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The role of erythrocyte distribution width, platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio in the development of graft rejection after keratoplasty

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Abstract

Objective: The aim of this research is to evaluate the pre-surgical Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Red Cell Distribution Width (RDW) in patients undergoing rekeratoplasty compared to control subjects.

Method: Penetrating keratoplasty (PKP) cases performed between 2013 and 2023 were evaluated retrospectively. The follow-up period was determined as at least 6 months. The first group consisted of 31 patients who underwent rekeratoplasty due to corneal graft rejection after PKP, and the second group consisted of 31 patients with the same diagnosis who did not experience graft rejection during their follow-up after PKP. NLR, PLR and RDW values from the preoperative complete blood count results of all patients were included in the study.

Results: Upon demographic analysis, it was evident that although no notable age discrepancy existed between the two categories, the prevalence of rekeratoplasty was considerably higher in males. Primary diagnoses of the disease did not appear to have a significant impact on the risk of rejection. There was no observable correlation between serum NLR, PLR and RDW levels and graft rejection. Nevertheless, a notable association was detected between RDW and the duration until the occurrence of the initial rejection episode.

Conclusion: High RDW value, which is an indicator of systemic inflammatory state, was found to be associated with the time to first rejection reaction in corneal transplant patients and graft rejection patients compared to controls, as in many diseases. RDW in terms of rejection reaction risk prediction and prognosis; it can be used as a useful, cheap and practical parameter.

Keywords: Penetrating keratoplasty, graft rejection, NLR, PLR, RDW.

INTRODUCTION

Penetrating keratoplasty (PKP) is a surgical procedure in which the diseased cornea of a patient is replaced with healthy, transparent corneal tissue obtained from a deceased donor. Corneal transplantation is considered the most successful type of allograft transplantation in humans in terms of outcomes (1). While it was traditionally performed as a penetrating (full-thickness) procedure for many years, advancements in surgical techniques have led to the development of lamellar keratoplasty methods. These novel techniques, tailored to the specific diagnosis, have significantly reduced the risk of tissue rejection (2,3). Nevertheless, PKP remains the first choice, particularly in conditions such as advanced keratoconus, keratitis sequelae, and bullous keratopathy.

Corneal transplantation differs from other organ transplants, such as liver, heart, and kidney transplants, because it does not rely on compatibility with major histocompatibility complex (MHC) class antigens (4). Consequently, the use of systemic immunosuppressive agents is not required. However, immune-mediated graft rejection remains one of the most significant causes of corneal graft failure.

Penetrating keratoplasty-related risk factors have been

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identified in the literatüre (3-6). Loss of corneal transparency and potential graft rejection can arise from various factors leading to local inflammation and vascularization in the graft tissue. Prolonged graft survival is noted, particularly in conditions like bullous keratopathy and keratoconus, where inflammation is less pronounced. The literature indicates that the occurrence of graft rejection following PKP varies between 4% and 20% (3).

The total count of white blood cells and their subtypes, as well as their rates (Neutrophil-to-lymphocyte ratio; NLR, platelet-to-lymphocyte ratio; PLR), are increasingly being used to indicate chronic inflammation (7-9). Neutrophils become activated during tissue damage and release enzymes like myeloperoxidase, acid phosphatase, and elastase. When inflammation occurs, the proportion of circulating leukocytes changes, often leading to neutrophilia and relative lymphopenia. Existing literature suggests that NLR and PLR play a significant prognostic role in hypertension, hepatic cirrhosis, diabetes mellitus, familial Mediterranean fever, cardiovascular diseases, and malignancies (10).

Erythrocyte distribution width (RDW) is a commonly used laboratory parameter to assess erythrocyte anisocytosis, which reflects the variation in the size of circulating red blood cells (11). Initially, RDW was primarily utilized in clinical applications to distinguish between different types of anemias. However, in numerous recent publications, an increase in RDW has been associated with various diseases such as vascular occlusive disease, heart failure, hypertension, ischemic heart disease, the active stage of inflammatory bowel disease, atherosclerosis, rheumatoid arthritis, and other conditions linked to progressive inflammation (12-16). Several studies have stated that inflammation and oxidative stress impact RDW (15). Furthermore, RDW has been shown to be indicative of elevated levels of hepcidin, IL-6, TNF-alpha, and other circulating cytokines in the bloodstream (11).

The aim of this study was to evaluate the preoperative NLR, PLR, and RDW rates of patients undergoing rekeratoplasty compared to control subjects. Additionally, it was also aimed to investigate the potential of these parameters as markers of inflammation and predictors of graft rejection in the process leading to rekeratoplasty.

METHOD

Ethics committee approval was received from Atatürk University Faculty of Medicine Ethics Committee (B.30.2.ATA.0.01.00/40). The study was retrospectively designed with scanned patient files. The ethics committee did not request a patient consent form for the study. In the study, the files of patients who underwent rekeratoplasty surgery between 2013 and 2023 were scanned in 2 months. All authors had access to information that could identify individual participants at or after data collection.

The files of patients who underwent penetrating keratoplasty and had a follow-up period of at least 6 months were retrospectively reviewed. The data of patients who underwent rekeratoplasty between April 2013 and May 2023 were reviewed between January 2024 and March 2024. All individuals who underwent PKP were screened and those who underwent rekeratoplasty were identified from this group. Patients' medical records were reviewed for age, gender, primary indication for PKP, follow-up period, and timing of first detection of symptoms associated with graft rejection.

The first group consisted of 31 patients who underwent rekeratoplasty due to corneal graft rejection after PKP, and the second group consisted of 31 patients with the same diagnosis who did not experience graft rejection during their follow-up after PKP. The primary surgical indications for rekeratoplasty were bullous keratopathy, keratoconus, and nonspecific vascularized scar (leucoma).

The same treatment protocol was applied to all patients. Following surgery, all patients received topical prednisolone, moxifloxacin, and autologous serum. Topical treatment was started immediately after surgery with all drops applied five times daily, antibiotic drops were discontinued at month 1, steroid drops were tapered to three times daily at month 3, twice daily at month 6, and once daily at month 12. Artificial tear drops were continued five times daily for at least 1 year. Corneal sutures were generally removed 12 months post-surgery. The corneal graft rejection was diagnosed based on characteristic findings during slit lamp biomicroscopy, such as hyperemia, rejection line, infiltrations, keratic precipitates, and graft edema.

The time frame for the initial identification of symptoms of graft rejection after undergoing PKP surgery was determined for the patients in the first group. The preoperative complete blood counts of all patients were recorded including NLR, PLR, and RDW values. NLR was calculated by dividing the number of neutrophils by the number of lymphocytes, and PLR by dividing the number of platelets by the number of lymphocytes.

The aim was to analyze the relationship between the systemic inflammation-related values and the timing of occurrence graft rejection following keratoplasty. Participants with preoperative glaucoma, endophthalmitis, inflammatory ocular diseases like severe dry eye and atopic keratoconjunctivitis, as well as those with a history of systemic infectious and inflammatory diseases, were excluded from the study.

RESULTS

The study consisted of a total of 62 participants, 31 patients who underwent re-keratoplasty formed the experimental group while the control group consisted of 31 patients who did not experience rejection after keratoplasty. The time from the first surgery to the first appearance of graft rejection symptoms in rekeratoplasty patients was calculated. The preoperative neutrophil (NE), lymphocyte (LY), platelet (PLT), NLR, PLR, and RDW values for all patients were determined.

The demographic data and the average NE, LY, PLT, RDW, NLR, and PLR values of the patients assessed before the operation are outlined in Table 1.

Table 1. The demographic and average hematological data of the patients

	Mean	Standard deviation	Median
Neutrophil	4.66	1.43	4.46
Lymphocyte	2.46	0.77	2.37
Erythrocyte distribution width	13.4	1.4	13.0
Platelet	265	65	268
Neutrophil-to-lymphocyte ratio	2.04	0.80	1.99
Platelet-to-Lymphocyte ratio	117.06	44.32	109.80
Age	53	19	54
The first rejection time (months)	38	32	32
Sex	Count	%	
Male	38	61.3%	
Female	24	38.7%	

The average ages of both groups were similar, but there was a significantly higher incidence of rekeratoplasty among male participants in the experimental group (p=0.01) (Table 2, Figure 1).

Table 2. Survival rate by gender

Case processing summary

Sex	Total Count	Count of events	Censored	
			Count	%
Male	38	24	14	36.8
Female	24	7	17	70.8
Overall	62	31	31	50.0

The NLR, PLR, and RDW values did not show any significant difference related to rejection risk. The mean survival time of the grafts was 62 months. Male patients developed rejection after an average of 49.339 months, while this period for female patients was an average of 60.602 months, and a statistically significant difference was detected between the two groups (p = 0.011) (Table 3).

There was no significant difference found when the diagnostic groups were compared in terms of their progression to rejection reaction and survival time (p= 0.907) (Table 4, Table 5, Figure 2).

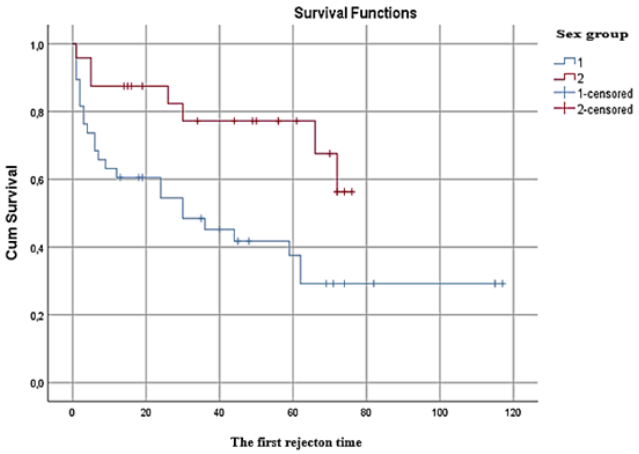

Figure 1. Survival rate by gender

Table 3. Means and medians of survival time by sex

Sex	Mean*				Median			
	Estimate	SE	95% CI		Esti-mate	SE	95% CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Male	49.339	8.139	33.387	65.291	30.000	14.187	2.193	57.807
Female	60.602	5.493	49.835	71.369				
Overall	62.233	6.701	49.099	75.368	62.000	13.295	35.942	88.058

*Estimation is limited to the largest survival time if it is censored; SE, Standard error,; CI, Confidence interval

Table 4. Case processing summary

Diagnostic groups	Total count	Count of events	Censored	
			Count	%
Leucoma	34	17	17	50.0
Keratoconus	12	6	6	50.0
Bullous keratopathy	16	8	8	50.0
Overall	62	31	31	50.0

Table 5. Means and medians of survival by diagnosis

Diagnostic groups	Mean*				Median			
	Estimate	SE	95% CI		Estimate	SE	95% CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Leucoma	64.062	9.088	46.249	81.876	72.000	30.537	12.148	131.852
Keratoconus	49.488	9.658	30.558	68.419	62.000	21.877	19.122	104.878
Bullous keratopathy	43.083	7.261	28.852	57.315	62.000	23.787	15.377	108.623
Overall	62.233	6.701	49.099	75.368	62.000	13.295	35.942	88.058

*Estimation is limited to the largest survival time if it is censored; SE, Standard error

The preoperative average NLR, PLR, and RDW measurements were analyzed for the patients. No significant correlation was observed between NLR (p=0.074) and PLR (p=0.111) values and the first rejection time (p> 0.05).

A significant result was found between RDW values and first rejection time. Patients with a RDW value greater than 13.431 have a 1.38-fold higher likelihood of experiencing rejection compared to those with a value below this threshold. The result is statistically significant (p=0.012) (Table 6).

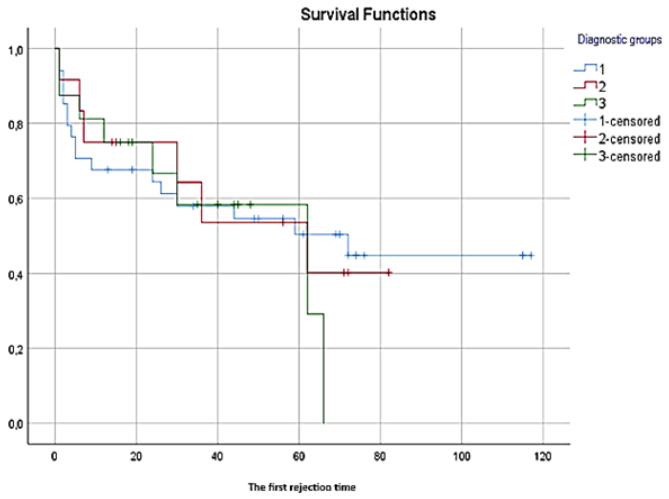

Figure 2. Survival rate by diagnostic groups

In individuals experiencing corneal transplant rejection and subsequent repeat corneal transplant surgery, an assessment was conducted on systemic inflammatory markers. The potential utilization of these markers as predictive indicators for assessing the risk in patients undergoing penetrating keratoplasty (PKP) was explored.

For this purpose, the retrospective analysis examined the demographic characteristics, primary indications, and preoperative systemic inflammatory indicators of patients who experienced graft rejection post- PKP and subsequently underwent rekeratoplasty. It also considered a control group of patients with a similar PKP diagnosis who did not experience graft rejection. NLR, PLR, and RDW values were assessed. The time taken for the first signs of graft rejection to appear after the initial surgery was calculated for the rekeratoplasty patients.

Upon demographic analysis, it was evident that although no notable age discrepancy existed between the two categories, the prevalence of rekeratoplasty was considerably higher in males.

Table 6. Variables in the equation

	Mean	B	SE	p	Exp (B)	95% CI for Exp (B)	
						Lower	Upper
RDW	13.431	0.324	0.129	0.012	1.383	1.075	1.780
NLR	2.037	0.434	0.243	0.074	1.544	0.959	2.485
PLR	117.058	-0.010	0.006	0.111	0.990	0.978	1.002

RDW, Erythrocyte distribution width; NLR, Neutrophil-to-Lymphocyte ratio; PLR: Platelet-to-Lymphocyte ratio, SE, Standard error

The main indications for primary keratoplasty were leukoma, bullous keratopathy, and keratoconus. It has been noted that the primary diagnoses of the condition did not show a substantial impact on the risk of rejection.

There was no observable correlation between serum NLR, PLR and RDW levels and graft rejection. Nevertheless, a notable association was detected between RDW and the duration until the occurrence of the initial rejection episode.

DISCUSSION

PKP is a crucial surgical procedure for treating corneal diseases or preventing vision loss. While visual complaints constitute an indication in most cases, it is also applied for therapeutic and/or tectonic purposes.

Advances in corneal transplant surgery and the increased availability of donor material have led to a rise in primary PKPs. Consequently, there has been an increase in rekeratoplasty procedures and graft failure has become a common cause of transplantation (17).

Recently, NLR, PLR, and RDW values are now accepted as simple markers for systemic inflammation. In some studies, NLR and PLR have been identified as prognostic markers in hypertension, diabetes mellitus, familial Mediterranean fever, cardiovascular diseases, hepatic cirrhosis, and malignancies (10). Moreover, several studies have highlighted the potential of RDW as an inflammation marker and showing its increase during inflammatory processes. In these studies, RDW was stated to be a prognostic factor in conditions such as stroke,

myocardial infarction, sepsis, and cancer (18,19).

The level of these inflammatory parameters has been studied in various eye diseases. Ozkok et al. investigated the relation between RDW values and visual potential in retinal vein occlusion (RVO), and they found a significant elevation in RVO cases compared to the control group. Higher RDW level was associated with lower best corrected visual acuity (20). Yingbo et al. examined the correlation between RDW and diabetic retinopathy (DRP), revealing a heightened incidence of DRP in diabetic patients with high RDW (21). Similarly, Pinna et al. found a substantial association between elevated RDW levels and non-arteritic anterior ischemic optic neuropathy (22).

Elbeyli et al. assessed RDW, PLR, and NLR parameters in patients with central retinal artery occlusion (CRAO), indicating that RDW seemed to outperform other inflammatory indices in predicting CRAO (23). In a study on PKP, Yıldız et al. evaluated systemic inflammatory parameters in patients who developed corneal graft rejection after PKP and found the NLR rate to be low in these patients (24).

In this study, in patients who had corneal graft rejection after PKP and underwent rekeratoplasty; systemic inflammatory parameters were evaluated. The usability of these parameters as prognosis indicators in PKP patients in terms of risk prediction was investigated. In terms of rekeratoplasty, no significant relationship was found between serum NLR, PLR, and RDW values and graft rejection. Again, it was observed that the primary diagnoses did not make a significant difference in terms of keratoplasty indications. The absence of difference in the keratoconus group, which has a low expected risk of graft rejection, may be attributed to the exclusion of patients who underwent DALK (deep anterior lamellar keratoplasty) from the study. While there was no demographic difference in age between the two groups in the study, it was observed that the rekeratoplasty rate was significantly higher in the male gender. This situation may be related to differences in the patients' immune systems. It also suggests that there may be differences between genders in terms of adherence to follow-up and treatment.

Limitations of the Study

The study has some limitations. Only PKP cases were included in the study and the number of cases was limited. Today, the preference of lamellar keratoplasty instead of PKP according to indication and the reduce in rejection rates have limited the number of patients. Due to the nature of the study, RDW values could not be evaluated during the period when the rejection reaction occurred and during follow-ups.

CONCLUSION

In conclusion, high RDW value, which is an indicator of systemic inflammatory state, was found to be associated with the time to first rejection reaction in corneal transplant patients and graft rejection patients compared to controls, as in many diseases. As far as is known, this is the first study investigating the relationship between the duration of graft rejection and RDW in corneal transplant patients. RDW, in terms of rejection reaction risk prediction and prognosis; can be used as a useful, cheap, and practical parameter. In order to better understand the role of these hematological parameters in graft rejection, comprehensive studies that include lamellar keratoplasties in addition to PKP are needed.

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