MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2023.10.39.43

OPEN ACCESS

Key Words
Neutrophil/lymphocyte ratio, pseudoexfoliation syndrome, pseudoexfoliation glaucoma

Corresponding Author
Rhutuja Deo,
Department of Ophthalmology, NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Digdoh Hills, Hingna Road, Nagpur-440019, India

Author Designation
[1]Associate Professor
[2]Professor and Head

Received: 22 July 2023
Accepted: 14 August 2023
Published: 18 September 2023

Citation: Rhutuja Deo and Rekha Khandelwal, 2023. Correlation of Blood Neutrophil/Lymphocyte Ratio and Pseudoexfoliation Syndrome. Res. J. Med. Sci., 17: 39-43, doi: 10.59218/makrjms.2023.10.39.43

Correlation of Blood Neutrophil/Lymphocyte Ratio and Pseudoexfoliation Syndrome

[1]Rhutuja Deo and [2]Rekha Khandelwal
[1,2]Department of Ophthalmology, NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Digdoh Hills, Hingna Road, Nagpur 440019, India

ABSTRACT
To study the relationship between blood neutrophil/lymphocyte ratio (NLR) and pseudoexfoliation syndrome. A cross sectional study was carried out at a tertiary care eye hospital attached to academic institute of Central India for a period of 12 months. Forty patients with pseudoexfoliation glaucoma (PXG), 47 patients of pseudoexfoliation syndrome (PXS) and 56 healthy controls were enrolled in this study. Complete ophthalmological examination and complete blood count measurements were performed. Patients with inflammatory, post-operative, traumatic or infective ocular conditions were excluded. Neutrophil/lymphocyte ratio was measured in all patients by Siemens ADVIA 2120 Heamatology system. The mean age in PXS was 69.09±5.88 years, 69.10±6.82 years in PXG and 67.43±5.39 years in control group. Metabolic comorbidities like diabetes and hypertension were similar in both the groups. The mean NLR values in PXS were 2.21±0.89, 2.57±1.47 in PXG and 1.68±0.27 in control group There was a statistically significant difference of NLR between PXS, PXG and control group ($p<0.0001$) obtained by ANOVA test. NLR was significantly higher in patients with pseudoexfoliation syndrome. This may indicate the heightened inflammatory response in ocular pseudoexfoliation syndrome further leading to pseudoexfoliation glaucoma.

INTRODUCTION

Pseudoexfoliation syndrome (PXS) is an age related, systemic disorder of the extracellular matrix characterised by abnormal basement membrane like material in extracellular and intraocular tissues. Clinically, the pseudoexfoliative material can be seen on anterior lens capsule, the pupillary ruff and other anterior segment structures[1]. It is also deposited in the skin, blood vessel walls and various organ systems. Pseudoexfoliation is strongly associated with secondary open angle glaucoma[2].

Though PXS occurs worldwide, its prevalence differs considerably. It is reported from 0-38% in different populations[3-6].

The pathophysiology of PXS is assumed to be multifactorial which includes genetic and non-genetic factors, trauma, viral infections and inflammation[7].

Oxidative stress, increased inflammatory activity, iris hypoperfusion, ocular ischemia and hypoxia were also considered to be related to the pathogenesis of PXS[8].

Inflammatory biomarkers such as interleukins IL-1α, IL6, IL8 and vascular endothelial adhesion molecule (ELAM-1) have also been associated with pseudoexfoliation[9]. Inflammation plays an important role in pathogenesis of PXS.

According to different studies performed previously, an increase in concentration of growth factors (e.g., basic fibroblast growth factor, hepatocyte growth factor, connective tissue growth factor, transforming growth factor beta1i.e. TGF-β1); vasoactive peptide endothelin-1a and oxidative stress markers (e.g. 8-isoprostaglandin-F2α) as well as a decrease in antioxidative protective factors (e.g. ascorbic acid) and an imbalance of MMPs and TIMPs has been observed in patients with PXS[10-14].

In recent years, inflammatory biomarkers are gaining diagnostic and clinical importance. Increased NLR IL1α, IL6, IL8 and increase in concentration of growth factors was also observed in PXS[9-15]. Association of altered neutrophil: lymphocyte ratio was also established in cardiovascular, cerebrovascular diseases and cancers[16].

Inflammation was an important biomarker found in age related macular degeneration, vascular diseases of retina[17-19] as well as diabetic retinopathy[17]. Very few studies have reported the use of NLR in PXS[15]. Thus, with previous studies indicating inflammation as a causative factor of PXS, this study was aimed to assess the relationship between neutrophil/ lymphocyte ratio (NLR) and pseudoexfoliation syndrome (PXS).

MATERIALS AND METHODS

A hospital based, observational study was conducted in a tertiary care hospital attached to an academic institute in Central India. After taking institutional ethics committee approval, a written informed consent was obtained from all subjects participating in the study.

All PXS patients who were referred from the peripheral outreach camps organized by the department of ophthalmology and preventive and social medicine over a period of 12 months were included in the study. Patients were referred for either cataract surgery or for the management of other ocular diseases to the tertiary care centre. All patients with PXS were included in the study. However, PXS patients with inflammatory or infective ocular conditions or chronic use of topical medication for PXG, long term steroid use, bleeding disorders, systemic infective conditions or malignancy were excluded from the study.

The patients underwent preliminary ophthalmic examination including visual acuity assessment using Snellen's chart at 6m, slit lamp biomicroscopy (Topcon, Oakland, NJ, USA), fundus examination using indirect ophthalmoscopy, optic disc evaluation using +78D lens (x16 magnification) and IOP measurement with a Goldmann applanation tonometer. The suspected glaucoma cases further underwent gonioscopy (4 mirror Sussmann goniolens) and perimetry on the Humphrey Visual Field analyser. The severity of PXG was not graded. This study was aimed to assess the correlation of pseudoexfoliation with elevated NLR irrespective of severity of PXG. The study was composed of 142 patients who were assigned to 3 groups:

- Age and sex matched control group (Control): 55
- Patients with pseudoexfoliation syndrome (PXS): 47
- Patients with pseudoexfoliation glaucoma (PXG): 40

PXS group included all the patients with the exfoliative material on the anterior lens capsule or pupillary margin, a normal optic disc, normal visual field findings and intraocular pressure <21 mm Hg[15].

PXG group included all patients with exfoliative material in the anterior chamber, an open angle on gonioscopy, IOP >21 mmHg, optic disc and visual field changes[15].

Age and sex matched control subjects were patients with no ocular disease except cataract and refractive error and no signs of pseudoexfoliation[15]:

Other variables were defined as follows:

- **Hypertension:** Chronic elevation in blood pressure (systolic $\geq$140 mmHg or diastolic $\geq$90 mmHg)[20]
- **Diabetes mellitus:** Criteria for the diagnosis included symptoms of diabetes plus a random blood glucose concentration $\geq$11.1 mmol L^{-1} ($\geq$200 mg dL^{-1})[21]

Neutrophil lymphocyte ratio (NLR): Complete blood count was done from the hospital's central pathology laboratory. The blood neutrophil and lymphocyte count were evaluated by Siemens ADVIA 2120 Haematology system. The NLR was calculated as the ratio of the neutrophil count to the lymphocyte count.

Statistical methods: The continuous variables were summarized in terms of mean and standard deviation, while categorical were expressed in terms of numbers and percentage. The continuous variables were compared across three groups using one-way analysis of variance and the paired comparison were obtained using Tukey's post-hoc test. The categorical variables were compared using Pearson's Chi-square test. The diagnostic parameters like accuracy, sensitivity and specificity of the cut-off values were obtained for the two study groups. The cut-off was determined using Youden Index. All the analyses were performed using SPSS ver. 20.0 (IBM Corp, USA) and the statistical significance was evaluated at 5% level

RESULTS

The study included 142 patients who were assigned to 3 groups. Pseudoexfoliation syndrome (PXS) group with 47 patients, pseudoexfoliation glaucoma (PXG) group with 40 patients and 55 patients in the age matched healthy control group (Table 1 and 2).

The mean age in the three groups were comparable and statistically non significant as shown in Table 3 compares neutrophil to lymphocyte ratio among the control, PXS and PXG groups. There was a statistically significant difference in the N: L Ratio of the control group as compared to the other groups ($p<0.0001$).

The same can be observed from Figure which shows a significant difference in NLR ($p = 0.014$) between the control and PXS group and a significant difference in NLR ($p<0.0001$) between the control and PXG group.

The IQR (interquartile range) and the 95% confidence interval (CI) values of the 3 groups are as below:

- CONTROL: IQR = 0.64, 95% CI: 1.61-1.72
- PXS: IQR = 0.88, 95% CI: 2.05-2.40
- PXG: IQR = 1.85, 95% CI: 2.10-3.04

DISCUSSIONS

In our study, we found that the mean NLR values in PXS were 2.21±0.89, 2.57±1.47 in PXG and 1.68±0.27 in control group. These findings are very similar to a study done by Kurtul *et al.* where the mean NLR values were 2.08±0.61 in PEXS, 2.20±0.58 IN PEXG and 1.51±0.57 in control group[17]. Another study done by Ozgonul *et al*.[22] compared neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in pseudoexfoliation syndrome. In this study the NLR values were 2.33±0.84 in PXS, 2.45±0.82 in PXG and 1.84±0.59 in control group.

In our study, we found that there was an association of elevated NLR and pseudoexfoliation. The pathogenesis of PXS includes many genetic and non-genetic factors. It has been related to oxidative stress, increased inflammatory activity, iris hypoperfusion, ocular ischemia and hypoxia[8,23,24].

There seems to be a role of local and chronic inflammation generated by complement pathways in the pathogenesis of PXS as suggested by Yildirim *et al.*[25].

Alpha 1 antitrypsin also inhibits the over expressed proteinases during inflammation. It also has a protective role against the proteolytic activity in inflammation in PXS as suggested by Cumurcu *et al.*[26] Some studies also postulate the relation of increased levels of homocysteine (Hcy) in PXS to chronic inflammation. Interleukins IL 1β, IL6, IL12, IL18 and C-reactive protein (CRP) are proinflammatory markers which stimulate increased release of Hcy[26].

Table 1: Comparison of mean age among groups

Parameters	Control (n = 56)	PXS (n = 47)	PXG (n = 40)	p-value
Age (years) [mean±SD]	67.43±5.39	69.09±5.88	69.10±6.82	0.270 (NS)*
Age group [No. (%)]				
51-60	9 (16.07)	5 (10.64)	6 (15.0)	0.343 (NS)
61-70	32 (57.14)	27 (57.45)	22 (55.0)	
71-80	15 (26.79)	13 (27.66)	8 (20.0)	
>80	0	2 (4.26)	4 (10.0)	

Table 2: Comparison of co morbidities among groups shows the prevalence of diabetes and hypertension which was similar in all the groups

Parameters	Control (n = 56)	PXS (n = 47)	PXG (n = 40)	p-value
Diabetes mellitus [No. (%)]				
Yes	3 (5.36)	3 (6.38)	1 (2.5)	0.690 (NS)
No	53 (94.64)	44 (93.62)	39 (97.5)	
Hypertension [No. (%)]				
Yes	11 (19.64)	6 (12.77)	5 (12.5)	0.526 (NS)

Table 3: Comparison of NLR in 3 groups

Parameters (Mean±SD)	Control (n = 56)	PXS (n = 47)	PXG (n = 40)	Control (n = 56)	p-value
Neutrophil count ($\times10^3$ µL)	0.57±0.05	0.63±0.08	0.65±0.11	0.57±0.05	<0.0001 (S)*
Lymphocyte count ($\times10^3$ µL)	0.35±0.05	0.31±0.08	0.29±0.09	0.35±0.05	0.001 (NS)*
N:L ratio	1.68±0.27	2.21±0.89	2.57±1.47	1.68±0.27	<0.0001 (S)*

There was a strong association between PXS and serum YKL-40, a new potential biomarker which has been found to have a role in pathogenesis of endothelial dysfunction[27]. Proinflammatory cytokines were also studied in early and late stages of PXS and PXG. A persistent pro inflammatory state in the anterior segment of early PXS would result due to stress conditions with severely impaired stress mechanisms[9]. This may be the reason for increased acute phase reactants and acute inflammatory markers in early PXS. CRP is also a highly sensitive predictive marker of inflammation and peripheral endothelial dysfunction; while TNF-α is a proinflammatory cytokine and both may be involved in the pathogenesis of PXS. Sorkhabi *et al.* concluded that increased levels of high sensitivity C reactive protein, as a marker of inflammation and tumour necrosis factor-α, a pro inflammatory cytokine were signs of inflammation in PXS[28].

All the above-mentioned biomarkers have been studied in relation to the pathogenesis of PXS. Thus we can say that neutrophil to lymphocyte ratio may be used as an inflammatory biomarker for diagnosis of PXS. Complete blood count and calculation of neutrophil lymphocyte ratio is a routine and cheap investigation. If NLR is proved as a suitable biomarker for pseudoexfoliation, an easy investigation that is complete blood count could aid us in diagnosing PXS at early stages.

In our study, we took neutrophil/lymphocyte ratio as a single indicator of inflammation as it was a simple, reliable, routinely done investigation without any extra burden to the laboratory as well as the patient. It was found to be statistically significant. Very few studies in literature have proved this correlation and thus our study may require further validation on large group studies.

One limitation of the study was availability of limited data on inflammatory biomarkers, cytokines and other markers which have an important role in the pathogenesis of pseudoexfoliation. The smaller sample size was another limitation. Hence, a large prospective study encompassing multiple inflammatory markers other than NLR is required to validate our results.

Pseudoexfoliation is considered to be a chronic inflammatory process eventually leading to pseudoexfoliative glaucoma. However, studies have shown that eyes with early PXS may have an ongoing pro-inflammatory state due to stress response. This chronic activation is hypothesized to contribute to the pathophysiology of glaucoma[9].

Activation of complement pathways also causes prolonged levels of subclinical inflammation. Even though pseudoexfoliation glaucoma results from a chronic fibrotic process, like all other such disorders, the initial phases of this disorder are characterized by elevated pro-inflammatory mediators[29,30,31].

This study aims to aid in early diagnosis of pseudoexfoliation if NLR can be proved as an effective biomarker for PXS in the acute phase. This could help in prompt intervention or treatment if required preventing further fibrotic processes leading to pseudoexfoliative glaucoma.

SUMMARY AND CONCLUSION

In our study, NLR was significantly raised in patients with pseudoexfoliation syndrome and pseudoexfoliation glaucoma in comparison to the control group. These increased parameters may indicate the heightened inflammatory response in PXS and may contribute in IOP elevations leading to pseudoexfoliation glaucoma. It can be considered as a single biomarker to predict pseudoexfoliation. Further studies will be required in future to evaluate its definite role.

REFERENCES

1. Ritch, R., 1996. Exfoliation syndrome. In: The Glaucomas, Ritch, R., M.B. Shields and T. Krupin, (Eds.)., St Louis, Mosby, pp: 993-1022.
2. Ritch, R. and U. Schlötzer-Schrehardt, 2001. Exfoliation (pseudoexfoliation) syndrome: Toward a new understanding. Acta Ophthalmologica Scand., 79: 213-217.
3. Forsius, H., 1979. Prevalence of pseudoexfoliation of the lens in finns, lapps, Icelanders, Eskimos and Russians. Trans Ophthalmol Soc U K., 99: 296-298.
4. Cashwell, L.F. and M.B. Shields, 1988. Exfoliation syndrome prevalence in a southeastern united states population. Arch. Ophthalmol., 106: 335-336.
5. Hiller, R., R.D. Sperduto and D.E. Krueger, 1982. Pseudoexfoliation, intraocular pressure and senile lens changes in a population-based survey. Arch. Ophthalmol., 100: 1080-1082.
6. Faulkner, H.W., 1971. Pseudo-exfoliation of the lens among the navajo Indians. Am. J. Ophthalmol., 72: 206-207.
7. Faschinger, C., O. Schmut, C. Wachswender and G. Mossböck, 2006. Glaukom und oxidativer stress: Determination of malondialdehyde-a product of lipid peroxidation. Der Ophthalmologe, 103: 953-959.
8. Sorkhabi, R., A. Ghorbanihaghjo and M.H. Ahoor, 2011. Oxidative stress in psudoexfoliation syndrome. Indian J. Ophthalmol., 23: 27-32.
9. Zenkel, M., P. Lewczuk, A. Jünemann, F.E. Kruse, G.O.H. Naumann and U. Schlötzer-Schrehardt, 2010. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. Am. J. Pathol., 176: 2868-2879.

10. Gartaganis, S.P., C.D. Georgakopoulos, A.M. Exarchou, E.K. Mela, F. Lamari and N.K. Karamanos, 2001. Increased aqueous humor basic fibroblast growth factor and hyaluronan levels in relation to the exfoliation syndrome and exfoliative glaucoma. Acta Ophthalmologica Scand., 79: 572-575.
11. Schlötzer-Schrehardt, U., M. Zenkel, M. Küchle, L.Y. Sakai and G.O.H. Naumann, 2001. Role of transforming growth factor-β1 and its latent form binding protein in pseudoexfoliation syndrome. Exp. Eye Res., 73: 765-780.
12. Ho, S.L., G.F Dogar, J. Wang, J. Crean and Q.D. Wu *et al*., 2005. Elevated aqueous humour tissue inhibitor of matrix metalloproteinase-1 and connective tissue growth factor in pseudoexfoliation syndrome. Br. J. Ophthalmol., 89: 169-173.
13. Hu, D.N. and R. Ritch, 2001. Hepatocyte growth factor is increased in the aqueous humor of glaucomatous eyes. J. Glaucoma, 10: 152-157.
14. Koliakos, G.G., 2003. 8-isoprostaglandin F_{2a} and ascorbic acid concentration in the aqueous humour of patients with exfoliation syndrome. Br. J. Ophthalmol., 87: 353-356.
15. Kurtul, B.E., P.A. Ozer and E.U. Kabatas, 2016. Elevated neutrophil-to-lymphocyte ratio in pseudoexfoliation syndrome. Eye, 30: 1045-1048
16. Schumacher, S., U. Schlötzer-Schrehardt, P. Martus, W. Lang and G. Naumann, 2001. Pseudoexfoliation syndrome and aneurysms of the abdominal aorta. Lancet, 357: 359-360.
17. Ulu, S.M., M. Dogan, A. Ahsen, A. Altug, K. Demir, G. Acartürk and S. Inan, 2013. Neutrophil-to-lymphocyte ratio as a quick and reliable predictive marker to diagnose the severity of diabetic retinopathy. Diabetes Technol. Ther., 15: 942-947.
18. Rodrigues, E.B., 2007. Inflammation in dry age-related macular degeneration. Ophthalmologica, 221: 143-152
19. Ilhan, N., M.C. Daglioglu, O. Ilhan, M. Coskun, E.A. Tuzcu, H. Kahraman and U. Keskin, 2014. Assessment of neutrophil/lymphocyte ratio in patients with age-related macular degeneration. Ocular Immunol. Inflammation, 23: 287-290.
20. Kasper, D., 2016. Hypertension. 19th Edn., McGraw Hill Education Medical, New York.
21. Kasper, D., 2016. Diabetes Mellitus. In: Harrison's Manual of Medicine, Harrison, T., D. Kasper, D. Longo, A. Fauci and S. Hauser, (Eds.)., McGraw Hill, New York.
22. Ozgonul, C., E. Sertoglu, T. Mumcuoglu, G. Ozge and G. Gokce, 2015. Prediction of pseudoexfoliation syndrome and pseudoexfoliation glaucoma by using neutrophil to lymphocyte ratio and platelet to lymphocyte ratio. Ocular Immunol. Inflammation, 24: 665-670.
23. Ocakoglu, O., N. Koyluoglu, A. Kayiran, N. Tamcelik and S. Ozkan, 2004. Microvascular blood flow of the optic nerve head and peripapillary retina in unilateral exfoliation syndrome. Acta Ophthalmologica Scand., 82: 49-53.
24. Sorkhabi, R., A. Ghorbanihaghjo, A. Javadzadeh, N. Rashtchizadeh and M. Moharrery, 2011. Oxidative DNA damage and total antioxidant status in glaucoma patients. Mol. Vis., 17: 41-46.
25. Yildirim, Z., F. Yildirim, N.I. Uçgun and A. Sepici-Dinçel, 2013. The role of the cytokines in the pathogenesis of pseudoexfoliation syndrome. Int. J. Ophthalmol., 6: 50-53.
26. Cumurcu, T., H. Ozyurt, H.D. Demir and H. Yardim, 2008. Serum alpha-1-antitriypsin levels in patients with pseudoexfolative syndrome. Curr. Eye Res., 33: 159-162.
27. Türkyılmaz, K., V. Öner, A. Kırbas, M.S. Sevim, B. Sekeryapan, G. Özgür and M. Durmus, 2013. Serum YKL-40 levels as a novel marker of inflammation and endothelial dysfunction in patients with pseudoexfoliation syndrome. Eye, 27: 854-859.
28. Sorkhabi, R., A. Ghorbanihaghjo, M. Ahoor, M. Nahaei and N. Rashtchizadeh, 2013. High-sensitivity C-reactive protein and tumor necrosis factor alpha in pseudoexfoliation syndrome. Oman Med. J., 28: 16-19.
29. Gauldie, J., M. Kolb and P.J. Sime, 2002. A new direction in the pathogenesis of pulmonary fibrosis. Respir Res., Vol. 3. 10.1186/rr158
30. J. Rheumatol., 1998. Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor and soluble GP130 in patients with systemic sclerosis. J. Rheumatol., 25: 308-313.
31. Sheppard, D., 2001. Pulmonary fibrosis: A cellular overreaction or a failure of communication? J. Clin. Invest., 107: 1501-1502.