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Research Article

Thiol-Disulfide Homeostasis and Ischemia Modified Albumin as a New Oxidative Stress Marker in Patients with Polycythemia Vera

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Abstract

Objectives: The present study aims to indicate both thiol-disulfide hemostasis and ischemia-modified albumin (IMA) levels in patients with Polycythemia Vera (PV).

Methods: In this prospective case-control study, 34 PV patients and 31 healthy control participants were included. Thiol levels were measured with a new modified colorimetric method. IMA levels were determined by cobalt binding test.

Results: Thiol levels were statistically significant between the groups. ($p<0.001$). IMA levels were also significanlty higher in PV group than healthy control subjects ($p<0.001$). We revealed that thiol and IMA levels were significantly higher in patients with PV in respect to the control groups.

Conclusion: The obtain results indicate that the oxidative balance is disturbed and changed towards the oxidant direction. The dynamic- thiol disulfide balance has moved to proliferation side in patients with PV, and may provide important contributions to the patient's follow-up and disease pathophysiology.

Keywords: Free radical, polycythemia vera, thiol-disulfide homeostasis, total antioxidant status, total oxidant status, ischemia -modified albumin

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Polycythemia Vera (PV) is a chronic myeloproliferative disease characterized by increased red blood cells or erythrocytosis originating from hematopoietic stem cells. [1-3] PV was first described in 1892 and its age-related incidence rate is 2.5-10/100,000 people.[4] The mean age at diagnosis is generally 60 years old in male patients. The diagnostic criteria of the World Health Organization (WHO) include elevated hemoglobin or hematocrit levels, abnormal results on bone marrow biopsy, and the presence of the JACK 2 genetic mutation, which is found in approximately 98% of cases. The majority of PV patients have a mutation in the Janus-type tyrosine kinase-2 (JAK2) gene. [3] A below-normal level of erythropoietin distinguishes PV from common secondary causes of erythrocytosis such as smoking, sleep apnea, and testosterone use.[5] The precise mechanism of uncontrolled cell growth in PV has not been clarified, although the consequences of tyrosine kinase activations due to JAK2-dependent mutation are known. In

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normal physiological conditions, free radicals and antioxidants are in balance, and by cause of the disruption of the balance to the free radical side, oxidative stress (OS) occurs.[6,7] OS indicator biomarkers can be used for new evaluations in laboratory methods used to determine the prognosis of diseases such as PV. Thiols are highly susceptible to radical damage by forming organic structures containing a sulfhydryl group attached to the carbon atom.[8] As a result of the abnormal increase in the free radical levels, thiol groups are transformed into disulfide bonds, which are covalent bonds.[9] The thiol- disulfide homeostasis is restored by reducing the disulfide structures formed to thiol (-SH) groups by reducing agents. Studies have shown that abnormal thiol-disulfide levels play a crucial role in the pathogenesis of many diseases.[11-20] Therefore, determination of dynamic thiol-disulfide balance can provide vital information about biochemical processes. The modification that occurs in albumin as a result of the increase in OS reduces the binding capacity of albumin. This condition, which occurs in albumin, is called ischemia-modified albumin (IMA) and is used as an indicator of OS.[21] In this study, it was aimed to determine the levels of systemic OS, thiol balance and IMA in PV patients.

Study Population

Thirty-four PV patients and 31 healthy subjects were included in this prospective case-control study. Age and gender were determined to be compatible among the study groups. Healthy controls were formed from individuals who did not have systemic disease and did not use drugs. Informed written consent was obtained from both groups. Other hematological disorders and malignancies except PV were eliminated the current study.

Sample Acquisition Procedure

Venous eight hours fasting blood specimens were taken into BD Vacutainer blood collection tubes (SST II Advance) from the study and healthy subjects. Then, whole working samples were centrifuged at 1500 x g for 10 min. The working specimens were then aliquoted into eppendorfs and stored in a refrigerator at -80°C.

Biochemical Parameters

TAS and TOS Measurement

Total antioxidant status (TAS) and Total oxidant status (TOS) levels were measured spectrophotometrically in the autoanalyzer as previously stated.[33] The oxidative stress index (OSI) value was calculated as the following formula.

OSI (arbitrary unit) =TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L)×100

Determination the Level of Thiol / Disulfide Homeostasis and IMA

Thiol/disulfide homeostasis was determined using the recently developed automated method using an Autocobas 501 (Roche-Hitachi, Mannheim, Germany) automated analyzer.[22] Firstly, disulfide structures were reduced to thiol structures with using sodium borohydride chemical. Secondly, to avoid the reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic) acid, excess sodium borohydride was extracted via formaldehyde chemical. Finally, the amount of disulfide was calculated as half the difference between total and native thiols. The autoanalyzer automatically recognizes lipemic and hemolytic serums and does not operate without approval. Therefore, hemolysis does not positively affect current results of the study.

Ischemia Modified Albumin (IMA) levels were analyzed using the Albumin cobalt binding assay previously described.[21] Briefly, known amounts of exogenous Co (CoCl2) were added to serum working specimens. By reason of the ischemic condition, transformed albumin binds to Co(II) to a lesser extent and excess (unbound) Co2+ is measured spectrophotometrically at 480 nm, forming a colored structure with dithiothreitol. The obtained results are given as absorbance units (ABSU).

Statistical Analysis

SPSS 22 (SPSS Inc., Chicago, IL, USA) program was used for the statistical analysis. The normal distribution of the groups was determined by the Shapiro-Wilk test. Nonparametric tests were used due to the study groups did not fit the normal distribution. Comparison of the two groups was performed with the Mann-Whitney U test. $P<0.05$ was considered statistically significant.

Results

This prospective study originated from 64 patients, 34 in the PV group and 31 in the healthy controls. There was no difference in age and gender in the patient and control subjects (Table 1).

Table 1. Demographic characteristics of the groups

Parameters	PV (n=34) n %	Control (n=31) n %	p
Gender			
Male	21 (61.8)	16 (51.6)	0.14*
Female	13 (38.2)	15 (48.4)	
Age	61.1±13.0	59.6±13.2	0.91¥

PV: Polycythemia Vera; *χ^2 test. ¥Student's t- test.

The PV group consisted of 21 (61.8%) male and 12 (55.2%) female, while the healthy subjects consisted of 11 (55%) male and 9 (45%) female. Leukocyte, Lactate dehydrogenase (LDH), Sedimentation, Hemoglobin parameters were statistically significant between the groups (p<0.05, Table 2).

Thiol levels were significantly lower in PV while disulfide levels were statistically significant with respect to the healthy subjects (p<0.001). Disulfide/native, disulfide/total and native/total levels were statistically significant between the study groups (p<0.001). The disulfide/native, disulfide/total and native/total ratios were statistically significant between the patient and healthy subjects (p<0.001). Furthermore, TAS, TOS and OSI levels were found statistically significant between the groups (p<0.001). In addition, IMA levels were high and significantly different between the groups (p<0.001, Table 3).

Discussion

This study indicated that thiol levels were considerably lower in PV patients than the healthy control subjects. Notwithstanding, disulfide levels were found significanlty high in PV patients compared to the controls. Another crucial finding of this study was that IMA levels were quite high and statistically significant in patients with PV compare to the healthy subjects. Moreover, in this study, it was stated that serum TAS levels were lower in PV patients, and TOS levels were higher than the healthy subjects. In this study, the ratios of disulfide/natural, disulfide/total and natural/

Table 2. Laboratory parameters of study and control groups

Parameters	PV Mean (n=34) (min-max)	Control Mean (n=31) (min-max)	p§
Lymphocyte (10^3µL)	1.87 (0.73-3.47)	1.79 (0.97-2.90)	0.909
Leukocyte (10^3µL)	9.27 (2.6-29.3)	7.09 (1.7-28.9)	0.007
Monocyte (10^3µL)	0.50 (0.12-1.45)	0.43 (0.16-1.03)	0.793
LDH (U/L)	264.1 (128-441)	186.2 (135-490)	<0.001
PLT (10^3µL)	339.4 (85-946)	212 (81-420)	0.002
Direct bilirubin (mg/dL)	0.15 (0.07-0.51)	0.13 (0.06-0.20)	0.378
Total bilirubin (mg/dL)	0.81 (0.3-3.4)	0.66 (0.27-1.2)	0.507
Sedimentation	9.26 (1-37)	15.9 (3-68)	0.010
Hemoglobin (g/dL)	15.5 (11.7-18.6)	13.4 (8.1-18.5)	0.003
MPV (fL)	9.3 (7.5-11.6)	9.7 (7.2-12.7)	0.255
PCT (%)	0.31 (0.09-0.90)	0.22 (0.08-0.42)	0.105
Glucose (mg/dL)	113.8 (85-159)	108.8 (75-135)	0.532
Albumin (g/dL)	42.24 (28.7-48.6)	41.5 (36-45.8)	0.213
CRP (mg/dL)	6.03 (0.24-49.7)	5.97 (0.71-16.7)	0.090

PV: Polycythemia Vera; LDH: Lactate Dehidrogenaz; PLT: Platelet; §Man-Whitney U test.

Table 3. Laboratory findings of the study groups

Parameter	PV Mean (n=34) (min-max)	Control Mean (n=31) (min-max)	p§
Total thiol (µmol/L)	473.6(347-666)	483.1 (443-535)	<0.001
Native thiol (µmol/L)	427.8 (296-616)	444.3 (405-492)	<0.001
Disulfide (µmol/L)	22.9 (11.5-37)	18.4 (10-25)	<0.001
Disulfide / Native thiol	5.46 (2.8-10.1)	4.1 (2.2-5.6)	0.008
Disulfide /Total thiol	4.8 (2.7-8.4)	3.8 (2.1-5.1)	0.008
Native thiol /Total thiol	90.2 (83.3.2-94.6)	92.3 (89.9-95.2)	0.008
IMA (ABSU)	1.14 (0.51-1.79)	0.95 (0.47-3.54)	<0.001
TAS (nmol Troloks/L	1.28 (0.68-1.87)	1.48 (1.1-1.97)	0.001
TOS (µmolH2 O2 Equiv./L	8.3 (4.6-12.6)	5.15 (7.2-3.1)	<0.001
OSI	0.66 (0.40-1.20)	0.35 (0.18-0.49)	<0.001

PV: Polycythemia Vera; TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; IMA: Ischemia Modified Albumin; §Man-Whitney U test.

total thiol levels were found a statistically significant in the PV group compared to the controls ($p<0.008$). To the best of our knowledge, this is the first study to indicate together with systemic oxidative stress, thiol-disulfide homeostasis and the levels of IMA.

Polycythemia vera (PV) is a chronic myeloproliferative disease that is characterized by an increase in red cell mass and the median age of patients diagnosed is usually 60 years and can be seen in any age group.[23]

Koyuncu et al. reported that they found native thiol, total thiol and disulfide levels to be higher and statistically significant in patients with PV than healthy subjects. In addition, they also indicated that IMA levels were higher and statistically significant in the PV group than in respect to the control. They think that the thiol-disulfide balance shifts towards proliferation due to the increase in IMA and thiol levels in patients with PV.[24]

Yilmaz et al. found that native thiol, total thiol and Native / total thiol levels decreased while disulfide levels increased in patients with PV than healthy subjects. They concluded that thiol-disulfide balance has shifted towards proliferation.[25] Musolino et al. found a significant serum levels of AOPPs and ds-nitrosylated proteins in patients with PV compared to the healthy volunteers. They think that OS could play a significant role in the physiopathology of myeloproliferative neoplasms.[26] Another study Blume et al. found an increased reduced glutathione (GSH) levels in the red blood cells of patients with myeloproliferative neoplasia with respect to the controls. They hypothesized that increased GSH levels due to the increased activity of glutathione synthetase.[27]

In the present study, it was found that thiol levels were lower and disulfide levels were higher in patients with PV compared to healthy control subjects. Decreased thiol levels may be due to the decrease in antioxidants containing sulfhydryl groups such as glutathione.[28] This reveals that OS may play an important role of the disease severity.

In the literature, Vener et al. reported that reactive oxygen species (ROS) levels were higher while TAS levels were lower in patients with primary and post–polycythemia vera myelofibrosis with respect to the controls. They assumed that increased ROS and decreased TAS levels due to the augmented oxidation associated with disease progression.[29] In the present study, serum TAS levels were lower in TOS and OSI levels were found to be higher in patients with PV compared to controls. We think that a decrease in antioxidant molecules will occur due to increased OS. Moreover, the decrease in antioxidant levels in PV patients may cause an increase in TOS and OSI levels due to increased OS. In addition, we hypothesized that decreased antioxidant capacity due to the increase in free radical levels may cause carcinogenesis. In the literature, several studies found serum IMA levels were higher in Polystemia Vera, chronic lymphocytic leukemia, cancer, psoriasis, diabetes mellitus compared to the healthy subjects.[28,30-32] They think that the increase in IMA levels occurs due to the increase in oxidative stress and the corresponding decrease in antioxidant levels. In line with the literature, serum IMA levels were found higher in patients with PV than healthy subjects. We hypothesized that the increase in IMA levels will make a significant contribution to free radical production under OS.

Conclusion

In conclusion, increased OS and consequently decreased antioxidant levels were found in patients with PV compared to the healthy subjects. This may have negatively affected the oxidant-antioxidant balance and caused the balance to be disrupted. Thiol levels were decreased and disulfide levels were increased in PV patients. Increased disulfide levels show that it causes a decrease in antioxidant levels. We think that the determination of thiol-disulfide levels may be a possible biomarker for early identification of PV and can be used in the follow-up and monitor of the disease.

Disclosures

Ethics Committee Approval: This study was approved by Hatay Mustafa Kemal University clinical Ethics Committee (protocol number: 2022/02).

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Conflict of Interest: None declared.

Authorship Contributions: Concept – H.E. Desing – H.E. Supervision – H.E.; Materials – R.C.; Data collection &/or processing – H.E., R.C., O.O.; Analysis and/or interpretation – H.E., R.C., O.O.; Literature search – H.E., R.C., S.C.T.; Writing – H.E.; Critical review – H.E., R.C., S.C.T., O.O.

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