



Thiol – Disulphide Homeostasis in Polycythemia Vera

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Abstract

Background: Thiol-disulphide homeostasis has vital role in cell signalling mechanisms, regulation of transcription factors and enzymatic activities, signal transduction and regulation of proliferation rate, apoptosis and detoxification and antioxidant protective mechanism.

Objective: This study aims to demonstrate in Polycythemia Vera (PV) patients the thioldisulphide homeostasis which is known to play a role in cell proliferation, apoptosis and various steps of cell cycle.

Design: Descriptive prospective cross-sectional study.

Settings: Yıldırım Beyazıt University Hospital Ankara, Turkey between 2016-2018.

Method: Forty-two PV patients and 43 healthy controls were included in the study. Serum total (-SH + -S-S-) and native (-SH) thiol levels were measured in all subjects. The amount of dynamic disulphide bonds and, the ratio of (-S-S-) and (-S-S-) × 100/(-SH), (-S-S-) × 100/(-SH + -S-S-), and -SH × 100/(-SH + -S-S-) were calculated with automatic method. The data obtained from the patient group were compared with the control group.

Main outcome measures: The amount of dynamic disulphide bonds and, the ratio of (-S-S-) and (-S-S-) × 100/(-SH), (-S-S-) × 100/(-SH + -S-S-), and -SH × 100/(-SH + -S-S-) were calculated with automatic method in PV patient and healthy control group.

Results: Both groups were similar in terms of age and gender distribution. Compared with the control group, PV group had significantly higher native thiol, total thiol and nativ/total thiol levels.

Limitation: The generalizability of the study's findings were limited by the small sample size.

Conclusions: In accordance with the nature of the disease, thiol balance in PV patients was in favor of proliferation. Increased total thiol (-SH + -S-S-), native thiol (-SH) levels and native thiol/total thiol ratio might be associated with uncontrolled proliferation. The balance of the thiol-disulphide homeostasis shifted to reductive thiol side in the PV. This change can provoke proliferation status of the disease and/or may be secondary to the disease.

Conflict of Interest: No conflict of interest was declared by the authors.

Keywords: Polycythemia Vera, thiol, disulphide bonds, myeloproliferative neoplasms

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Polistemia Vera Olgularında Thiol-Disülfid Homeostasis

Öz

Giriş: Bu çalışmanın amacı, Polistemia Vera (PV) olgularında; hücre siklusunun çeşitli basamaklarında, apoptozis ve hücre proliferasyonunda önemli rol oynadığı bilinen tiyol-disülfid homeostasisini incelemeyi amaçladık.

Yöntemler: Kırkiki PV olgusu 43 sağlıklı kontrol grubuyla karşılaştırıldı. Serum total (-SH + -S-S-) ve nativ (-SH) tiyol seviyeleri her iki grupta ölçüldü. Dinamik disülfid bağlarının yüzdesi ve (-S-S-) and (-S-S-) × 100/(-SH), (-S-S-) × 100/(-SH + -S-S-), ve -SH × 100/(-SH + -S-S-) oranları Erel ve arkadaşlarının yeni metodu ile hesaplandı. PV olguları ve sağlıklı kontrol grubunun verileri kıyaslandı.

Sonuçlar: Her iki grubun yaş ve cinsiyet dağılımları benzerdi. PV grubunda nativ tiyol, total tiyol ve nativ/total tiyol seviyeleri istatistiksel olarak anlamlı artmıştı.

Tartışma: PV hastalığının doğasıyla uyumlu olarak, PV olgularında dengenin proliferasyon yönüne kaydığı gözlemlendi. Total tiyol (-SH + -S-S-), nativ tiyol (-SH) ve nativ /total tiyol oranındaki artışı PV hastalığındaki kontrolsüz proliferasyonla açıklayabiliriz. PV olgularında tiyol/disülfid dengesinin indirgeyici tiyol tarafında olduğunu gösterdik. Tüm bu değişiklikler PV hastalığının patofizyolojisinde olan kontrolsüz proliferasyonla ilişkili olabilir.

Anahtar kelimeler: Polistemia vera, tiyol, disülfid bağ, myeloproliferatif hastalık.

INTRODUCTION

Thiols are organic compounds which contain sulphhydryl group. Albumin and proteins constitute the major part of plasma thiol while low molecular weight thiols such as cystein, cystein glycine, glutathione, homocystein and gamma glutamyl cystein constitute a lower proportion^{1,2}. These compounds have high reduction capacity and are known as good nucleophiles because they can easily get into many reactions³. Thiols are open to both reversible and irreversible modifications and can provide new end products by forming reversible bonds with disulphides as a result of oxidation reactions. The formed disulphide bonds can be reduced back to thiols. Thus, thiol-disulphide homeostasis is maintained. Thiol-disulphide homeostasis has vital role in cell signalling mechanisms, regulation of transcription factors and enzymatic activities, signal transduction and regulation of proliferation rate, apoptosis and detoxification and antioxidant protective mechanisms⁴. Increased intracellular thiol has been associated with proliferation while thiol levels have been found to be decreased in apoptosis.

Thus thiols are important compounds in cell cycle regulation and the control of cell division⁵. Additionally, it has been shown that some chemotherapeutic agents which use the thioredoxin system exert their anti-cancer effects through thiol homeostasis⁶.

Thiol-disulphide homeostasis show different pattern in various diseases. In degenerative vascular diseases such as coronary atherosclerosis^{7,8}, diabetes mellitus⁹, preeclampsia¹⁰ and cerebral ischemia¹¹, thiol levels are decreased and disulphides are found to be increased. On the other hand, in proliferative diseases, thiols are expected to be increased¹²⁻¹⁴.

Chronic Myeloproliferative disorders (CMPD) comprise a group of diseases in which one or more of pluripotent hematopoietic stem cells in the bone marrow have proliferation advantage. Disregulation of proliferation cycle of hematopoietic stem cells have a role in the exaggerated production of mature blood cells. Polycytemia Vera (PV) is one of the CMPD and is characterized by clonal proliferation of erythroid lineage and presence of JAK 2 V617F mutation in most cases¹⁵.

There is in fact proliferation in all three lineages and continuous proliferation which may result in post-PV myelofibrosis and transformation to acute leukemia¹⁶⁻²⁰. Various abnormalities have been discovered in the signal transduction pathways in the hematopoietic stem cell cycle of PV patients²¹. Although it is well known that tyrosine kinase activation results in JAK2V617F mutation and increased sensitivity to hematopoietic growth hormones and cytokines, the exact mechanism of uncontrolled cell proliferation in PV is still incompletely understood²². To our knowledge, thiol-disulphide homeostasis has not been studied in PV patients before. In this study we have aimed to investigate the levels of native thiol, total thiol and disulphide, and the ratios of disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol in PV patients using a novel, automated method that determines dynamic thiol/disulphide homeostasis.

METHODS

Study population

Forty-two patients diagnosed with PV and 43 healthy volunteers were enrolled in the study. Forty-two patients with PV were compared with 43 healthy control cases. The control group was constituted of cases who applied for check up and who did not have any systemic diseases or drug use. Patients with diabetes, severe renal or liver diseases, active infectious or inflammatory diseases, previous stroke, rheumatologic diseases, or malignancy were excluded from the study.

This study has been designed in accordance with 2013 Brazil version of Helsinki Declaration and was approved by the local Ethics Committee. All participants have provided written consent.

Biochemical parameters

Venous blood samples were taken from each patient into tubes containing ethylenediamine

tetraacetic acid (EDTA) after 8 hours of fasting. Collected samples were immediately centrifuged at 3483,6 g value for 10 minutes to separate the serum, then the samples were stored at -80°C until analysis.

Thereafter, all parameters were analyzed at the same time. The thiol/disulphide homeostasis were determined with the more recently developed automated method. Firstly, short disulfide bonds were reduced with sodium borohydride to form free functional thiol groups. To prevent reduction of DTNB (5,5'-dithiobis-[2-nitrobenzoic] acid), reductive sodium borohydride was removed and consumed with formaldehyde. All of the thiol groups, including reduced and native thiol, were measured after DTNB reduction. The dynamic disulfide value was defined as half of the difference between total and native thiols. After determining native and total thiols, disulphide level, disulphide/total thiol percent ratios, native thiol/total thiol percent ratios and disulphide/native thiol percent ratios were calculated². Measurements were made by an Autocobas 501 autoanalyser (Roche-Hitachi, Mannheim, Germany). The analyzer automatically detects lipemic-icteric and hemolytic serums and does not work without approval. Hemolysis does not interfere positively with results.

Statistical Analysis

Normality distributions of study groups were evaluated by the Kolmogorov-Smirnov test. The parametric values were given as mean \pm SD, non-parametric values were given as median (Inter Quartile Range). Comparisons were done with Student's t-test in cases of normal distribution and with Mann-Whitney U test in cases of abnormal distribution. The Spearman and polyserial correlation coefficients were calculated to evaluate the relationship between the measurements. The P value <.05 was regarded as significantly.

Ethics Statement

Ethics committee approval was received for this study from the Institutional Review Board of Ankara Atatürk Training and Research Hospital (No. K-18-727). Written informed consent was obtained from all patients and controls.

RESULTS

The median age of the study cases was 59.7 (14.6) years (range 30-83 years) and the median age of the control cases was 58.2 (13.4) years (range 43-84). There was no differences between the median age of the control cases and the median age of the study cases. Gender of cases were 18 males and 24 females. The distribution of the genders was similar in the control cases and study cases. There were 11 (18.3%) cases with thrombosis history and 6 (10%) cases with hemorrhage history in cases of PV. There were 38 (90%) cases with JAK positivity in the PV group. There were 31 (51.7%) cases with splenomegaly in the PV group. The mean spleen size of the PV group was measured as 127 (30.8) mm.

Mean native thiol levels (SH), total thiol levels (SH+SS) and the native thiol/total thiol ratio (SH/ SH+SS) were higher in PV group compared to the control group (P< .001, P = .001 and P = .02, respectively), which is described in Table 1 and Figure 1.

The basal complete blood counts and white blood cell differential were compared in PV and control groups. The age and gender distribution was similar between the groups. The mean levels of hemoglobin, white blood cell count and platelets were statistically significantly higher in the PV group as expected (Table 1).

Table 1: The PV and Control group complete blood count values and thiols levels.

	PV (n=42)	Control (n=43)	P value
Age	59.7 (14.6)	59.5 (9.6)	0.920
Hemoglobin (g/dl)	18.2 (2.3)	13.2 (1.7)	<.001
Red Blood Cell (RBC) (M/ uL)	6.14 (1.25)	5,2 (0.98)	.165
Mean Corpuscular Vol. (MCV) (fL)	93 (12)	89 (8)	.321
Platelet (K/uL)	500 (295)	350 (185)	<.001
White Blood Cells (K/uL)	12230 (3337)	7500 (1800)	<.001
Basophils (K/uL)	70 (130)	10 (2.3)	<.001
Lymphocytes (K/uL)	2098 (714)	2010 (658)	.402
Native Thiol (µmol/L)	471.3 (55.2)	428.6 (37.8)	<.001
Total thiol (µmol/L)	509.2 (61.2)	470.0 (38.5)	.001
Disulfide (µmol/L)	19.8 (11.4)	20.7 (6.4)	.648
%SS/SH (µmol/L)	4.27 (2.46)	4.76 (2.66)	.106
%SS/totalSH (µmol/L)	3.82 (2.00)	4.41 (1.38)	.119
%SH/totalSH (µmol/L)	92.73 (4.32)	91.31 (4.43)	.020

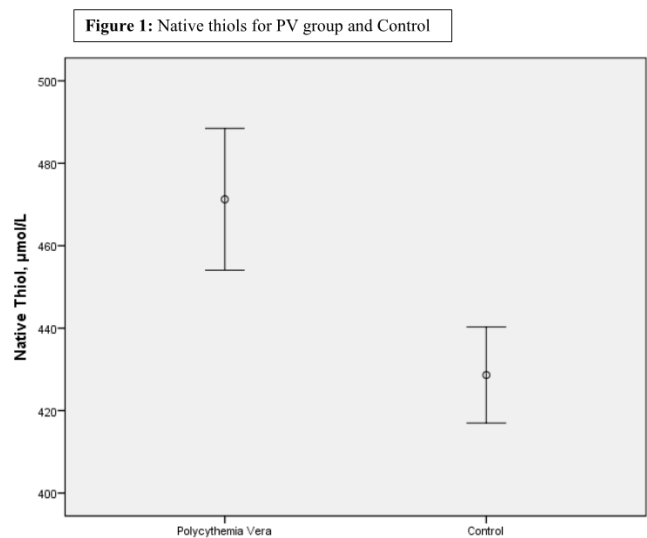


Figure 1: Native thiols for PV group and Control

There was no statistically significant difference between thiol and disulfide values among those with and without JAK positivity in the PV group. Those with and without hemorrhage history or thrombosis history. Among PV cases, cases with a history of hemorrhage or thrombosis compared cases with no history of hemorrhage or thrombosis and, there was no statistically significant difference between thiol and disulfide values. There was no statistically significant correlation between spleen size and thiol-disulfide parameters of PV group.

DISCUSSION

The results of this study have shown that both native and total thiol levels as well as the native/ total thiol ratio (SH/SH+SS) are elevated in PV patients indicating that thiol disulphide homeostasis has shifted towards proliferation. To our knowledge, this is the first study in the literature investigating the thiol disulphide homeostasis in PV. One study has demonstrated increased levels of reduced glutathione (GSH) which is one of the low molecular weight thiols, in the red blood cells of patients with myeloproliferative neoplasia compared with healthy controls. Additionally, they noted that the elevated GSH containing compounds is independent of the age of red blood cell²³. Additionally, in another study demonstrated previous studies which utilized Erel and Neşelioğlu method, found that plasma thiol disulphide homeostasis was increased in degenerative diseases, such as diabetes, obesity, pneumonia, and in the case of asthma, whereas it was reduced in proliferative diseases such as multiple myeloma, renal cell carcinoma, lung cancer, and renal cancer²⁴⁻³⁰. In this study we could not find any differences between the PV and control groups in terms of the amount of plasma disulphide bonds. This indicates clearly that the balance has shifted towards proliferation.

In summary; thiol-disulphide homeostasis was enlarged and the balance shifted to reductive thiol side in the disease. This change can provoke proliferation status of the disease and/or may be secondary to the disease.

Conflicts of interest: The authors have no conflict of interests to declare.

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