THIOL-DISULPHIDE HOMEOSTASIS IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

TIOL-DISULFIDNA HOMEOSTAZA KOD PACIJENATA
SA ESENCIJALNOM TROMBOCITEMIJOM

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Summary

Background: This study aimed to show the status of thiol-disulphide homeostasis in essential thrombocytosis patients, which is known to play a role in platelet function.
Methods: The study included 27 ET patients and a control group of 36 healthy subjects. Serum total (–SH + –S–S–) and native (–SH) thiol levels were measured in all subjects using an automatic method.
Results: Age and gender distribution were similar in both groups. Compared with the control group, in the ET group, there were increased native thiol and total thiol levels (p = 0.001, p = 0.046). There was no correlation between thiol, total thiol and disulphide ratios with Jak2 mutation, hemorrhage and thrombosis. A positive correlation was determined between thrombosis and thiol disulphide homeostasis (p = 0.058). The study results showed that thiol-disulphide homeostasis shifted to the proliferative side in ET, in which ineffective erythropoiesis was predominant. It is also known that platelets are more active in ET cases and thiol disulphide balance is important in platelet function.
Conclusions: This result suggests that thrombotic complications may be reduced if the formation is achieved of mechanisms (oxidation mechanisms) that will trigger the increase of disulphide groups. However, more extensive research is needed on this subject.

Keywords: essential thrombocythemia, thiol, disulphide bonds, myeloproliferative neoplasms

Kratak sadržaj

Uvod: Ova studija je imala za cilj da pokaže stanje homeostaze tiol-disulfida kod pacijenata sa esencijalnom trombocitozom, za koje je poznato da imaju ulogu u funkciji trombocita.
Metode: Studija je obuhvatila 27 ET pacijenata i kontrolnu grupu od 36 zdravih subjekata. Izmereni su serumski ukupni (–SH + –S–S–) i prirodni (–SH) nivoi tiola kod svih subjekata koristeci automatsku metodu.
Rezultati: Distribucija starosti i pola bila je slična u obe grupe. U poređenju sa kontrolnom grupom, u ET grupi povecani su izvorni tiol i ukupni nivoi tiola (p = 0,001, p = 0,046). Nije bilo korelacije između tiola, ukupnog tiola i disulfidnih racija sa Jak2 mutacijom, krvarenjem i trombozom. Utvrđena je pozitivna korelacija između tromboze i tiol-disulfidne homeostaze (p = 0,058). Rezultati studije pokazali su da se tiol-disulfidna homeostaza pomera na proliferativnu stranu u ET, u kojoj je dominantna neefikasna eritropoeza. Takođe je poznato da su trombociti aktivniji u ET slučajevima, a da je balans tiol-disulfida važan u funkciji trombocita.
Zaključak: Ovaj rezultat sugeriše da se trombotičke komplikacije mogu smanjiti ako se postigne formacija mehanizama (oksidacionih mehanizama) koji ce izazvati povećanje disulfidnih grupa. Međutim, potrebno je detaljnije istraživanje o ovoj temi.

Ključne reči: esencijalna trombocitemija, tiol, disulfidne veze, mieloproliferativne neoplazme

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Introduction

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder, the diagnosis of which is based on the exclusion of other myeloproliferative neoplasms. Almost half of ET patients are asymptomatic. In symptomatic cases, vasomotor symptoms (a headache, dizziness, vision loss, palpitation, atypical chest pain, distal paresthesia, erythromelalgia) thrombotic and hemorrhagic attacks can be seen (1). These complications are often non-fatal. Leukemic and myelofibrotic transformation incidence is low. When these are considered, life expectancy is high in ET patients (2, 3).

ET is a disease with bone marrow myeloproliferation in which monoclonal proliferation of megakaryocytes is more distinct. Although the pathogenesis of ET is not clearly known, 90% of cases have acquired somatic driver mutations such as JAK2, CALR, and MPL. Hypersensitivity to cytokines such as erythropoietin (EPO), thrombopoietin (TPO), interleukin 3 (IL-3) and stem cell factor (SCF) is known in the etiopathogenesis of ET. Increased free radicals are also known to increase the risk of genetic mutation in neoplastic processes (4–6). In recent investigations, the etiopathogenesis of ET may refer to an increase in oxidative stress parameters in terms of neoplastic transformation (4). Oxidant and antioxidant balance in the human body is important in sustaining homeostasis and maintaining cell function (4). Proteins, lipids, and DNA are the target molecules in oxidative damage. Disulphide bonds formed in newly synthesized proteins in the endoplasmic reticulum are important for protein structure and stability (5). The role of thiol-disulphide reactions with dynamic disulphide bond rearrangement in the function of intracellular proteins and platelets is important (7). The protein disulphide isomerase enzyme and sulfhydryl groups on the platelet surface have been shown to play a role in platelet aggregation, adhesion and secretion (7).

Thiols are organic compounds known as mercaptans. Most of the plasma thiol pool is formed of albumin thiols, and a small proportion is low-molecular-weight thiols such as cysteine, cysteinylglycine, glutathione, homocysteine. Thiols can enter oxidation reactions via oxidants, and disulphide bonds can form. The resulting disulphide bonds are reversible and are the earliest finding of protein oxidation (8). The reversible reduction between thiol groups and disulphide bonds results in the protection of the dynamic thiol-disulphide homeostasis. Dynamic thiol-disulphide homeostasis has an important role in cell signalling mechanisms, transcription factors, regulation of enzymatic reactions, signal transduction, antioxidant protection and detoxification (8, 9). Abnormalities in dynamic thiol-disulphide homeostasis have been proven in solid organ malignancies such as prostate cancer (10), hematological malignancies such as multiple myeloma (11), cardiovascular diseases (12), polycystic ovary syndrome (13) and diabetes mellitus (14). The dynamic thiol-disulphide homeostasis status was first detected in 1979. A recent novel method, developed by Erel and Neselioglu, can measure variable levels separately and additively (15), thereby allowing evaluation both individually and holistically.

An excessive increase in free radicals or deterioration in antioxidant systems are known to cause structural damage to DNA, changes in lipid and protein functions, and membrane damage. These changes increase the risk of mutation and the process of neoplastic transformation. Based on this hypothesis, the aim of this study was to investigate comparisons of plasma thiol-disulphide homeostasis of ET patients with a control group using a new method (15).

Materials and Methods

Study population

The study included 27 patients diagnosed with ET and 36 healthy volunteers. The control group comprised cases who applied for a check-up and had no systemic disease or drug use. Patients with diabetes, severe renal or liver diseases, active infectious or inflammatory diseases, rheumatological diseases, or malignancy were excluded from the study. The study was designed in accordance with the 2013 Brazil version of the Helsinki Declaration and was approved by the Local Ethics Committee. All participants provided written informed consent.

Biochemical parameters

Venous blood samples were taken from each patient into tubes containing ethylenediamine tetraacetic acid (EDTA) after 8 hours of fasting. The collected samples were immediately centrifuged at 1500 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 was determined with the recently-developed automated method. First, short disulphide 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 disulphide value was defined as half of the difference between total and native thiols. After determining native and total thiols, the disulphide level, disulphide/total thiol percentage ratios, native thiol/total thiol percentage ratios and disulphide/native thiol percentage ratios were

calculated (15). Measurements were taken with 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 the results. Real-time quantitative PCR (qPCR) is used for JAK2 V617F quantification.

Statistical analysis

Conformity of the data to a normal distribution was evaluated using the Kolmogorov-Smirnov test. The parametric values were given as mean ± standard deviation (SD), and non-parametric values were given as median (Interquartile Range). Comparisons were made with the Student's t-test in cases of normal distribution and with the Mann-Whitney U test in cases of skewed distribution. The Spearman and polyserial correlation coefficients were calculated to evaluate the relationship between the measurements. A value of $p < 0.05$ was regarded as statistically significant.

Results

The mean age was 57.52 ± 15.36 years in the 27 ET cases and 60.25 ± 9.61 years in the 36 healthy subjects in the control group. There was no statistically significant difference between the age and gender distributions of the ET group and the control group. The female/male ratio in the ET group was 13/14 (39.1%/60.9%), and the female/male ratio in the control group was 16/20 (44%/56%). There were 13 (56.5%) cases with splenomegaly, 13 (56.5%) with Jak 2 mutation, 4 cases (17.4%) with hemorrhage and 6 cases (26.1%) with a history of thrombosis.

Mean native thiol levels (SH), total thiol levels (SH + SS) and the native thiol/total thiol ratio (SH / SH + SS) levels were measured both in the ET group and in the control group. The laboratory findings and thiol level measurements are shown in *Table I*.

The mean values of native thiol levels (SH), total thiol levels (SH + SS) and the native thiol/total thiol ratio (SH / SH + SS) in the ET group were compared with those of the control group ($p = 0.001$, $p = 0.046$ and $p = 0.062$, respectively) (*Table I*). The age and gender distribution was similar between the groups. The basal complete blood counts and white blood cell/platelets differential were compared in the ET and control groups. The mean levels of platelets and white blood cell count were statistically significantly higher in the ET group as expected (*Table I*). There was a tendency for a decrease in disulphide level (p: 0.056).

There was no statistically significant relationship between JAK 2 mutation positivity and a history of hemorrhage or thrombosis and mean native thiol levels (SH), total thiol levels (SH + SS) and the native

Table I Laboratory and clinical demographic findings in ET and control group.

	ET (n=27)	Healthy control groups (n=36)	P value
Age (year)	57.52 ± 15.36	60.25 ± 9.61	0.421
Gender (M/F)	14/13	20/16	0.121
Haemoglobin (gr/dL)	14.31 ± 1.65	13.9 ± 1.42	< 0.041
Thrombocyte $\times 10^6$/L	910 ± 364	375 ± 148	< 0.001
Leukocyte $\times 10^6$/L	10.860 ± 6.400	$6,200 \pm 2,560$	< 0.035
Basophils $\times 10^6$/L	80 ± 110	30 ± 50	< 0.02
Lymphocytes $\times 10^6$/L	2.097 ± 708	1.670 ± 580	< 0.027
Native thiol	458.8 (85.9)	417.9 (36.1)	0.001
Total thiol	484.80 ± 76.37	452.52 ± 30.02	0.046
Disulphide	18.60 ± 7.35	20.21 ± 6.15	0.062
Albumin	4.1 ± 0.31	3.9 ± 0.52	0.621
% SS/SH	3.59 (2.53)	4.89 (2.91)	0.113
% SS/total SH	4.54 ± 2.27	4.49 ± 1.42	0.387
% SH/total SH	92.49 ± 4.61	91.06 ± 2.81	0.132

* P: positive, N: negative

thiol/total thiol ratio (SH / SH + SS) in the ET group. There was no statistically significant correlation between spleen size and thiol-disulphide parameters of the ET group. There was a tendency for a positive correlation between the levels of native and total thiols in patients with thrombosis in ET cases (p = 0.048).

Discussion

The results of this study showed that native and total thiol levels were significantly higher in ET cases when compared with the control group. There was a tendency to a decrease in disulphide levels. This study can be considered of value because it is the first study showing thiol-disulphide homeostasis using the Erel method (15) in ET cases.

In ET cases, there is predominance of mega-karyocytopoesis with abnormal proliferation of mega-karyocytes. In this clonal megakaryocyte proliferation, paracrine stimulation is known to be mediated by bone marrow and peripheral blood cytokines. In ET, there are known to be abnormalities in platelet function, structure and lifetime (16). When compared with normal platelets, ET platelets are large, imma-ture and sensitive to activation. The different states of abnormal platelets may be active, desensitizing, and resting states (17, 18). In ET cases, epinephrine-induced platelet aggregation due to loss of alpha 2 adrenergic receptors is frequently observed (19, 20). In addition, defects in arachidonic acid metabolism and platelet receptor abnormalities such as GP2B3A or acquired deficiency of dense granule pools may be seen (21, 22). In a previous experimental study, it was suggested that in platelet aggregation induced by arachidonic acid, the added thiol reagents (reagent) increase the effect of arachidonic acid or do not antagonize it (23). In ET cases, it is known that P-selectin, thrombospondin and active fibrinogen receptor glycoprotein 2b3a are increased, but the relationship with thrombosis is variable. Studies suggesting that cytoreductive treatment reduces the incidence of thrombosis in ET cases have suggested that platelet count in thrombosis may be a risk factor (24). In the current cases, no relationship was deter-mined between the presence of thrombo-hemor-rhagic complication and plasma thiol-disulphide levels.

Glutathione is an important regulator of the cellular redox environment (modulator). Almost all glutathione is found intracellularly and in reduced form (25). Other low molecular weight thiols are found in plasma, especially in disulphide forms (25, 26). Plasma glutathione levels and GSH / GSSG rates have been shown to differ in cases involving fasting, alcoholism, cirrhosis, and malignancy (7, 27, 28). It was suggested in one study that to produce and maintain free thiols in the active regions of the platelet protein disulphide isomerase, the thiol disulphide exchange mechanism could be the redox mechanism. It has also been reported that plasma GSH can work together in the cellular redox mecha-nism under the control of the enzyme platelet protein disulfidase. Reduction of disulphide levels in fibri-nogen receptors with reducing agents induces platelet aggregation (29). In the current study, there was a positive correlation between total and native thiols and thrombosis. The role of thiols and di-sulphides in platelet function is an area that has not yet been explored. Several recent studies have suggested that rearrangement of thiol groups and disulphide bonds is part of the platelet stimulation process in various platelet responses such as aggregation and secretion (30–32). Previous reports have demonstrated the role of platelet surface sulfhydryl groups in the platelet response, and several groups of sulfhydryls on the platelet surface have been classified. In some experimental studies, the presence of a thiol-disulphide exchange reaction in platelet surface proteins during aggregation has been suggested. Thiols can participate in reactions according to thiol-disulphide exchange modification, which can regulate sulfhydryl-dependent pathways in platelet activation.

The platelet fibrinogen receptors, 2b and 3 in-tegrin, both contain disulphide bonds. These integrins have high cysteine content. It is thought that cysteine in 2b and 3 integrins is bound to disulphide bonds, but in recent reports it has been suggested that some exist as free thiols (25).

In conclusion, it can be considered of interest that there were significantly high levels of native and total thiol levels in ET patients and there was a tendency for a positive correlation between thiol in-crease and thrombosis. Furthermore, if the mecha-nisms (oxidation mechanisms) that will trigger the increase of disulphide groups can be produced, perhaps the possibility of thrombotic complications may also be reduced. However, there is a need for more extensive research on this subject.

Conceptualization: ASY. Data curation: ASY, MA, ÖE. Formal analysis: ASY, MA, ÖE. Investigation: AŞY, MA, ÖE. Writing – original draft: AŞY, SA, İD, MA, ÖE. Writing - review & editing: AŞY, ŞMB, İD, ÖE

Financial disclosure

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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