), true positives (TPR=true positive rate) versus the fraction of false (FPR=false positive rate).

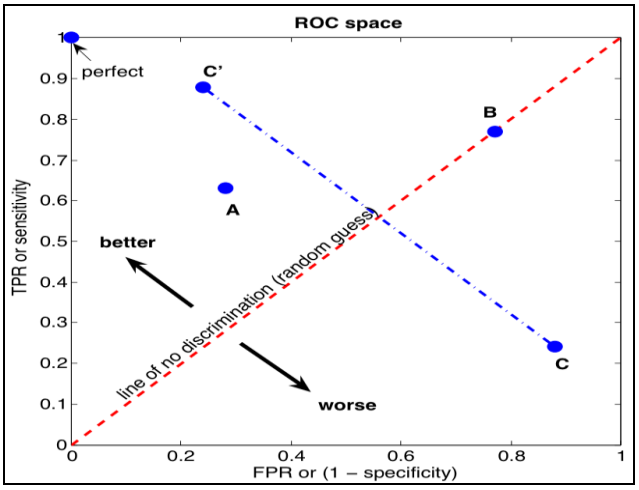

Figure 1: ROC space.

An ROC space is defined by FPR and TPR as x and y axes respectively, which depicts relative trade-offs between true positive (benefits) and false positive (costs). Since TPR is equivalent with sensitivity and FPR is equal to 1 - specificity, the ROC graph is sometimes called the sensitivity versus (1 - specificity) plot. Each prediction result or one instance of a confusion matrix represents one point in the ROC space. The best possible prediction method would yield a point in the upper left corner or coordinate (0, 1) of the ROC space, representing 100% sensitivity (no false negatives) and 100% specificity (no false positives). The (0,1) point is also called a perfect classification. A completely random guess would give a point along a diagonal line (the so-called line of no-discrimination) from the left bottom to the top right corners. An intuitive example of random guessing is a decision by flipping coins (head or tail).

Level of significance

"P" is level of significance, p>0.05 not significant, p<0.05 significant, p<0.01 highly significant, and p<0.001 very highly significant

Sensitivity

$$Sensitivity = \frac{TP}{TP + FN} \times 100$$

Specificity

$$Specificity = \frac{TN}{TN + FP} \times 100$$

RESULTS

The present study was conducted in the department of ophthalmology in collaboration with department of pathology, Rama Medical College, Kanpur to explore the association between platelet aggregation parameters and primary open angle glaucoma (POAG) and correlate them with severity of POAG. All the patients attending glaucoma clinic diagnosed as glaucoma were invited to participate in the study, of these 60 patients fulfilling the inclusion criteria were enrolled in the study as cases while 60 healthy individuals were enrolled as controls.

In majority of cases of glaucoma, early changes in visual field like isopter contraction and baring of blind spot were observed (73.3%), only 10 (16.7%) cases were diagnosed having paracentral scotoma and in only 6 (10.0%) end stage visual field defects like small island of central vision (tubular vision) were observed on perimetry (Table 2).

Table 2: Distribution of cases according to severity of glaucoma (perimetry).

S. no.	Severity	Number	Percentage
1	Early changes in visual field	44	73.3
2	Paracentral scotoma	10	16.7
3	End stage visual field defects	6	10.0

All the controls had normal retinal nerve fibre imaging while all the cases showed thinning of retinal nerve fibres.

Fundus C: D ratio findings of all the subjects in control group were within normal limits.

Out of 120 eyes of patients of glaucoma, only 56 (46.7%) eyes had normal C: D ratio (0.3-0.4) fundus examination findings. Mild (0.5-0.6), moderate (0.7) and severe (0.8-0.9) C: D ratio findings were seen in 14.2%, 35.0% and 4.2% eyes respectively (Table 3).

In platelet parameters and POAG comparison, only PCT showed significant association with POAG, rest of the platelet aggregation parameters did not show any significant association with POAG.

Table 3: Fundus C: D ratio findings of eyes of glaucoma cases.

S. no.	Fundus C: D ratio findings	Number	Percentage
1	Within normal limits (0.3-0.4)	56	46.7
2	Mild (0.5-0.6)	17	14.2
3	Moderate (0.7)	42	35.0
4	Severe (0.8-0.9)	5	4.2

Difference in platelet counts of cases (155900±35356) and controls (155800±47510) was not found to be significant statistically.

Though platelet distribution width of controls (14.43±4.60%) was higher as compared to cases (13.16±3.37%) yet this difference was not found to be significant statistically.

PCT of controls was significantly higher as compared to cases (0.247±0.200% versus 0.168±0.046%).

Mean platelet volume of controls was higher as compared to cases (10.82±1.32 versus 10.51±1.81 fentolitre) but this difference was not found to be significant statistically.

Difference in platelet-large cell ratio between cases (40.17±3.14%) and controls (40.24±4.82%) was not found to be significant statistically (Table 4).

Based on the direction of assessment, PCT was evaluated for prediction of glaucoma at a cut-off with a lower value indicating positive result. Area under curve was 0.689 (indicating a projected accuracy of approximately 68.9%). A cut off of PCT <0.203 was found to be 83.3% sensitive and 53.3% specific.

Severity of POAG did not show any significant association with any of the platelet aggregation parameters (Table 5).

Table 4: Association of POAG with platelet aggregation parameters.

S. no.	Parameters	Cases (n=60)		Controls (n=60)		Student 't' test	
		Mean	SD	Mean	SD	't'	'p'
1	Platelet (per microliter)	155900	35356	155800	47510	0.013	0.990
2	PCT (%)	0.168	0.046	0.247	0.200	-2.959	0.004
3	PDW (%)	13.16	3.37	14.43	4.60	-1.715	0.089
4	MPV (femtolitre)	10.51	1.81	10.82	1.30	-1.093	0.276
5	PLCR (%)	40.17	3.14	40.24	4.82	-0.094	0.925

Table 5: Association of severity of POAG with platelet aggregation parameters.

S. no.	Parameters	Changes in VF (n=44)		Scotoma (n=10)		End stage VF (n=6)		ANOVA
		Mean	SD	Mean	SD	Mean	SD	
1	Platelet (per microliter)	160341	29607	150900	43773	131667	53242	F=1.914; p=0.157
2	PDW (%)	13.15	3.49	12.56	2.80	14.27	3.54	F=0.476; p=0.624
3	PCT (%)	0.18	0.04	0.16	0.05	0.13	0.08	F=3.024; p=0.056
4	MPV (femtolitre)	10.50	2.01	10.84	0.72	10.02	1.63	F=0.381; p=0.685
5	PLCR (%)	39.96	3.56	40.96	1.69	40.37	0.90	F=0.414; p=0.663

DISCUSSION

Glaucoma is a multifactorial ocular condition characterized by progressive optic neuropathy, leading to irreversible vision loss. It happens to be a leading cause of blindness worldwide. The World Health Organization (WHO) estimates that glaucoma affects around 76 million people globally, a number projected to increase to over 111 million by 2040 due to aging populations.[4] The economic burden includes direct medical costs, productivity loss, and the need for long-term care in advanced stages. Public health strategies emphasizing early diagnosis and effective management are essential to mitigate the impact of glaucoma. Glaucoma is primarily associated with elevated IOP, although NTG exists where IOP is within normal limits. The disease manifests in several forms, with the most common being POAG and ACG. Early detection and treatment are crucial to prevent permanent vision impairment.

The pathogenesis of glaucoma is complex and involves multiple mechanisms. Elevated IOP is a significant risk factor, but other elements like vascular dysregulation, genetic predisposition, and systemic conditions contribute to the disease's development and progression.[5-8]

Recent research has highlighted potential links between glaucoma and coagulation abnormalities. Coagulation and fibrinolytic systems' imbalances can contribute to vascular dysregulation, influencing ocular blood flow and IOP, thus impacting glaucoma pathogenesis.[9] Platelets aggregation parameters are routine haematological parameters that are essential markers of coagulation. A possible role of platelet aggregates in pathogenesis and progression of glaucoma has been mooted since a long period.[10-13] However, this relationship has remained ignored for a long period, only to be revived in last few years with a few studies evaluating relationship of platelet parameters with glaucoma severity and pathogenesis.[14] Over the years, it has also been seen that glaucoma medication have an anti-platelet effect indicating a possible relationship between glaucoma progression and platelet aggregates.[16] Despite the evidence of these direct and indirect relationships, role of platelet aggregates in primary open angle glaucoma has remained a relatively less explored area of research. Hence, the present study was planned with an aim to study the association of platelet aggregation parameters with primary open angle glaucoma and its severity.

In the present study, we did not find a significant association of different platelet parameters with severity of POAG either. However, Ma et al found significant differences in platelet parameters, platelet count, PDW, MPV and P-LCR but did not find it significant for PCT.[15] Moreover, for platelet count and PCT the differences among different severity grades of POAG were non-linear in nature and were dominantly guided by extremely higher or lower values in severe glaucoma cases. It may also be noted that for most of the cases with mild to moderate grade of POAG, mean platelet aggregate parameters were close to their values in controls. These trends strengthen our proposition that platelet aggregate parameters in glaucoma must be viewed in context with the duration and pattern of antiglaucoma medications and should not be viewed only as the cause but should be viewed as an effect only. These findings also explain our findings which had a dominance of cases with mild glaucoma only and hence their failure to achieve a statistically significant difference when compared to controls. In the study by Maiya and Prakruthi too, irregular trends in platelet aggregate parameters were seen among different severity grades.[17] They found mean plateletcrit values to be minimum and same in severe and mild grade and maximum in moderate grade. Similarly, platelet count values were higher in severe (2.796±0.42 l/cumm) followed by mild (2.789±0.485 l/cumm) and minimum in moderate (2.621±0.361 l/cumm) POAG groups. Similar irregular trends were also seen for MPV and PDW too, thus ruling out a significant linear association between severity of glaucoma and platelet aggregates. However, Ma et al in another study found PDW and MPV to have a significant incremental trend with increasing severity of glaucoma but they did not record a significant decreasing trend for platelet count.[19] Lubis et al similar to the present study, did not find a significant association between glaucoma severity and any of the platelet parameters studied.[18] Li et al in their study among PACG patients too, did not find a significant association of any of the platelet parameters studied with severity of glaucoma.[16]

The findings of the present study, thus highlight the need to study platelet aggregate parameters in glaucoma patients with various perspectives and in more comprehensive manner. They show the need to study the role of confounding factors like duration of disease, type and pattern of antiglaucoma medication used and other systemic and incidental factors that could contribute to changes in platelet aggregate parameters, especially in view of inconsistencies in literature and most of the existing literature depicting these relationships being guided by a dominance of severe glaucoma cases. Further studies on a larger sample size in both cross-sectional and longitudinal nature are affected. Comprehensive recording of variables that may influence both glaucoma progression and platelet aggregate parameters with special reference to assessment of level of oxidative stress should be carried out to understand this relationship further.

Limitations

On the basis of our observations, we make the following limitations. Further studies on larger sample size with adequate representation of different severity grades of glaucoma must be carried out. Further studies should also take into account the type, duration and pattern of anti-glaucoma medication use as an independent factor related with changes in platelet aggregation parameters. Longitudinal studies on a larger sample size with a long duration of follow-up are recommended to study the association between progression of glaucoma and changes in platelet aggregation parameters.

CONCLUSION

The present study was conducted to explore the association between platelet aggregation parameters and POAG and correlate them with severity of POAG. Sixty diagnosed cases of glaucoma and sixty healthy controls were enrolled in the study. Age of subjects enrolled ranged between 41 to 60 years, majority were males (57.5%). Cases and controls were comparable for age and gender ratio. Following were the major findings of the present study.

Poor visual acuity (6/36 and poor) was observed in higher proportion of eyes of glaucoma cases as compared to controls (65% versus 35.0%), intra-ocular pressure (by non-contact tonometery) of cases was significantly higher than controls (23.03±7.82 versus 13.78±2.75%). Out of 60 cases of glaucoma, scotoma and end stage visual field defects were observed in 16.7% and 10.0% cases; all the controls had normal visual field, though higher level of platelet counts were observed among cases as compared to controls yet this difference was not found to be significant statistically; PDW, MPV and PLCR levels of cases was found to be lower among cases as compared to controls but differences were not statistically significant; PCT levels of

cases were significantly lower as compared to controls (0.168±0.046 versus 0.247±0.200%). On receiver operator curve, cut-off of PCT level <0.203 was found to be 83.3% sensitive and 53.3% specific for predicting glaucoma. Severity of glaucoma (in terms of changes in visual field by perimetery) did not show any significant association with platelet aggregation parameters.

In the present study, association of POAG with only PCT platelet aggregation parameter was observed, rest of the parameters did not show any significant association. Further studies with larger sample size might help to establish association of POAG with platelet aggregation parameter.

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