Can Serum B12 Level Be Used As An Effective Differential Criterion in Differential Diagnosis of Primary and Secondary Polycythemia?

Serum B12 Düzeyi Primer ve Sekonder Polisitemi Ayrıcı Tanısında Etkin Bir Ayrıcı Tanı Kriteri Olarak Kullanılabilir mi?

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Öz

Amaç: Polisitemia vera'da (PV) JAK2 V617F mutasyonu %95 oranında saptanmaktadır. Fakat bu test hem yüksek maliyetli hem de zaman alan bir tetkiktir. Bu çalışmada sekonder polisitemiyi (SP) dışlayabilmek için JAK2 V617F mutasyonuna ilave olarak kullanılabilecek hızlı, ucuz ve kolay ulaşılabilen bir tetkik araştırılması planlanmıştır.
Hastalar ve Yöntem: Bu çalışmaya Dünya Sağlık Örgütünün 2016 tanı kriterlerine göre tanı almış 39 PV hastası ve 51 SP hastası alındı. Hastaların demografik özellikleri, fizik muayene bulguları ve laboratuvar sonuçları tespit edildi. Bu iki grup arasında istatiksel analiz yapıldı.
Bulgular: PV grubunda, serum B12 düzeyi, eritrosit sayısı, lökosit sayısı, nötrofil sayısı, trombosit sayısı ve laktat dehidrogenaz düzeyi SP grubundan istatiksel olarak anlamlı derecede yüksek bulunmuştur. Serum B12 düzeyinin 343 pg/mL'nin üzerinde olması PV-SP ayrımı için eşik değer olarak saptanmıştır.
Sonuç: Yüksek serum B12 düzeyi PV ile ilişkili bulunmuştur. Fakat serum B12 düzeyinin primer ve sekonder polisitemi ayrıcı tanısında kullanılabilmesi için bu çalışmada ki verileri destekleyen daha geniş hasta serileri ile yapılan prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Polisitemi, primer, sekonder, vitamin B12, eritrosit

Abstract

Aim: JAK2 V617F mutation is determined in 95% of polycythemia vera (PV) patients. However, this test is a high-cost and time-consuming examination. In this study, a rapid, inexpensive and easily available test that can be used in addition to JAK2 V617F mutation is planned to exclude secondary polycythemia (SP).
Patients and Methods: A total of 39 PV patients and 51 SP patients, who were diagnosed according to 2016 diagnostic criteria of World Health Organization, have been included in this study. Demographics, physical examination findings and laboratory results of patients have been determined. A statistical analysis was performed between these two groups.
Results: In PV group, serum vitamin B12 level, erythrocyte count, leukocyte count, neutrophil count, platelet count and lactate dehydrogenase levels were determined to be statistically significantly higher than SP group. Serum vitamin B12 level above 343 pg/mL has been determined as the threshold value for the differentiation of PV from SP.
Conclusion: High serum vitamin B12 level was determined to be associated with PV. However, there is a need for prospective studies performed with larger patient series supporting the data obtained in this study in order for serum vitamin B12 level to be used in the differential diagnosis of primary and secondary polycythemia.

Key words: Polycythemia, primary, secondary, vitamin B12, erythrocyte

INTRODUCTION

Polycythemia means to the increase in erythrocyte mass. Hemoglobin (Hb) levels above >16.5 g/dL in men and >16.0 g/dL in women, and hematocrit (Hct) levels above >49% in men and > 48% in women have been defined as polycythemia (1). Absolute polycythemia is examined under two topics which are primary and secondary polycythemia. While primary polycythemia is developed due to a mutation in erythrocyte precursors, secondary polycythemia (SP) is developed due to increased erythropoietin (EPO) level (2). While EPO increases are often observed due to hypoxemia, they may also be presented due to renal transplant, EPO-producing tumors, renal artery stenosis and hydronephrosis (3-6). JAK2 V617F mutation is observed in about 95% of polycythemia vera (PV) patients (7). This mutation is used as a major criterion in the diagnosis of PV (1). Increased EPO and absence of JAK2 V617F mutation should suggest SP in cases with polycythemia. The patients

who were considered to have SP should be evaluated with regard to smoking, the altitude of their residence, pulmonary and cardiac diseases that could cause hypoxia (8). The rate of normal oxygen saturation, and normal pulmonary and cardiac evaluation are observed in SP due to high altitude. Different from other secondary polycythemias, these findings complicate the differentiation of high altitude SP from PV.

The purpose of this study is to evaluate serum vitamin B12 level in primary and secondary polycythemia patients at the time of diagnosis.

PATIENTS AND METHODS

A total of 90 patients, consisting of 39 patients diagnosed with PV and 51 patients diagnosed with SP, who were followed in Hematology Clinic between years 2010-2018, has been included in this retrospective study. Hemoglobin (Hb) levels of >16 g/dl in women and Hb >16,5 g/dl in men were acknowledged as polycythemia. Criteria determined by World Health Organization on 2016 were used as PV diagnosis criteria. Patients for whom PV diagnosis was excluded were recognized to have SP. Demographic data, physical examination findings, serum vitamin B12 levels, complete blood count results, JAK2 V617F mutation status, EPO levels and oxygen saturation of patients have been determined at the time of diagnosis. Serum vitamin B12 levels at >300 pg/ml were considered normal, between 200-300 pg/ml were considered borderline, and <200 pg/ml were considered to be low.

While our study expresses the continuous variables with their descriptive characteristics as mean, standard deviation, minimum and maximum values, the categorical variables are expressed in terms of frequencies and percentages. While analyzing the data, Kolmogorov-Smirnov normality test was used to determine the suitability of the continuous variables for normal distribution. The data that were normally distributed were analyzed by using independent-samples t-test, while the non-normally distributed data were analyzed by using Mann-Whitney U test. The threshold values of B12 were determined by ROC curve analysis. A p value of <0.05 was accepted to be statistically significant. The analyses were carried out using SPSS 24. Approval was received from the Noninvasive Clinical Research Ethics Board at Van Yüzüncü Yıl University with the date 23.11.2018 and approval number 09.

RESULTS

A total of 90 patients, consisting of 39 PV and 51 SP patients, have been included in this study. Mean age was 55.49±11.18 in PV group, 22 (56.4%) of these patients were male and 17 (43.6%) were female. In SP group, mean age was 38.14±13.52, and 48 (94.1%) of patients were male and 3 (5.9%) were female. 30.8% of patients in PV group was determined to have splenomegaly and 23.1% was determined to have hepatomegaly. While no splenomegaly was determined in SP group, hepatomegaly was determined in 3.9% of the patients in this group. Patient characteristics are presented in Table 1 (Table 1). SP reasons were determined as smoking (66.7%), high altitude (27.4%) and chronic obstructive pulmonary disease (5.9%).

Mean Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and EPO levels were determined to be statistically significantly lower in PV group compared to SP group (p<0.05). Red blood cell (RBC), red cell distribution width (RDW), white blood cell (WBC), neutrophil count, platelet count and lactate dehydrogenase (LDH) were determined to be higher in PV group compared to SP group (p<0.05). Lymphocyte count, mean platelet volume (MPV), folic acid, indirect bilirubin and oxygen saturation levels were determined to be similar in both groups (p>0.05).

Table 1. Patient characteristics

	PV (%)	SP (%)
Age	55.49±11.18	38.14±13.52
Male	22 (56.4)	48 (94.1)
Female	17 (43.6)	3 (5.9)
Smoking		
Yes	16 (41)	34 (66.7)
No	23 (59)	17 (33.3)
Splenomegaly		
Yes	12 (30.8)	0 (0)
None	27 (69.2)	51 (100)
Hepatomegaly		
Yes	9 (23.1)	2 (3.9)
None	30 (76.9)	49 (96.1)
Thrombosis		
Yes	10 (25.6)	2 (3.9)
None	29 (74.4)	49 (96.1)
Erythromelalgia		
Yes	21 (53.8)	27 (52.9)
None	18 (46.2)	24 (47.1)
JAK2 V617F		
Positive	34 (87.2)	0 (0)
Negative	5 (12.8)	51 (100)

The relation between PV and SP groups with regard to laboratory parameters is demonstrated in Table 2 (Table 2).

Mean B12 level was 463±269 (pg/mL) in PV group while it was found as 307±147(pg/mL) in SP group. This difference was determined to be statistically significant (p:0.002). While serum vitamin B12 level was determined as >300 pg/mL in 70% of PV patients, it was determined above >300 pg/ml in 30% of SP patients. The ROC analysis of the curve (AUC) of 0.697 (Figure 1). A cut-off value of 343 pg/mL for B12 was able to estimate primary and secondary PV separation with 64% and 75%, respectively (likelihood ratio-LR = 2.54).

Table 2. The relation between PV and SP according to laboratory values

	PV (Mean±SD)	SP (Mean±SD)	P value
Hemoglobin (g/dl)	18.14±1.35	18.39±0.86	0.016
MCV (fL)	83.18±12.03	90.04±3.54	0.000
MCH (pg)	26.28±4.92	29.55±1.44	0.000
MCHC (g/dL)	31.36±1.66	32.65±0.95	0.004
RBC (million/µL)	6.93±1.34	6.12±0.37	0.000
RDW-SD (fL)	59.25±7.07	55.45±3.63	0.006
WBC (/µL)	11,246±3499	7,931±1677	0.000
Neutrophil count (/µL)	8,092±3,449	4,405±1420	0.000
Lymphocyte count (/µL)	2,141±813	2,630±715	0.230
Platelet count (/µL)	455,256±236,157	233,333±51,676	0.000
MPV (fL)	8.74±0.89	8.75±0.74	0.129
B12 (pg/mL)	463±269	307±147	0.002
Folic acid (ng/mL)	6.34±3.56	6.09±2.35	0.073
LDH (U/L)	361±140	206±66	0.000
Indirect bilirubin (mg/dL)	0.56±0.28	0.73±1.26	0.283
Erythropoietin (mU/mL)	3.93±4.03	10.42±8.19	0.004
Oxygen saturation (%)	94.31±2.31	94.45±3.04	0.562

EPO: erythropoietin, LDH: lactate dehydrogenase, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MPV: mean platelet volume, RBC: red blood cell, RDW: red cell distribution width, WBC: white blood cell
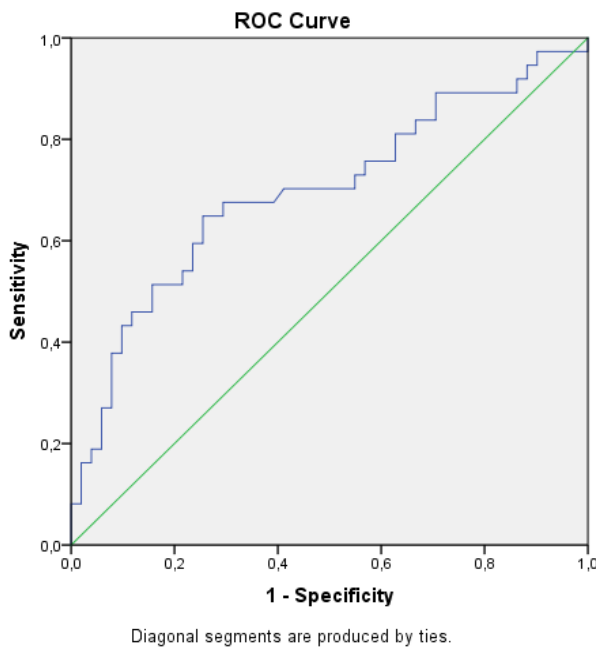
Figure 1. ROC analysis for B12

DISCUSSION

Vitamin B12 is a water soluble vitamin present in lipid, carbohydrate and protein metabolism. This vitamin is particularly important for normal growth and development, bone marrow function and nervous system function. The reasons of vitamin B12 deficiency, clinical manifestations induced by its deficiency, and its treatment have been described extensively (9). Total serum vitamin B12, including both bound and free forms, are measured in Vitamin B12 analyses. Most of Vitamin B12 (70% - 90%) is bound to haptocorrin (HC), and its metabolically active form (10%-30%) is bound to transcobalamin (10). Increased Vitamin B12 levels is a result of one or several of the following mechanisms: It may be explained with increased plasma level of transcobalamin I / III produced by myeloid, hepatic and other cell types, increased hepatic cytolysis, decreased vitamin B12 clearance by the liver, decreased transcobalamin II production by the liver and thus decreased transfer into peripheral tissues, increased gastrointestinal

intake or therapeutic applications (11). Circulating cobalamin-binding proteins and antibodies that cause high plasma cobalamin levels have also been reported (12).

High serum vitamin B12 levels are not associated with symptoms or toxicity, but while some can be associated with some conditions that are completely benign (e.g., parenteral vitamin B12 injection, use of vitamin B12 preparations), others may be a sign of serious diseases. When an underlying malignant disease is determined to be the reason of high vitamin B12 level, malignancy is generally at a stage not determined before and/or at a non-metastatic stage (11). High serum levels of vitamin B12 have been shown to be associated with diseases such as renal failure, cancer, hematologic malignancy (e.g. acute or chronic leukemia), myeloproliferative disorders (polycythemia vera, essential thrombocytosis, chronic myeloid leukemia, primary myelofibrosis), hypereosinophilic syndrome and hepatic disease (e.g. cirrhosis, hepatitis, hepatocellular carcinoma and metastatic liver tumors) (13, 14).

It has been reported that serum vitamin B12 levels are determined to be high in 30%-50% of polycythemia vera patients (15, 16). In our study, serum vitamin B12 level was determined to be above >300 pg/mL in 70% of PV patients. While these increases are less dramatic compared to chronic myeloid leukemia, high plasma concentration of cobalamin is also due to increased HC. Increased vitamin B12 levels (>900 ng/ml) or increased unsaturated vitamin B12 binding capacity (>2200 ng/ml) were indicated in PV diagnostic criteria described by Polycythemia Vera Study Group on 1975 (17). High vitamin B12 level is not among the new diagnostic criteria of today (1). In our study, vitamin B12 was determined to be higher in PV group. There are no other studies comparing B12 levels in PV and SP in the literature. This is the first study showing that B12 is higher in PV patients compared to SP patients. Vitamin B12 threshold level that could be used in the differentiation of two groups was determined as >343 pg/mL. Furthermore, leukocyte count, neutrophil count and platelet count was determined to be high in PV patients, as it was expected from PV.

Smoking causes tissue hypoxia by mechanisms such as increased carboxyhemoglobin in the blood, causing lung disease, and increased erythrocyte oxidant damage in the pulmonary circulation (18). These will lead to erythrocytosis as a result of inadequate oxygenation of blood circulation through the lungs and consequent increased production of erythropoietin (19). We found that cigarette smoking and high altitude play a role in the etiology of 94.1% of the SP. Oxygen saturation was determined to be similar in PV and SP groups. The geographic area of our study has a high altitude. High altitude polycythemia patients often apply to our clinic. JAK2 V617F mutation is checked in patients with normal oxygen saturation and no cardiopulmonary diseases. While this test is not easily accessible, it is also an expensive and time-consuming method. For this reason, new approaches are needed to differentiate high altitude polycythemia patients. Serum vitamin B12 levels may be an additional test to help JAK2 V617F mutation in the diagnosis of polycythemia. According to the results of our study, high vitamin B12 levels, leukocytosis, neutrophilia and thrombocytosis have supporting attributions for PV.

The limitations of our study are the low number of patients, that it is a retrospective study, the fact that mean age was not similar between two groups, and different gender distribution in these groups.

In conclusion, serum vitamin B12 levels were determined to be higher in PV patients compared to SP patients. There is a need for prospective studies performed with a larger number of patients on this subject in order for serum vitamin B12 level to be used as one of the criteria for differential diagnosis of PV and SP.

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