Evaluatıon of Neutrophıl-To-Lymphocyte Ratıo and Mean Platelet Volume In Patıents With Hyperthyroıdısm

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ABSTRACT

Both Lymphocytes and platelets play an important role in the pathogenesis of Graves' disease (GD), a chronic inflammatory autoimmune disease. The mean platelet volume (MPV), which is considered as an indirect indicator of platelet function, may be increased as an inflammatory marker in GD. The aim of this study is to prove that the autoimmune, chronic inflammatory pathogenesis of GD can be demonstrated by evaluating the whole blood count parameters

A total of 75 patients of which 41 were diagnosed as GD and 34 were diagnosed as TNG were included in the study. Complete blood count parameters were evaluated in both groups before treatment and after treatment when euthyroidism was achieved.

In GD group, pretreatment MPV values were significantly higher than TNG group (p: 0.021). There was a statistically significant decrease in NLR in GD group compared to TNG group after treatment (p: 0,025). Although there was no statistically significant difference, lymphocyte count was higher in GD group compared to TNG group before treatment. The monocyte count was significantly higher in the GD group compared to the TNG group before treatment (p: 0.006). There was no correlation between MPV and free T3, free T4, TSH values before and after treatment in GD group.

These results suggest that autoimmunity and inflammation in GD are reflected in whole blood count parameters and that MPV elevation in GD is related to autoimmunity rather than the metabolic effect of thyroid hormones.

Key Words: Graves'Disease; Toxic nodular goiter; Mean platelet volume; Neutrophil-lymphocyte ratio

Introduction

Hyperthyroidism occurs because of excessive thyroid hormone synthesis and secretion by the thyroid gland. The two most common causes of hyperthyroidism are; Graves disease (GD) and toxic nodular goiter (TNG), respectively (1).

GD is an autoimmune disease of the thyroid which is characterized by hyperthyroidism, diffuse goiter, ophthalmopathy and dermopathy. GD has IgG-type antibodies (TRAb) that bind to and activate the thyrotropin receptor (TSH receptor). These TRAb autoantibodies stimulate follicle hypertrophy, hyperplasia and increase the synthesis of thyroid hormones (2). TNG, the second most common cause of hyperthyroidism after Graves' disease, is usually caused by activating mutations of the somatic thyrotropin receptor or stimulatory G protein alpha-subunit. Thyroid hormones are synthesized and secreted autonomously due to that activating mutations (3, 4).

The main pathology in autoimmune thyroid diseases such as GD, is the body's inadequate immune response to its own tissues accompanied by chronic inflammation. However, autoantibodies against specific cell antigens are also seen in these diseases. Lymphocytes have important roles in the autoimmune process associated with GD. Activation of T lymphocytes and monocytes infiltrating the thyroid gland secretes some inflammatory mediators. The deterioration of the immune system regulatory functions of the leukocytes, their activations, changes in the number of these cells and the mediators they secrete play a crucial ya da critical role in disease pathogenesis. B lymphocytes proliferation or activation are stimulated by increased activity of T-helper cells in peripheral blood. B lymphocytes are transformed into plasma cells that produce antibodies. These antibodies increase the production of thyroid hormones by activation of TSH receptor and consequently cause hyperthyroidism (5). Lymphocytes and platelets play an important role in immunological and inflammatory processes. Platelets interact with

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the immune system cells (neutrophils, monocytes, lymphocytes and dendritic cells) in inflammation. It has been suggested that this interaction can occur through direct contact with receptors or indirectly through soluble mediators. Platelets cause the migration and adhesion of T cells, B cells and NK cells by expression and secretion of adhesion molecules and secretion of chemotactic substances. The effects of platelets enhancing immune cell function are very important for the development of autoimmune thyroid diseases. Mean platelet volume (MPV) is an indicator of thrombocyte size. The synthesis and release of proinflammatory, prothrombotic agents in response to changes in the microenvironment causes an increase in platelet volume. Therefore, MPV is accepted as an indirect indicator of platelet function (5, 6).

The number of leukocytes and subgroups (neutrophils, lymphocytes, monocytes, basophils, eosinophils), which are important components of inflammation, can now be easily determined by whole blood counting devices (7).The neutrophil to lymphocyte ratio (NLR), which is measured by dividing the number of neutrophils by lymphocytes, is used performed to evaluate the inflammatory response in many chronic diseases (8, 9). We believe that the autoimmune and chronic inflammatory pathogenesis of GD will be reflected in complete blood count parameters.

In our study, we investigated MPV and NLR values in patients with GD and TNG before start of treatment and after treatment when euthyroidism was achieved. The aim of this study is to prove that the autoimmune, chronic inflammatory pathogenesis of GD can be demonstrated by evaluating the whole blood count parameters.

Material and Method

This retrospective study included 75 hyperthyroidism patients admitted to the endocrinology clinic between January 2010 and April 2019. Patients were divided into two groups as GD or TNG. Forty-one patients were diagnosed as GD and 34 were diagnosed TNG. After the approval of the ethics committee, the records of the patients were evaluated retrospectively.

Patients who were between 18-80 years of age at diagnosis, who met the diagnostic criteria for toxic nodular goiter or graves' disease and whose TSH values were suppressed before treatment were included in the study. Graves' disease and toxic

nodular goiter were diagnosed by thyroid function hormone tests, radioactive iodine uptake test, thyroid ultrasonography and thyroid scintigraphy.

Hyperthyroidic patients who didn't meet the diagnostic criteria for toxic nodular goiter or graves' disease, who were not within the specified age range at the first diagnosis, who used antithyroid drugs at the time of diagnosis, those with had high or normal TSH values before treatment, who had any hematologic disease that might cause changes in the complete blood count parameters, patients with any malignancy, rheumatologic disease, active or chronic infectious disease, diabetes mellitus, active thyroid orbitopathy and smokers were excluded.

Complete blood count (hemogram-CBC) tests and thyroid function tests (TSH, free T4, free T3) results before and after treatment of patients were recorded. Complete blood count tests with nihon kohden celltac G device and thyroid function tests (TSH, free T4, free T3) with Architect CI16200 device were performed. NLR was measured by dividing the neutrophil count by the lymphocyte count. For TSH, free T4 and free T3, values between 0.35-4.94 μIU /mL, 0.7 - 1.48ng / dL, 1.71 - 3.71 pg /mL were accepted as normal.

Statistical Analysis: Descriptive statistics for continuous variables were expressed as mean±standard deviation. Independent sample t tests were used to compare the means of the continuous variables between groups. Paired t test was used to compare pre- and post-treatment values of the groups. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value between MPV and thyroid hormone in GD. Statistical significance was taken as 5% in the calculations and SPSS for Windows version 23.0 package program was used for statistical analysis.

Results

75 patients were enrolled in the study. The GD group comprised of 34 women and 7 men and the TNG group comprised of 26 women and 8 men. We did not find gender or age differences between the groups.

TSH, free T3, free T4, neutrophil, lymphocyte, platelet, monocyte, MPV and NLR values of all patients ya da individuals before treatment are shown demonstrated ya da represented in Table 1.

The pretreatment MPV and monocyte levels were significantly different between the groups and were higher in the GD group (p= 0.021 and p=

Table 1. Complete blood count (hemogram) tests and thyroid function tests (TSH, free T4, free T3) results of patients before treatment

	TNG Mean± Std. Dev.(n=34)	GD Mean±Std.Dev.(n=41)	*p
PLT (103/mm3)	264711.76±62708.68	267482.92±60317.47	0.846
MPV (fL)	8.41±0.80	8.92±1.02	0.021
Lyn (103/uL)	2227.94±644.94	2472.92±811.09	0.158
Mono (103/uL)	489.41±132.70	625.12±134.89	0.006
Neut (103/uL)	4066.47±1155.75	4271.21±1763.21	0.563
fT3 (pg / mL)	4.16±1.72	9.81±6.88	0.001
fT4 (ng / dL)	1.13±0.39	1.97±0.67	0.001
TSH (µIU / mL)	0.038±0.06	0.012±0.04	0.051
NLR	1.96±0.78	1.85±0.91	0.613

*: Comparison results by independent t-test TSH, f T3: free T3, f T4: free T4, Neut: Neutrophil, Lyn: Lymphocyte, PLT: Platelet, Mono: Monocyte, MPV: Mean platelet volüme and NLR: Neutrophil-lymphocyte ratio, TNG: Toxic noduler guatr group, GD: Graves disease group

Table 2. NLR values between the groups before and after treatment

	TNG Mean± Std. Dev.(n=34)	GD Mean± Std.Dev.(n=41)	*p
NLR Before	1.96±0.78	1.85±0.91	0.613
NLR After	2.01±0.53	1.65±0.37	0.025

*: Comparison results by paired t-test, NLR: Neutrophil-lymphocyte ratio,
TNG: Toxic noduler guatr group, GD: Graves disease group

0.006, respectively). In receiver operating characteristic (ROC) curve analysis, the MPV for thyroid hormone was found to be 8.35 fL with 66%, sensitivity and 50% specificity in GD group (Figure 1).

Although there was no statistically significant difference, lymphocyte count was higher in GD group compared to TNG group before treatment. Before treatment, there was no difference in NLR between the groups; after treatment, NLR was significantly lower in GD patients (p= 0.613 p= 0.025, respectively) (Table 2).

There was a significant decrease in monocyte count in GD group after treatment compared to before treatment (p= 0.001).

There was no correlation between MPV and free T3, free T4, TSH values before and after treatment in GD group.

Discussion

It is determined that in the pathophysiology of GD platelets also play an important role such as lymphocytes (5, 6).

Studies showing platelet activation in autoimmune thyroid diseases are available in the literature. For example, Tomczyńska et al. demonstrated a significantly higher level of platelet activation in autoimmune thyroid diseases using flow cytometry technique and kinetic measurement of aggregation. Because of the similar results in groups with Hashimoto's thyroiditis and GD, they suggest that platelet activation was due to inflammation and autoimmune processes rather than thyroid hormone disorders (10).

MPV, which is an indicator of platelet thrombocyte size, is calculated by complete blood count devices. During inflammation; active platelets which synthesize and secrete proinflammatory, prothrombotic agents, are larger in size. Therefore, MPV is thought to be a marker that can easily detect inflammation and activation in inflammatory diseases (5, 11).

Panzer et al. performed a study comparing the MPV and platelet counts of patients with hyperthyroidism before and after antithyroid treatment Increased platelet count and decreased MPV levels were observed after 3 weeks of therapy (12).

In another study, Bagir et al. observed patients with GD who were euthyroid after received antithyroid drug treatment for at least 1 year and
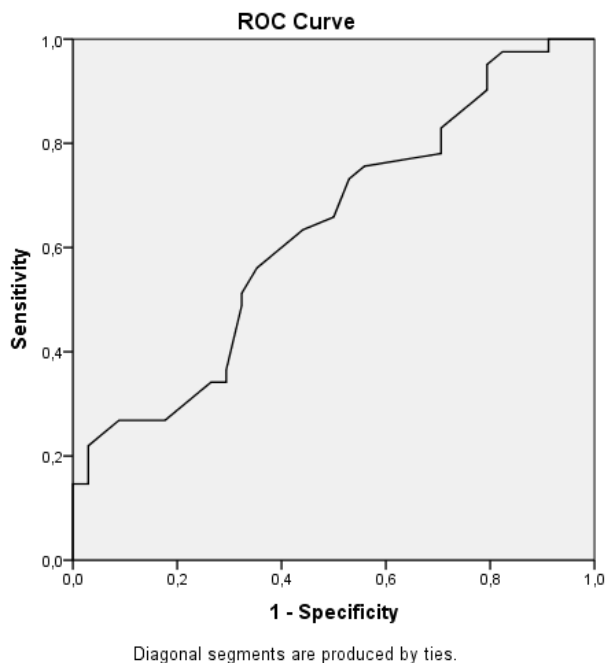
Fig. 1. Receiver operating characteristic (ROC) curve for MPV and thyroid hormone in the Graves' *disease* group. The optimal cut-off value was 8.35 (sensitivity 66%, specificity 50%)

who were followed for at least 6 months after discontinuation of their medication. MPV, which was found to be similar after discontinuation of the antithyroid drug, was found higher in the relapse group than in the remission group within six months following antithyroid drug treatment withdrawal (11). However, these results were attributed to hypermetabolism or hyperthyroidism rather than autoimmunity in GD in both studies (11, 12).

In our study, before treatment MPV values were significantly elevated in GD patients. However, there was no statistically significant difference in MPV levels before and after treatment in the GD group. In addition, there was no correlation between MPV and free T3, free T4 or TSH values before and after treatment in the GD group. These results suggest that MPV elevation in GD may be associated with autoimmunity rather than the metabolic effect of thyroid hormones.

Neutrophils and lymphocytes, which also play a role in GD pathogenesis, are important components of inflammation (7).

NLR, calculated by dividing the neutrophil count by lymphocyte count, is a simple, inexpensive and easily accessible inflammatory response marker. Atılgan et al. found a significantly higher NLR and MPV values in patients with active thyroid orbitopathy (TO) compared to both inactive thyroid orbitopathy and control group. It was

suggested that high NLR and MPV can be an indicator of active inflammation in patients with TO. Also in this study MPV levels in patients with inactive TO were demonstrated to be significantly higher than in the control group. These results have been reported to be evidence of the presence of a permanent, subclinical systemic inflammation even in the inactive stage of the disease (13).

Similarly, Celik T. investigated the NLR levels in Graves' patients with thyroid ophthalmopathy and reported higher NLR in patients with thyroid ophthalmopathy than the control group. In this study NLR levels was also found to be significantly higher in patients with active thyroid ophthalmopathy than inactive ones (14).

Turan E., evaluated NLR and hematological parameters in Graves' disease. Contrary to expectations, the study showed that NLR decreased in the patients with GD. This result was attributed to the possible increase of lymphocytes in GD and the suppressive effect of antithyroid drugs on lymphocytes. When the pre- and post-treatment groups were compared, the NLR was lower and the monocyte count was higher in the pretreatment group (7).

In this study, a statistically significant decrease in NLR was observed in GD group compared to TNG group after treatment. However, there was no significant difference between the two groups in the NLR before treatment. Although there was no statistically significant difference, we think that the higher lymphocyte count in the GD group compared to the TNG group was caused by this.

Similar to the other study, in our study, the number of monocytes was significantly higher in the pretreatment GD group and was significantly reduced after treatment.

In our study, MPV values were higher in GD group compared to TNG group before treatment. Since there is no correlation between MPV and thyroid hormones, we have attributed this finding to autoimmunity in GD rather than hypermetabolism.

The limitations of this study were that it was designed retrospectively and involved the low numbers of cases. The most important limitation of our study was the lack of measurement of inflammatory markers such as tumor necrosis factor alfa, interleukin 6, interleukin 1 beta, C-reactive protein and erythrocyte sedimentation rate simultaneously with complete blood count. Demonstrating the relationship between these inflammatory markers and hematological indices could make an important contribution to the

verification of our results. Prospective studies involving larger patient populations are needed to investigate the effects of GD on whole blood count parameters.

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