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Platelet indices in graves disease, especially plateletcrit

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Abstract

Graves' disease is an autoimmune thyrotoxicosis caused by thyroid receptor antibody and occurs in 1% of the population. Autoimmune events develop against cells in various tissues, including platelets. Platelet lymphocyte interaction can regulate these events. We investigated this relationship in our study. We compared leukocyte and platelet indices in 66 Graves patients and 38 control patients from hemogram parameters prospectively. Lymphocyte count, neutrophil count, platelet lymphocyte ratio, neutrophil lymphocyte ratio was insignificant between the two groups (p: 0.51, 0.13, 0.19, 0.097, respectively). Platelet count, PDW and PCT were significantly higher in Graves patients (p: 0.02, 0.03, 0.00, respectively), and MPV was significantly lower (p: 0.007). In the ROC curve analysis, the PCT threshold value was 0.24. Platelet indices in graves patients and especially PCT were more correlated with the disease than other hemogram parameters. This finding may help to clarify the pathogenesis of the disease.

Keywords: Graves disease, platelet indices, plateletcrit

Introduction

Graves' disease (GD) is an autoimmune disease characterized by the presence of circulating organ-specific antibodies (TSH receptor antibody). An abnormal humoral and cellular immune response to thyroid antigens is responsible for the pathogenesis of Graves [1]. Antibodies do not destroy thyroid cells, they cause hyperthyroidism by stimulating their metabolic activity and growth through TSH receptor [2].

The autoimmune reaction is likely to result from the major histocompotiability genes (human leukocyte antigen), thyroid-specific genes, polymorphism in immunoglobulin genes and this is exposed by an exogenous (infective, toxic, pathological) or endogenous (age, gender, genetic) factor [1]. The excessive inflammatory factor production is responsible for the chronic course of the disease, immunocompetent cell stimulation and tissue damage [3].

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There are publications that suggest that blood platelets modulate lymphocyte activity, increase endothelium adhesion, and support rolling in Graves' disease [4,5]. Both blood platelets and lymphocytes have significant effects on inflammation, immunology and homeostasis. These events are critical for the development of thyroid autoimmune disease [6].The aim of this study was to find out which hemogram indexes were significant in Graves' disease.

New automated hematological analyzers can measure hemogram parameters including platelet indices at low cost. The main platelet indices are platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). MPV represents the mean volume of blood platelets and PDW reflects the heterogeneity of platelet volume [7]. The platelet mass and PCT are calculated from PC and MPV [8].

Materials and Methods

This study was a cross-sectional, case–control study. The design of the study was approved by the Ethical Committee and Institutional Review Board of Van Regional Training Hospital. The study was conducted in Van Regional Training Hospital, departments of Endocrinology and Internal Medicine. Written informed consents were obtained from participants.

Sixty six patients clinically diagnosed as Graves Disease were recruited from the Endocrinology Department of Van Regional Training Hospital between December between May 2014 and May 2015. Thirty-eight age-matched patients, who have been admitted to internal medicine outpatient clinic of the hospital during the same period but have not been diagnosed with any disease, were enrolled to the study as the control group (Table 1, 2). Exclusion criteria for the patient group were as follow; having another thyrotoxicosis other than Graves, being younger than 18 years of age and older than 60 years, the presence of inflammatory disease, myeloproliferative disorders, taking anticoagulation medicine, malignancy and infection. The exclusion criteria for the control group were being younger than 18 years of age and older than 60 years, having any chronic disease or infection and taking medication.

The diagnosis of Graves was performed by performing thyroid function tests, thyroid ultrasound and thyroid radioactive iodine uptake. Afterwards they were included in the study.

Blood samples were obtained from the patients at the time of diagnosis and from the healthy group at the time of application. The samples were studied in the Automated Hematology Analyzer Sysmex XN 1000 device in the following first hour.

Platelet lymphocyte ratio (PLR) was calculated by dividing the platelet count by the number of lymphocytes and neutrophil lymphocyte ratio (NLR) by dividing the neutrophil count by the number of lymphocytes. Free triiodothyronine (fT3), thyroid stimulating hormone (TSH), and free thyroxine (fT4) were studied in cobas 8000 modular analyzer with chemiluminescent method. The threshold values determined by the laboratory for TSH, fT3, fT4 were 0.27-4.2 -22IU/ml, 3.1-6.8 pmol/l and 12-22 pmol/l respectively.

Statistical Analysis

SPSS software version 20 (SPSS, Chicago, IL, USA) was used for statistical analyses. Values were reported as means ± standard deviation or medians (minimum–maximum. Proportions were compared by using the Chi-square test or Fisher's exact test. The level of significance was established by using the student t-test for normally distributed values and Mann Whitney U test for abnormally distributed values. Correlation analysis was determined by using Spearman's rank correlation coefficİent. A receiver-operating characteristic (ROC) analysis was conducted to specify the best cut-off value to predict the outcome. Differences were reflected as significant at $p<0.05$.

Results

There were 66 patients in the Graves Disease group and 38 healthy persons in the control group. Characteristics of the groups are given in Table 1-2.

Table 1. Gender comparison between groups; Chi-square test

		Groups		P value
		Control	Graves	
Gender	Female	33	48	0.095
	Male	5	18	
	Total	38	66	

Table 2. Age comparison between groups; Independent t test

Group	Age	N	Mean	S.D(±))	P Value
Control	19-57	38	29.1842	8.6	0.10
Graves	18-60	66	32.0000	8.4	

There was not any significant difference in patients' age and gender between the groups (GD:18-60 vs control:19-5; p=0.38 and 0.063 respectively).

Hemoglobin, Lymphocyte count, Neutrophil count, Platelet lymphocyte ratio, Neutrophil lymphocyte ratio was insignificant between the two groups (p: 0.37, 0.51, 0.13, 0.19, 0.097, respectively). Platelet count, PDW and PCT was significantly higher in Graves patients (p: 0.02, 0.03, 0.00, respectively) and MPV, RDW and MCV were significantly lower in Graves disease (p: 0.007, 0.00, 0.00 respectively) (Table 3).

Table 3. Comparison of hemogram parameters between groups; Independent t test

Groups	Indexes/PLR,NLR	N	Mean	Std. Deviation (±)	P value
Control group	Lymphocyt count/ mm³	37	2191.62	617.016	0.51
Graves Disease		66	2273.03	588.712	
Control group	Platelet count/ mm³	37	271270.27	71426.982	0.02
Graves Disease		66	304530.30	66786.968	
Control group	Neutrophil count/ mm³	37	4019.46	1407.898	0.13
Graves Disease		66	3623.79	1218.978	
Control group	Hb (g/dl)	37	14.2054	1.29935	0.37
Graves Disease		66	14.4742	1.53896	
Control group	MPV (%)	38	10.245	1.0462	0.007
Graves Disease		66	9.705	0.9151	
Control group	PDW (fl)	36	15.286	1.7327	0.03
Graves Disease		64	15.795	0.4871	
Control group	PCT (%)	36	2.0378	1.22870	0.00
Graves Disease		63	2.8586	0.73045	
Control group	RDW (%)	37	41.046	2.8476	0.00
Graves Disease		65	37.608	2.5512	
Control group	MCV (fl)	38	85.042	4.4069	0.00
Graves Disease		66	80.306	6.3603	
Control group	Platelet lymphocyte ratio	37	129.7265	39.81329	0.19
Graves Disease		66	140.1730	38.66315	
Control group	Neutrophil lympho-cyte ratio 0.097	37	1.9505	0.83490	0.097
Graves Disease			1.6894	0.71334	

In the ROC curve analysis, the area under the curve (AUC) of the PCT was 71% and the diagnostic threshold value for Graves Disease was 0.24 (sensitivity: 76%, specificity 56%) (figure 1). In the ROC curve analysis of RDW, MCV, MPV and PDW, the AUCs were lower than the AUC of the PCT (AUC: 16%, 26%, 34% and 46%, respectively).

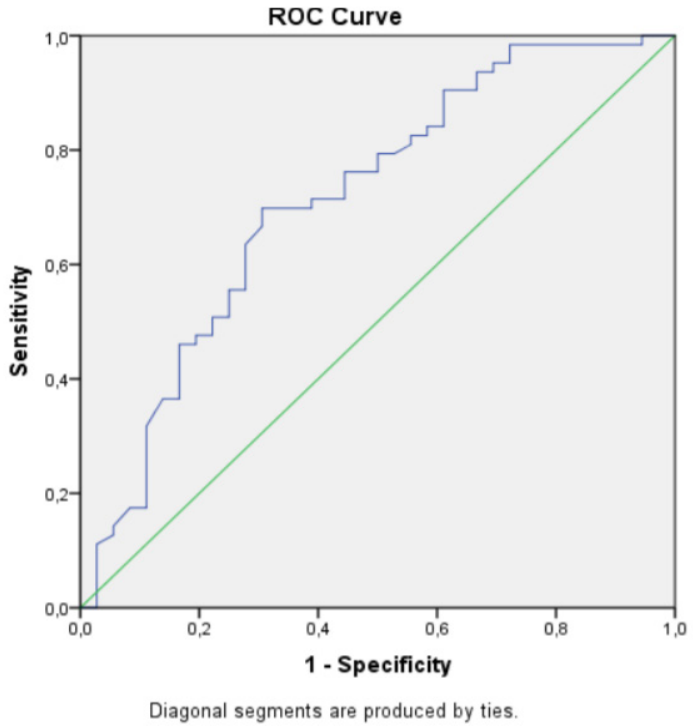

Figure 1. ROC analysis curve for PCT

Discussion

It is known that an inflammation is involved in the pathogenesis of Graves' disease (GD). Recently, the investigation of hemogram parameters as a marker of inflammation in the diseases in which inflammation is involved attracted great interest. In our study, we found that platelet indices were the mostly affected ones among hemogram parameters in GD.

Platelets actively respond to micro-environmental change by proinflammatory and prothrombotic agent synthesis and release. These processes result in an increase in the mean platelet volume (MPV) due to the transformation of the platelet shape from the discoid to the spheric. MPV is an indicator of platelet activity. There are studies showing that platelet number, shape and size have changed with the level of autoantibody and inflammatory mediator [9-11].

Tomczyńska et al. investigated the platelet activity in Hashimoto and Graves' patients and found higher than control group [12]. Platelet changes, such as lower platelet counts, increased MPV together with the shortened platelet lifespan, were observed in GD previously [13]. There is evidence that platelet count, size and life span decrease in the development phase of Graves' disease [14]. In study of Kuznik et al., platelet counts in GD patients were significantly lower than healthy controls [4]. Bagir et al. compared recurrent GD with remission and found MPV significantly higher in the recurrent group and attributed this state to hypermetabolism [15]. In contrast we found that the platelet count (PC), was significantly higher and mean platelet volume (MPV) was significantly lower in the patient group (304530/mm³ vs 271270/mm³; p: 0.02, 9.7 vs 10.2; p:0.007 respectively) There was no significant difference in platelet lymphocyte ratio (PLR) (p: 0.19).

There is evidence that platelet survival and number can increase rapidly after hyperthyroidism treatment [16]. On the other hand, significant reduction in MPV is reported in inflammatory bowel diseases [17-19] and in rheumatologic diseases [20]. Kayahan et al. found lower MPV in inflammation in the study of ulcerative colitis patients and they explained this as the measurement of small inactive platelets due to the rapid clearance of large active platelets from the circulation [21]. On the other hand, cytokines released in inflammation are likely to increase the number of smaller platelets by stimulating megakaryopoiesis. Our study supports these findings.

There are conflicting studies that show a relationship between inflammatory diseases such as hepatic steatosis, rheumatic diseases and MPV. MPV increases in some but decreases in others. It has been shown that in Hashimoto, another autoimmune thyroid disease, MPV decreased parallel to endothelial inflammatory dysfunction [11].

In our study, we found that MPV was significantly lower in the patient group than in the control group. Since our patients were examined at the time of initial diagnosis, our findings were consistent with the above-mentioned MPV decrease in the GD development stage. However, we found an insignificant yet a positive correlation between fT4 and MPV (p: 0.06, pearson: +0.22). With this finding, we concluded that thyroid hormone increase, in other words hypermetabolism, could increase MPV in accordance with claims of Bagir et al. but according to our clear result we suggest the hypothesis that autoimmunity may make a clear fall in MPV.

Plateletcrit (PCT) gives more precise information than PLT count and MPV because it is determined with the combination of PC and MPV (PCxMPV) [22]. The PCT, giving an idea of platelet mass, had the highest sensitivity and specificity among the indices ın our study (MPV, PDW, PLR, and NLR) and significantly higher in the patient group (0.28 ± 0.07, 0.2 ± 0.1; p: 0.00, respectively).

The PDW, which measured platelet heterogeneity, was significantly higher in patients with GD (15.79 ± 0.48, 15.28 ± 1.73; p: 0.03, respectively). Our PDW and PC results also support hypermetabolism. In PCT, MPV also has an effect and it is much more meaningful in our ROC curve analysis, therefore we suggest that PCT measurement is more valuable for autoimmunity. We found that 0.24 could be used as the threshold value (sensitivity 76%, specificity 56%).

Many studies have pointed out that platelet lymphocyte interaction plays an important role in inflammation. It is suggested that platelets can affect lymphocytes directly through receptors and through mediators indirectly [9,10]. There are several epidemiological studies showing the lymphocytosis in autoimmune thyroid patients [4,23,24]. In our study, we found no significant difference for LC between the two groups. Kuznik et al. found that LC was

significantly lower in patients with GD than healthy patients [4].

GD is closely related to the redistribution of white blood cells in which leukopenia with absolute or relative neutropenia and relative lymphocytosis is seen [25]. Reduction of neutrophils associated with hyperthyroidism may be due to decreased circulation time of neutrophils and decreased bone marrow granulocyte reserve [26,27]. In the study of Kyritsi et al., neutropenic patients were screened and 43.6% of them had thyroid disease (4.1% Graves). Besides, there was a negative correlation between sT3 and neutrophil counts in the same study [28]. In our study, the NC of the two groups was similar, but it was lower in patient than in the healthy control group, and we also found a negative correlation between sT4, sT3 and NC (p:0.023 pearson: -0,28, p:0.027 pearson: -0 ,27, respectively).

In another study, patients with and without Graves ophthalmopathy were compared, NLR was significantly higher in patients with ophthalmopathy (2.72 vs 1.98 p: 0.012) [29]. In our study, we compared patients with and without Graves regardless of ophthalmopathy and we found no significant difference, further to that the NLR was slightly lower in patients with Graves (1.68 vs. 1.95 p: 0.097). We commented that this may be due to frequent NC deficits associated with the disease.

Conclusion

The literature review indicates that our study is the first study to investigate the detailed platelet indices in GD and to show that PCT is meaningful. The fact that it was prospective and that the examination was made at the time of the first diagnosis increases the value of the study. The critical aspect of our study is the small number of cases.

As a result, it may be appropriate to use PCT as an indicator of indirect inflammation in GD. This index can be used as a parameter to diagnose the disease. It can assist the monitoring of the disease. It may also be useful in other diseases with inflammation in the pathogenesis. There is a need for prospective studies using larger series to elucidate and investigate some conflicting results in the literature.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
This study received no financial support.

Ethical approval
We state that the study has been approved by the the ethic committee of Van Regional Research and Education Hospital have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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