Dicle Tıp Dergisi / Dicle Med J (2019) 46 (2) : 315 - 320

www.diclemedj.org

Original Article / Özgün Araştırma

Thiol – Disulphide Homeostasis in Polycythemia Vera

Aysun Şentürk Yıkılmaz[1], Şule Mine Bakanay[2], Sema Akinci[3], Senem Maral[4], Fuad Mustafayev[5], Murat Alisik[6], Özcan Erel[7], İmdat Dilek[8]

1 Department of Hematology, Yıldırım Beyazıt University Medical Faculty, Ankara, Turkey ORCID: 0000-0001-5281-5955
2 Department of Hematology, Yıldırım Beyazıt University Medical Faculty, Ankara, Turkey ORCID: 0000-0003-3941-0368
3 Department of Hematology , Ataturk Training and Research Hospital, Ankara, Turkey ORCID: 0000-0003-4237-3342
4 Department of Hematology , Ataturk Training and Research Hospital, Ankara, Turkey ORCID: 0000-0003-4766-1861
5 Department of Internal Medicine, Ataturk Training and Research Hospital,Erzurum, Turkey ORCID: 0000-0003-0254-1402
6 Department of Medical Biochemistry, Yildirim Beyazıt University Medical Faculty,Ankara, Turkey ORCID: 0000-0003-0434-3206
7 Department of Medical Biochemistry, Yildirim Beyazıt University Medical Faculty,Ankara, Turkey ORCID: 0000-0002-2996-3236 8 Department of Hematology, Yıldırım Beyazıt University Medical Faculty, Ankara, Turkey ORCID: 0000-0001-8316-4460

Received: 13.12.2018; Revised: 06.03.2019; Accepted: 02.04.2019

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: Yildirim 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 patieint 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 provocate 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

DOI: 10.5798/dicletip.574893

Yazışma Adresi / Correspondence: *Aysun Şentürk Yıkılmaz, Department of Hematology,Yıldırım Beyazıt University, Bilkent Yolu 3. Km. Çankaya Ankara 06010, Turkey, e-mail: senturkaysun@gmail.com*

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 istatiksel 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 sulphydryl 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 reactions[3]. 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 enzymmatic activities, signal transduction and regulation of proliferation rate, apoptosis and detoxification and antioxidant protective mechanisms[4]. 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 division[5]. Additionally, it has been shown that some chemotherapeutic agents which use the thioredoxin system exert their anti-cancer effects through thiol homeostasis[6].

Thiol-disulphide homeostasis show different pattern in various diseases. In degenerative vascular diseases such as coronary atherosclerosis[7,8], diabetes mellitus[9], preeclampsia[10] and cerebral ischemia[11], thiol levels are decreased and disulphides are found to be increased. On the other hand, in proliferative diseases, thiols are expected to be increased[12-14].

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. Policytemia 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 cases[15].

There is in fact proliferation in all three lineages and continuous proliferation which may result in post-PV myelofibrosis and transformation to acute leukemia[16-20]. Various abnormalities have been discovered in the signal transduction pathways in the hematopoietic stem cell cycle of PV patients[21]. 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 understood[22]. 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^{o}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 bods 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 dinamic 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 calculated[2]. 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 Analyzis

Normality distributions of study groups were evaluated by the Kolmogorov-Smirnov test. The parametric values were given as mean ± 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 Corpusculer 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
Disülfide (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

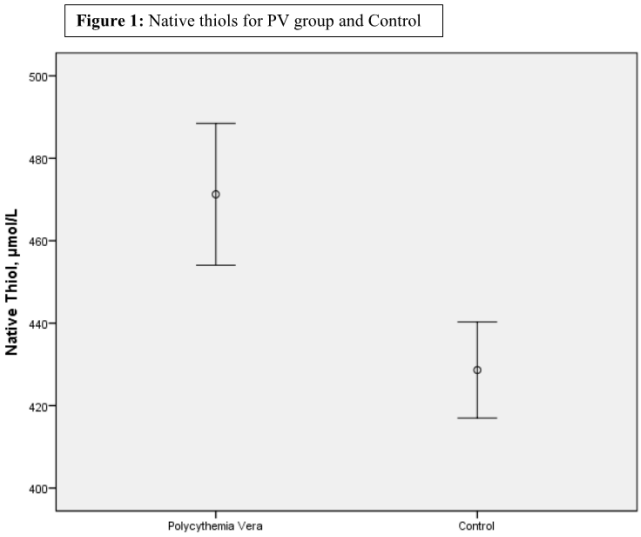

Figure 1: Native thiols for PV group and Control

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 cell[23]. 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 cancer[24-30]. 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 provocate 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.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Ruben A. Mesa, Murray N. Silverstein, et all. Population-based incidence and survival figures in essential thrombocythemia and agnogenic myeloid metaplasia: an Olmsted County Study, 1976-1995. Am J Hematol. 1999; 61: 10-5.
2. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014; 47: 326-32.
3. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. Free Radic Biol Med. 2013; 65: 244-53.
4. Kundi H, Erel Ö, Balun A, et all. Association of thiol/disulfide ratio with syntax score in patients with NSTEMI. Scand Cardiovasc J. 2015; 49: 95-100.
5. Li W, Busu C, Circu ML, Aw TY. Glutathione in cerebral microvascular endothelial biology and pathobiology: implications for brain homeostasis. Int J Cell Biol. 2012: 434971.
6. Wondrak GT. Redox-directed cancer therapeutics: molecular mechanisms and opportunities. Antioxid Redox Signal. 2009; 11: 3013-69.
7. Kundi H, Ates I, Kiziltunc E, et all. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med. 2015; 33: 1567-71.
8. Ateş İ, Ozkayar N, Altay M, et all. Is disulphide/thiol ratio related to blood pressure in masked hypertension? Clin Exp Hypertens. 2016; 38: 150-4
9. Ates I, Kaplan M, Yuksel M, et all. Determination of thiol/disulphide homeostasis in type 1 diabetes mellitus and the factors associated with thiol oxidation. Endocrine. 2016; 51: 47-51.
10. Korkmaz V, Kurdoglu Z, Alisik M, et all. Impairment of thiol-disulphide homeostasis in preeclampsia. J Matern Fetal Neonatal Med. 2016; 3: 1-6
11. Bektas H, Vural G, Gumusyayla S et all. Dynamic thiol-disulphide homeostasis in acute ischemic stroke patients. Acta Neurol Belg. 2016; 116: 489-94.
12. Prabhu A, Sarcar B, Kahali S, et all. Cysteine catabolism: a novel metabolic pathway contributing to glioblastoma growth. Cancer Res. 2014; 74: 787–96.
13. Kirlin WG, Cai J, Thompson SA, et all. Glutathione redox potential in response to differentiation and enzyme inducers. Free Radic Biol Med. 1999; 27: 1208–18.
14. Nkabyo YS, Ziegler TR, Gu LH, et all. Glutathione and thioredoxin redox during differentiation in human colon epithelial (Caco-2) cells. Am J Physiol Gastrointest Liver Physiol 2002; 283: 1352–9.

15. Michiels J.J.,Berneman Z.,Schroyens W, et all. Changing Concepts of Diagnostic Criteria of Myeloproliferative Disorders and the Molecular Etiology and Classification of Myeloproliferative Neoplasms: From Dameshek 1950 to Vainchenker 2005 and Beyond, Acta Haematologica. 2015; 133: 36-51.
16. Cao HC, Lin J, Qian J, et all. Detection of the JAK2 mutation in myeloproliferative neoplasms by asymmetric PCR with unlabeled probe and high-resolution melt analysis. J Clin Lab Anal. 2011; 25: 300-4. doi: 10.1002/jcla.20474.
17. Andersen CL, Bjørn ME, McMullin MF, et all l. Circulating YKL-40 in patients with essential thrombocythemia and polycythemia vera treated with the novel histone deacetylase inhibitor vorinostat. Leuk Res. 2014; 38: 816-21.
18. Bjørn ME, Andersen CL, Jensen MK, et all. Circulating YKL-40 in myelofibrosis a potential novel biomarker of disease activity and the inflammatory state. Eur J Haematol. 2014; 93: 224-8.
19. Klampfl T, Gisslinger H, Harutyunyan AS, et all. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013; 369: 2379-90.
20. Bruchova H, Yoon D, Agarwal AM, et all. Erythropoiesis in polycythemia vera is hyper-proliferative and has accelerated maturation. Blood Cells Mol Dis. 2009; 43: 81-7.
21. Laubach JP, Fu P, Jiang X, et all. Polycythemia vera erythroid precursors exhibit increased proliferation and apoptosis resistance associated with abnormal RAS and PI3K pathway activation. Exp Hematol. 2009; 37: 1411-22.
22. Blume KG, Paniker NV, Beutler E. Enzymes of glutathione synthesis in patients with myeloproliferative disorders. Clin Chim Acta. 1973; 45: 281-5.
23. Ates I, Kaplan M, Yuksel M, et all. Determination of thiol/disulphide homeostasis in type 1 diabetes mellitus and the factors associated with thiol oxidation. Endocrine. 2016; 1: 47-51.
24. Yıldırım F, Sermetow K, Aycicek A, et all. Increased oxidative stress in preschool children exposed to passive smoking. J Pediatr (Rio J). 2011; 87: 523-8.
25. Ozler S, Oztas E, Erel O, et al. Impact of Gestational Diabetes Mellitus and Maternal Obesity on Cord Blood Dynamic Thiol/Disulfide Homeostasis. Fetal Pediatr Pathol. 2017; 36: 8-15. doi: 10.1080/15513815.2016.1223237.
26. Babaoglu E, Kilic H, Hezer H, et all. Comparison of thiol/disulphide homeostasis parameters in patients with COPD, asthma and ACOS. Eur Rev Med Pharmacol Sci. 2016; 20: 1537-43.
27. Hanikoglu F, Hanikoglu A, Kucuksayan E, et al. Dynamic Thiol/Disulphide Homeostasis Before and After Radical Prostatectomy in Patients with Prostate Cancer. Free Radic Res. 2016; 50: S79-S84
28. Dirican N, Dirican A, Sen O, et al. Thiol/disulfide homeostasis: A prognostic biomarker for patients with advanced non-small cell lung cancer? Redox Rep. 2016; 21: 197-203.
29. Guney T, Kanat IF, Alkan A, et al. Assessment of serum thiol/disulfide homeostasis in multiple myeloma patients by a new method. Redox Report. 2017;22: 246-51. doi: 10.1080/13510002.2016.1180100.
30. Aldemir M, Karaguzel E, Okulu E, et all. Evaluation of oxidative stress status and antioxidant capacity in patients with renal cell carcinoma. Cent European J Urol. 2015; 68: 415-20.