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PERIPHERAL BLOOD PARAMETERS IN PATIENTS WITH VERSUS WITHOUT INCIDENTAL POLYCYTHEMIA: A CROSS-SECTIONAL ANALYSIS WITH EMPHASIS ON NLR, PLR, VITAMIN D AND VITAMIN B12 LEVELS

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ABSTRACT

Objective: To investigate routine peripheral blood parameters in patients with versus without secondary polycythemia with emphasis on NLR, PLR, and levels of Vit D and Vit B12

Methods: A total of 367 internal medicine outpatients (mean±SD age: 49.5±17.4 years, 66.2% were males) with (n=176) and without (n=191) secondary polycythemia were included in this cross-sectional study. Data on patient demographics, smoking status, peripheral blood parameters, NLR and PLR and Vit D and Vit B12 levels were recorded in both groups.

Results: Patients with vs. without secondary polycythemia had significantly higher median(min-max) values for WBC (8.5(4.5-22.6) vs. 7.2(3.1-18.7) $x10^9$/L, p<0.001), neutrophil (4.7(1.6-557) vs. 4.1(0.7-55.2) cells/mm^3, p<0.001) and lymphocyte (2.5(0.8-9.6) vs. 2.2(0.5-7.2)cells/µL, p<0.001) and significantly lower platelet (246(11.5-1476) vs. 263(140-456) cells/mm^3, p=0.032) and PLR (99.1(8.2-1093.3) vs. 116.4(25.5-447), p<0.001). Vit B12 levels were negatively correlated with ferritin levels (r=-0.536, p<0.05) only in patients with polycythemia, and with NLR (r=-0.148, p<0.05) and PLR (r=-0.228, p<0.01) only in patients without polycythemia.

Conclusion: In conclusion, our findings revealed the association of secondary polycythemia with significantly lower platelet counts and PLR but no significant difference between patients with and without secondary polycythemia in terms of NLR, Vit B12 and Vit D values. Our findings emphasize the direct negative correlation between hemoglobin levels and PLR as well as the lower likelihood of coagulation activation and thrombotic processes in patients with secondary polycythemia.

Keywords: *Secondary polycythemia; peripheral blood; PLR; NLR; Vitamin B12*

1. INTRODUCTION

Polycythemia, defined by the increase of hematocrit or hemoglobin, is caused by either a clonal myeloproliferative disorder (polycythemia vera [PV]) or a nonclonal erythropoietin-driven elevation in red blood cell mass (secondary polycythemia) (Köhler & Dellweg, 2010; Spivak, 2018; Tefferi et al., 2005).

Secondary polycythemia occurs most commonly as a response to chronic hypoxemia due to obstructive sleep apnea (OSA), obesity hypoventilation syndrome, and chronic obstructive pulmonary disease (COPD) (Nagalla, 2020).

Complete blood count (CBC) is a an easily measurable, available, and reliable laboratory test providing measurements such as the differential white blood cell counts that can be used to calculate the neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR) as easily measured, reproducible, and inexpensive markers of the systemic inflammatory immune response (Akbas et al., 2016; Kholief et al., 2019; Tefferi et al., 2005; Tulgar et al., 2016).

NLR denotes the ratio of neutrophils, representing the active nonspecific inflammatory mediator initiating the first line of defense, to lymphocytes representing the regulatory or protective component of inflammation (Bhutta et al., 2011; Kholief et al., 2019). It provides prognostic as well as diagnostic information about subclinical inflammation beyond conventional risk factors and it is a reliable marker of low-grade systemic inflammation in various clinical conditions (Balta et al., 2016; Ozturk et al., 2015; Tulgar et al., 2016). Platelets and lymphocytes are important blood parameters related to immune surveillance, and the PLR plays an important role in cytokine-dependent immune response and considered a more sensitive marker of systemic inflammation in various conditions (Kholief et al., 2019; Taşoğlu et al., 2014; Turkmen et al., 2013).

Although association of PLR and NLR with inflammation and numerous diseases was reported, there are limited data on PLR and NLR as well as laboratory markers of coagulation activation, or Vitamin D (Vit D) and Vitamin B12 (Vit B12) status in patients with secondary polycythemia (Nadeem et al., 2013; Tulgar et al., 2016).

Therefore, this study was designed to investigate routine peripheral blood parameters in patients with versus without secondary polycythemia with special emphasis on NLR, PLR and levels of Vit D and Vit B12.

2. METHODS

2.1 Study population

A total of 367 internal medicine outpatients (mean±SD age: 49.5±17.4 years, 66.2% were males) were included in this cross-sectional study and divided into two groups based on presence of secondary polycythemia including those with polycythemia (n=176) and those without polycythemia (n=191).

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the “Declaration of Helsinki” and approved by the institutional ethics committee.

2.2. Assessments

Data on patient demographics (age, gender), smoking status and laboratory parameters including WBC ($x10^9$/L), neutrophil (cells/mm^3), lymphocyte (cells/µL) and platelet (cells/mm^3) counts, hemoglobin (g/dL), hematocrit (%),glucose (g/dL), albumin (g/dL), urea (mg/dL), creatinine (mg/dL), eGFR, Fe (µg/dL), TIBC (µg/dL), ferritin (ng/mL), vitamin B12 (pg/mL), vitamin D (ng/mL) and TSH (mIU/L), NLR and PLR were recorded in both groups. Laboratory parameters including Vit B12 (cut-off 187-800 pg/mL), Vit D (cut-off 20-80 ng/mL), NLR (cut off males: 0.43~2.75 and females: 0.37~2.87) and PLR (cut off males: 36.63~149.13 females: 43.36~172.68) were also evaluated in terms of reference ranges (abnormal ≥cut-off value vs. normal: <cut-off value) in study groups.

Polycythemia was considered when there was a hemoglobin level of greater than 16.0g/dL.

2.3. Statistical analysis

Statistical analysis was made using MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Chi square test, Yates Continuity Correction and Fisher Exact test were used for analysis of categorical data. Mann-Whitney U test were used for analysis of the parametric variables. Correlation analysis was performed with Spearman Rho correlation test. Data were expressed as "mean ± standard deviation (SD), median (min-max) and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. Patient demographics and smoking status in study groups

In the polycythemia group compared with absence of polycythemia, patients were younger (median (min-max age: 46.0(15-99) vs. 53.0(17-86) years, p=0.002), the percentage of males were higher (93.2 vs. 41.4%, $p<0.001$) and smoking was more frequent (85.8 vs. 18.3%, $p<0.001$) (Table 1).

3.2. Laboratory parameters in study groups

Patients with vs. without polycythemia had significantly higher median (min-max) values for WBC (8.5(4.5-22.6) vs. 7.2(3.1-18.7) $x10^9$/L, $p<0.001$), neutrophil (4.7(1.6-557) vs. 4.1(0.7-55.2) cells/mm^3, $p<0.001$), lymphocyte (2.5(0.8-9.6) vs. 2.2(0.5-7.2)cells/µL, $p<0.001$), Hb (16.8(16-19.5) vs. 13.5(7.2-15.9) g/dL, $p<0.001$), hematocrit (49.4(25.5-165) vs. 40.8(24.5-49.3) %, $p<0.001$) and Fe (117(60-133) vs. 71.5(3.6-205)µg/dL, p=0.005), whereas platelet (246(11.5-1476) vs. 263(140-456) cells/mm^3, p=0.032), urea (33(3.4-246) vs. 37(12-234) mg/dL, $p<0.001$) and PLR (99.1(8.2-1093.3) vs. 116.4(25.5-447), $p<0.001$) were significantly lower in patients with polycythemia compared those without polycythemia (Table 2).

Overall, VitB12 levels were abnormal in 27% of patients with no significant difference in patients with vs. without polycythemia (26.7 vs. 27.0%, respectively). Vit D levels were abnormal in 66.7% of patients with no significant difference in patients with vs. without polycythemia (80.0 vs. 65.6%, respectively). NLR and PLR levels were abnormal in 21.6% and 16.9% of patients, respectively and with no significant difference in patients with vs. without polycythemia (20.6 vs. 22.5% and 14.3 vs. 19.4%, respectively) (Table 2).

3.3. Correlations of Vit D, Vit B12, NLR and PLR with other laboratory parameters

Vit D levels were positively correlated with albumin levels ($r=0.557$, $p<0.05$) and Vit B12 levels were negatively correlated with ferritin levels ($r=-0.536$, $p<0.05$) only in patients with polycythemia, while Vit B12 levels were negatively correlated with NLR ($r=-0.148$, $p<0.05$) and PLR ($r=-0.228$, $p<0.01$) only in patients without polycythemia (Table 3).

Both in patients with and without polycythemia, NLR and PLR values were correlated positively with each other ($r=0.565$, $p<0.01$ and $r=0.616$, $p<0.01$, respectively) and negatively with hemoglobin levels ($r=-0.169$, $p<0.05$ and $r=-0.248$, $p<0.01$, respectively) (Table 3).

4. DISCUSSION

Our findings revealed increased likelihood of younger age, male gender and active smoking and lower platelet counts and PLR values in patients with polycythemia as compared to those without

polycythemia. No significant difference was noted between patients with and without polycythemia in terms of Vit D levels, Vit B12 levels and NLR values.

Albeit not significant, a tendency for lower Vit B12 levels and increased likelihood of abnormal Vit D levels were noted in the polycythemia group. NLR and PLR were correlated positively with each other and negatively with hemoglobin levels, regardless of the presence of polycythemia.

Our findings revealed association of secondary polycythemia with lower PLR but higher hematocrit levels. This seems notable given that patients with markedly elevated hematocrit due to PV are considered to be at increased risk of arterial thrombosis and venous thromboembolism (VTE) (Folsom et al., 2020; Griesshammer et al., 2019; Marchioli et al., 2013), while an increase in PLR, but to a lesser extent NLR, has been suggested amongst the useful clinical predictors of VTE (Ferroni et al., 2015; Gürsoy et al., 2014; Li et al., 2009).

Notably, patients with PV are considered to be more symptomatic and have a higher requirement of phlebotomy and a higher thrombotic tendency as compared to the secondary polycythemia (Nevrekar et al., 2019). The conditions most frequently associated with secondary polycythemia (COPD and OSA) are associated with states of oxygen deprivation and thus depend on increased hemoglobin levels for adequate oxygen delivery in response to chronically low oxygen saturation (Nadeem et al., 2013). Accordingly, phlebotomy-based reduction of the compensatory elevated hemoglobin level in patients with secondary polycythemia is considered harmful and not to be balanced by a benefit for VTE risk reduction (Nadeem et al., 2013).

In a past study with 26 patients with PV and 11 patients with smoker's polycythemia, authors reported that patients with PV vs. smokers' polycythemia had a greater number of thromboembolic events per patient and more peripheral arterial thromboemboli (Schwarcz et al., 1993). Given that smoking was evident in majority of patients with secondary polycythemia in the current study along with lower platelet counts and PLR in patients with vs. without polycythemia, our findings support that smokers' polycythemia does not represent a hypercoagulable state equivalent to that of PV (Schwarcz et al., 1993).

Indeed, data from past studies indicated significantly higher WBC, neutrophil, basophil, eosinophil, lymphocyte and NLR but similar platelet and PLR values in smokers when compared to non-smokers (Khand et al., 2015; Tulgar et al., 2016). Although higher WBC and lymphocyte counts in our secondary polycythemia group may also be linked to direct effects of smoking in this group, it should be noted that our findings revealed similar NLR but lower platelet and PLR values in patients with vs. without secondary polycythemia.

Although no significant difference was noted in terms of Vit B12 and Vit D levels between patients with and without secondary polycythemia in the current study, there was a tendency for lower Vit B12 levels and higher rate of abnormally low Vit D levels in the former group. These findings are notable given that Vit D levels were positively correlated with albumin levels and Vit B12 levels were negatively correlated with ferritin levels only in patients with secondary polycythemia, while Vit B12 levels were negatively correlated both with NLR and PLR only in patients without secondary polycythemia.

In fact, while the association of vitamin D deficiency with several many chronic inflammatory diseases has been reported, the relationship between vitamin D and inflammatory markers remain inconclusive (Akbas et al., 2016; Pludowski et al., 2013; Shab-Bidar et al., 2012; Wamberg et al., 2013). Our findings revealed no correlation of Vit D levels with NLR or PLR regardless of presence of

polycythemia. Nonetheless, PLR and NLR were reported to be significantly higher in patients with lower 25(OH)D levels, and PLR was found to be an independent predictor of 25(OH)D levels (Akbas et al., 2016). This seems notable given the higher rate of Vit D insufficiency whereas lower PLR values noted in our patients with secondary polycythemia, which seems to emphasize the direct negative correlation between hemoglobin levels and PLR.

Certain limitations to this study should be considered. First, potential lack of generalizability is an important limitation due to single-center study design with relatively small sample size. Second, the cross-sectional design made it impossible to establish any cause and effect relationships.

5. CONCLUSION

In conclusion, our findings revealed the association of secondary polycythemia with significantly lower platelet counts and PLR but no significant difference between patients with and without secondary polycythemia in terms of NLR, Vit B12 and Vit D values. Our findings emphasize the direct negative correlation between hemoglobin levels and PLR as well as the lower likelihood of coagulation activation and thrombotic processes in patients with secondary polycythemia.

Conflict of interest

The authors declare that they have no conflict of interest

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None

Data availability statement

Data available on request from the authors

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