Red cell distribution width levels in patients with pseudoexfoliation syndrome and pseudoexfoliation glaucoma

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Purpose: The purpose of this study was to assess the levels of red cell distribution width (RDW) in patients with pseudoexfoliation syndrome (PEX) and pseudoexfoliation glaucoma (PEXG), and to compare their RDW values with healthy controls. Methods: 40 patients with PEX, 40 with PEXG, and 80 control subjects were enrolled in this study. Complete ophthalmologic examination and complete blood count measurements were performed on all subjects. Complete blood counts were performed within one hour of blood collection. Results: RDW levels were significantly higher in patients with PEX and PEXG than in controls (P = 0.027 and P < 0.001, respectively). Furthermore, a significant difference was found in RDW values between PEXG and PEX groups (P = 0.016). RDW levels were gradually increased from control group to PEXG group (P < 0.001). Multivariate logistic regression analysis revealed that RDW was independently associated with the presence of PEX/PEXG (odds ratio 1.765, 95% confidence interval (CI) 1.095–2.867, P = 0.013). Conclusion: We conclude that RDW may be a useful marker for predicting the presence of PEX and progression to PEXG.

Key words: Inflammation, neutrophil, pseudoexfoliation, red cell distribution width

Pseudoexfoliation syndrome (PEX) was initially described by a Finnish ophthalmologist, John Lindberg, in 1917. It represents a common age-related systemic disorder affecting approximately 10% to 20% of the general population over the age of 60 and involving both genetic and non-genetic factors in its etiopathogenesis. In view of the complex inheritance of PEX syndrome, it must be assumed that additional genetic (modifying genes) and/or environmental (fibrotic triggers) factors influence the manifestation of the disease. Candidate factors, which might stimulate the synthesis of abnormal pseudoexfoliation fibrils, include profibrotic growth factors (TGF-β1), cytokines (IL-6), and amino acids (homocysteine), as well as various stress conditions such as oxidative stress, UV radiation, and hypoxia. It is characterized by pathologic accumulation of an abnormal fibrillar extracellular material in the anterior segment of the eye and in various extracellular tissues. Progressive obstruction of the aqueous humor outflow pathways by abnormal pseudoexfoliation material deposits cause chronic pressure elevation, optic nerve damage, and subsequent development of open angle glaucoma in eyes with PEX syndrome, a condition known as pseudoexfoliation glaucoma (PEXG). It accounts for a majority of glaucoma cases in some countries and for approximately 25% of open angle glaucoma worldwide. Based on the systemic nature of underlying connective tissue disorder, PEX syndrome has been associated not only with glaucoma but also with a broad spectrum of other ocular, surgical, and systemic complications including cardiovascular and cerebrovascular diseases.

Red blood cell distribution width (RDW), an index of variation of erythrocyte volume (i.e., anisocytosis), is conventionally included in a standard complete blood count (CBC). In brief, this parameter is automatically calculated by dividing the standard deviation (SD) of erythrocyte volume from the mean corpuscular volume (MCV). The result of this straightforward equation is then multiplied by 100 to express results in percentage (%). According to the “Holy Bible” of laboratory medicine (i.e., Henry’s Clinical Diagnosis and Management by Laboratory Methods), the conventional reference range of RDW is roughly comprised between 12% and 15%.

Studies have reported that increased RDW levels were significantly associated with adverse events and poor prognosis in various cardiovascular and cerebrovascular diseases. Given the association of PEX with various diseases, particularly cardiovascular or cerebrovascular diseases, and the role of inflammation and oxidative stress in the pathogenesis of PEX, RDW may be increased in PEX/PEXG patients. Increased RDW has been associated with the presence of retinopathy and allergic conjunctivitis. A study conducted by Bengi Ece Kurtul and Emrah Utuku Kabatas showed increased RDW with PEX and PEXG. We also hypothesized that RDW may be...
related with the presence of PEX or PEXG. Accordingly, the aim of this study was to evaluate serum levels of RDW in patients with PEX/PEXG in comparison with those in healthy subjects.

Methods

This case–control study was conducted at the postgraduate department of ophthalmology, Government Medical College, Srinagar from October 2018 to October 2020. Forty patients with PEX, 40 with PEXG, and 80 controls were included in the study.

Patients with pseudoexfoliation syndrome (PEX): These patients exhibited deposits of exfoliative material on the anterior lens capsule and/or iris during slit-lamp examination, in one or both eyes with a normal optic disc and visual field findings in patients with an intraocular pressure (IOP) of <21 mmHg.

Patients with pseudoexfoliation glaucoma (PEXG): In addition to exfoliative material, these patients showed the characteristic optic disc damage and visual field findings, and an IOP >21 mmHg without any treatment.

Control subjects had no history of ocular disease (except for refractive error, strabismus, and cataract) and no evidence of exfoliation material on the anterior lens capsule or pupillary margin. They had normal optic disc and visual fields.

Patients who experienced any of the following conditions were excluded: systemic infectious, inflammatory, oncological, hematological, or rheumatologic diseases; renal failure; liver or thyroid dysfunction; chronic obstructive pulmonary disease; chronic or recurrent inflammatory eye disease; ocular trauma; ocular infection; and missing laboratory data.

Laboratory parameters

Blood samples were collected in all patients after fasting for at least eight hours. Complete blood count (CBC) parameters were analyzed immediately after collection using an ASPEN PE-6000 PROCAN analyzer.

Statistical analysis

Data was entered into Microsoft Excel 2010 and analyzed using SPSS version 23. Categorical variables were summarized as frequencies and percentages, whereas continuous variables were summarized as mean and standard deviation. We used logistic regression for calculating odds ratio. Chi-squared test and t-test were used wherever appropriate. P < 0.05 were considered to be statistically significant.

Results

The mean age of cases (PEX/PEXG) was 65.38 ± 6.790 years and mean age of controls was 63.54 ± 5.953 years. Majority of the patients were males in both study groups. There was no statistically significant difference between the two study groups with respect to age and sex. The demographic characteristics are given in Tables 1a and 1b.

Lymphocyte counts were gradually decreased from control to PXG groups. The neutrophil/lymphocyte ratio (NLR) values were gradually increased from control to PXG groups (P = 0.005 and P < 0.001, respectively).

The RDW values were gradually increased from control to PEXG groups. There was a significant difference in RDW levels between PEX and control groups (P = 0.027) and PEXG and control groups (P < 0.001). Furthermore, a significant difference was found in RDW values between PEXG and PXG groups (P = 0.016). The description of laboratory parameters is shown in Table 2 and Figs. 1 and 2.

Multivariate logistic regression analysis comparing PEX and PEXG patients with controls indicated that elevated RDW level was a significant risk factor for PEX/PEXG status (odds ratio = 1.765; 95% confidence interval (CI) = 1.095–2.867, P = 0.013).

Discussion

The present study showed higher RDW levels in PEX/PEXG groups than the control group. Furthermore, RDW was independently associated with the presence of PEX/PEXG. PEX is generally considered as a complex, multifactorial, late-onset disease involving a combination of genetic and non-genetic factors in its etiopathogenesis.[20] Although the incidence of PEX syndrome is clearly influenced by environmental factors, there is a strong genetic component to the disease.[21,22] A genome-wide association study in Scandinavian populations identified lysyl-oxidase-like-1 (LOXL1) gene on chromosome 15q24.1 as a major contributor and principal genetic risk factor for PEX and PXFG30, a finding that has been replicated in multiple populations worldwide.[23-27]

Table 1a: Showing mean age of study groups

| Disease  | n  | Mean Age (Years) | SD  | 95% CI | P     |
|---------|----|------------------|-----|--------|-------|
| Age     | Cases | 80   | 65.38 | 6.790 | -0.157 to 3.832 | 0.071* |
| Controls| 80   | 63.54 | 5.953 |       |       |

*: No statistically significant difference (P>0.05); n=Number of patients; SD=Standard deviation; CI=Confidence interval

Table 1b: Showing gender distribution of study groups

| Gender   | No. | %    | Cases (PEX/PEXG) | Controls | Pearson Chi-Squared Test |
|----------|-----|------|------------------|----------|-------------------------|
| Males    | 59  | 73.75| 49               | 10       | 2.849                   | 0.091* |
| Females  | 21  | 26.25| 31               | 8        |                         |
| Total    | 80  | 100  | 80               | 10       |                         |

*: No statistically significant difference (P>0.05)

Table 2: Showing laboratory parameters of study groups

| Characteristic | Controls (n=80) | PEX (n=40) | PEXG (n=40) | P     |
|----------------|----------------|------------|-------------|-------|
| Neutrophil count (x10^9/L) | 3.24±1.02 | 3.60±1.12 | 3.63±0.76 | 0.165 |
| Lymphocyte count (x10^9/L) | 2.21±0.52 | 1.86±0.62 | 1.79±0.72 | 0.005 |
| NLR            | 1.49±0.67 | 1.98±0.74 | 2.06±0.48 | <0.001 |
| RDW (%)        | 13.10±0.96 | 13.85±1.09 | 14.90±1.23 | <0.001 |

PEX: Pseudoexfoliation syndrome; PXG: Pseudoexfoliation glaucoma; NLR: Neutrophil/lymphocyte ratio; RDW: Red blood cell distribution width
In view of the complex inheritance of PEX, it must be assumed that additional genetic (modifying genes) and/or environmental (fibrotic triggers) factors influence the manifestation of the disease. Candidate factors, which might stimulate the synthesis of abnormal pseudoxfoliation fibrils, include profibrotic growth factors (TGF-β1), cytokines (IL-6), and amino acids (homocysteine), as well as various stress conditions such as oxidative stress, UV radiation, and hypoxia.

Stress-induced, temporally restricted subclinical inflammation in anterior segment tissues is detected during the early stages of the fibrotic PEX process. According to numerous studies performed previously, inflammatory markers (e.g., alpha-1 antitrypsin, interleukin-6, high-sensitivity C-reactive protein [hs-CRP], and tumor necrosis factor-alpha) and growth factors (e.g., basic fibroblast growth factor, hepatocyte growth factor, connective tissue growth factor, transforming growth factor beta 1, and tumor necrosis factor-beta) were reported to be increased in PEX patients.

The underlying mechanisms between RDW and PEX/PEXG have not yet been clearly demonstrated. Some possible mechanisms may be suggested for this association. One of the possible mechanisms may be related to increased inflammatory activity. It is well known that PEX is related to inflammatory reactions. Previous studies demonstrated that PEX is associated with elevated inflammatory markers. In the present study, we have further found that RDW levels were significantly higher in PEX/PEXG group than in control group. It was shown that elevated inflammatory cytokines suppress the maturation of erythrocytes, allowing juvenile erythrocytes to enter into circulation and thereby leading to an increase in heterogeneity of the size and resulting in elevated RDW levels.

The other mechanism is based on the relationship between elevated RDW and increased oxidative stress. Elevated RDW levels may be related to increased oxidative stress in the presence of PEX/PEXG. An increase in oxidative stress markers (e.g., 8-isoprostaglandin-F2α) and a decrease in antioxidative protective factors (e.g., ascorbic acid) were observed in patients with PEX. Increasing evidence suggests that the oxidative-antioxidative balance is disturbed in patients with PEX/PEXG, both in the anterior segment and throughout the body, and that the resulting oxidative stress constitutes a major mechanism involved in the pathophysiology of this fibrotic process. It is well known that oxidative stress directly damages erythrocytes and leads to shortened erythrocyte survival, resulting in elevated RDW levels.

**Conclusion**

This study demonstrated that the red cell distribution width, the parameter reflecting inflammation, and oxidative stress were significantly higher in patients with PEX/PEXG. Oxidative stress, therefore, appears to represent a modifiable risk factor in the management of patients with PEX/PEXG. We conclude that RDW may be a useful marker for predicting the presence of PEX and progression to PEXG.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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