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The Relation Between Thiol/Disulphide Homeostasis and Pseudotumor Cerebri

Tiyol/Disülfid Homeostazisi ve Bening İntrakranial Hipertansiyon Arasındaki İlişki

Gülhan SARIÇAM,[a]
Hatice Ferhan KÖMÜRCÜ[b]

[a]Clinic of Neurology, Ankara Ulus State Hospital
[b]Clinic of Neurology, Ankara Atatürk Training and Research Hospital, Ankara

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Correspondence:
Gülhan SARIÇAM
Ankara Ulus State Hospital
Clinic of Neurology, Ankara, TURKEY/TÜRKİYE
gulhansar01@hotmail.com

ABSTRACT Objective: The aim of this study is to investigate serum dynamic Thiol-Disulfide homeostasis and selenium levels as a new oxidative stress parameter in patients with pseudotumor cerebri. **Material and Methods:** A total of 55 participants, including twenty patients with pseudotumor cerebri (PTC) and thirty five healthy volunteers, were included in the study. Total Thiol (TT) (-SH+-S-S) and Native Thiol (NT) (-SH) levels were measured in serum samples of all PTC patients and healthy volunteers participating in the study. Dynamic disulfide bond (DDsB) levels (-S-S-) and (-S-S-)/(-SH), (-S-S-) /(-SH+-S-S-), (-SH)/(-SH+-S-S-) ratios were calculated and selenium levels were measured. The results of patients with PTC and healthy volunteers were compared. **Results:** TT, NT, DDsB levels and (-S-S-)/(-SH), (-S-S-)/(-SH+-S-S-) and (-SH)/(-SH+-S-S-) ratios were not statistically significant between patients with PTC and healthy volunteers. In the PTC patient group, we found a high negative lineer correlation between age and the NT (r: -0.726; p<0.001) and TT (r: -0.722; p <0.001) levels. In the control group, we found moderate negative lineer correlation between age and NT (r:-0.532; p<0.001) and TT (r:-0.568; p<0.001). **Conclusion:** We investigated oxidative stress exposure using dynamic thiol/disulfide homeostasis and selenium levels in patients with PTC. We suggest that age may also be an important parameter in the change of antioxidant balance.

Keywords: Pseudotumor cerebri; oxidative stress; dynamic thiol/disulfide homeostasis

ÖZET Amaç: Bu çalışmanın amacı psödotümör serebri tanılı hastalarda yeni bir oksidatif stress parametresi olarak serum dinamik tiyol-disülfid homeostazisini ve selenyum düzeylerini araştırmaktır. **Gereç ve Yöntemler:** 20 psödotümör serebri (PTC) tanılı hasta, 35 sağlıklı gönüllü olmak üzere toplam 55 katılımcı çalışmaya dahil edildi. Çalışmaya katılan tüm PTC tanılı hastaların ve sağlıklı gönüllülerin serumda total tiyol (-SH+-S-S) ve native tiyol (-SH) düzeyleri ölçüldü. Dinamik disülfid bağ düzeyi (-S-S-) ve (-S-S-)/(-SH), (-S-S-)/(-SH+-S-S-) ve (-SH)/(-SH+-S-S-) oranları hesaplandı ve selenyum düzeyleri ölçüldü. Elde edilen veriler PTC tanılı hastalar ve sağlıklı gönüllüler arasında kıyaslandı. **Bulgular:** PTC tanılı hastalar ile sağlıklı gönüllüler arasında total tiyol, native tiyol, dinamik disülfid bağ düzeyi ve (-S-S-)/(-SH), (-S-S-)/(-SH+-S-S-) ve (-SH)/(-SH+-S-S-) oranları arasında istatistiksel olarak anlamlı bir fark bulunamadı. Psödotümörlü hasta grubunda yaş ile native tiyol (r:-0,726; p<0,001) ve toplam tiyol (r:-0,722; p<0,001) seviyeleri arasında kuvvetli düzeyde negatif yönde doğrusal ilişki saptandı. Kontrol grubunda yaş ile native tiyol (r:-0,532; p<0,001) ve toplam tiyol (r:-0,568; p<0,001) arasında orta düzeyde negatif yönde doğrusal bir ilişki bulundu. **Sonuç:** Psödotümör serebri tanısı olan hastalada dinamik tiol/disülfid homeostazı ve selenyum düzeylerini çalışarak bu hastalarda oksidatif strese maruziyeti araştırdık. Antioksidan dengenin değişmesinde yaşın da önemli olabilecek bir parametre olduğunu düşündük.

Anahtar Kelimeler: Psödotümör serebri; dinamik tiyol-disülfid homeostazisi; oksidatif stres

Pseudotumor cerebri syndrome (PTC) is a rare disease defined by the presence of elevated intracranial pressure. The syndrome can lead to severe headache, impaired visual acuity and papilledema. The most

powerful risk factors are obesity and female gender. In the general population, incidence of PTC is estimated between 1-2 per 100,000. However this ratio can reach to 19 per 100,000 in women aged 20-44 years.[1]

Despite the large number of hypotheses and publications in the last decade, the etiology of the disease is still not explained. Several mechanisms have been proposed to explain the pathogenesis of the PTC. Cerebrospinal fluid drainage-production imbalances, aquaporin receptors, the effect of corticosteroids, obesity and chronic inflammation, abnormal metabolism of vitamin A, stenosis in the cerebral venous sinuses are the most common reasons for the pathogenesis of PTC.[2-4]

Previously, it has been shown that oxidative stress levels were increased in patients with diabetes mellitus, alzheimer, peripheral vascular diseases, cardiovascular diseases and many chronic diseases.[5,6] It is thought that oxidative stress may increase in the cases of PTC which is not yet clearly identified as etiologically.

The effects of glucocorticoids and mineralocorticoids in the development of PTC has also been demonstrated previously. Endocrinologic pathologies such as Addison's disease, cushing syndrome and adrenal hyperplasia also cause PTC.[7,8] There is increasing evidence that high plasma cortisol level has dysfunctions of 11β-hydroxy steroid dehydrogenase type 1, although it is not a typical feature. It has been detected that 11β-hydroxy steroid dehydrogenase type 1 (11β-HSD1) activity is regulated by inflammatory cytokines (TNFα, IL-1β, IL-6) and adipokines (leptin).[9]

It is known that one of the most powerful and consistent risk factors in PTC is obesity.[10] Obesity is a proinflammatory situation and hormones such as leptin and adiponectin are secreted depending on the increase of fat tissue.[11] As a result of clinical studies, it was explained that in obese cases, DNA damage increased and antioxidant capacity decreased.[12,13]

The oxidative effects of free radicals from all these inflammatory processes are inhibited by the antioxidant system.[14] Depending on the oxidative stress, the structures and functions of proteins, lipids, nucleic acids and enzymes are impaired in the body.[15] The role of inflammation, free radicals and oxidative stress in pseudotumor cerebri pathophysiology is not known exactly and trials on this subject is limited. We consider that if the role of oxidation in the etiology of PTC is understood, it may be helpful to develop the prevention and treatment of disease.

In this study, we aimed to investigate thiol-disulfide homeostasis which plays an important role in antioxidant defense and detoxification in patients with PTC by using a new method, and investigate whether there is a change in serum selenium level.

MATERIAL AND METHODS

All patients provided written informed consent before enrollment. All procedures comply with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Ankara Atatürk Training and Research Hospital, Ankara, Turkey.

This prospective study included 20 patients admitted with pseudotumor cerebri and 35 healthy volunteers with normal neurological examination for the control group. The patients, included to this study, was diagnosed based on the Dandy criteria. Detailed neurological examinations and routine laboratory tests of all participants were performed. All patients had bilateral papillary edema on fundus examination and visual fields were limited. The results of cranial magnetic resonance imaging (MRI) and magnetic resonance (MR) venography were normal. Lumber puncture is applied to patients and the average cerebro spinal fluid (CSF) opening pressure is measured as 27 mmHg. Newly diagnosed patients who have not started treatment are included the study.

Participants with chronic diseases that would affect the results of the study such as diabetes mellitus, hypertension, cancer, rheumatic diseases, atherosclerotic heart disease and peripheral vascular diseases, as well as smokers, alcoholics and anyone taking antioxidant supplements, were excluded from the study. Serum disulfide (-SS-), native thiol

(-SH), total thiol (-SH + -SS-), disulfide/native thiol (-SS-)/(-SH), disulfide/total thiol (-SS-)/(-SH+-SS-), native thiol/total thiol (-SH)/(-SH+-SS), and selenium levels were measured for patients and control grups.

Healthy controls, peripheral blood samples were obtained after 12 hours fasting. Ten ml plain tubes and 2-ml vacuum tubes containing ethylene diamine tetra acetic acid (EDTA) were used. Blood samples were centrifuged at 1,500 g for 10 minutes. Serum samples, which were obtained for thiol and disulfide (DS) studies were immediately frozen at –80°C until analysis. Native thiol (NT), total thiol (TT) and disulphide amounts of the PTC and healthy controls were examined in the same session with the same serum samples.

Serum NT, TT, and DS levels (μmol/L) were measured using the new, cost-effective spectrophotometric method described previously by Erel and Neselioglu.[16] Briefly, reducible DS bonds were first reduced to form free functional thiol groups. Formaldehyde was used to remove unused and consumed sodium borohydride, and after the reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), all thiol groups including both reduced and native groups were determined. The amount of active DS was determined by taking half of the difference between serum TT and NT groups. After the calculation of native and total thiols (-SH), DS amounts (–S-S), the percentage of disulfide/total thiol (DTT) ratios, the proportion of native thiol/total thiol (NTT) ratios and the percentage of disulfide/native thiol (DNT) ratios were calculated (%).[17]

STATISTICAL ANALYSIS

When the data are evaluated, frequency distributions for categorical variables and descriptive statistics for numerical variables (Mean±SD) are given. The Kolmogorov Smirnov normality test was applied to the variables to be able to decide which analyzes to apply. As a result of the test, all variables were assumed to be normality assumptions ($p>0.05$) and parametric tests were used in this comparison. Whether or not there was any difference between the two independent groups was examined by the Independent Sample T test. The Pearson Correlation Coefficient is used to determine the degree of non-causal relationship between two numerical variables.

RESULTS

The patient group with PTC consisted of 17 (85.0%) women and 3 (15%) men. The control group consisted of 24 (68.6%) healty women and 11 (31.4%) healty men. There was no statistically significant difference between the PTC and control groups in gender ($p>0.05$). The mean age of the patient group was 45.35 and the mean age of the normal group was 40.43 years. There was no statistically significant difference between control and PTC patients in age ($p>0.05$).

No statistically significant difference was observed between the control and patient groups when compared to the native thiol, disulfide, total thiol, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol and selenium averages (independent sampling test) ($p>0.05$) (Table 1).

TABLE 1: Evaluation of variables in groups.

Variables	Control (N:35) Mean±SD	Patient (N:20) Mean±SD	t	p
Native Thiol	452,93 ± 44,35	454,78±67,740	-0,109	0,914
Disulfide	21,05 ± 5,59	19,12±3,943	1,365	0,178
Total Thiol	495,03 ± 48,38	493,01±68,356	0,128	0,899
Disulfide/Native Thiol	0,05 ± 0,01	0,04±0,011	1,097	0,278
Disulfide/Total Thiol	0,04 ± 0,01	0,04±0,009	1,083	0,284
Native Thiol/ Total Thiol	0,92 ± 0,02	0,92±0,019	-1,083	0,284
Selenium	63,74 ± 10,67	64,36±18,848	-0,153	0,879

TABLE 2: Investigation of the relationship between age and variables.

		Age	
		Control	Patient
Native Thiol	R	-0,532	-0,726
	P	**0,001****	**0,000*****
Disulfide	R	-0,345	-0,018
	P	**0,042***	0,939
Total Thiol	R	-0,568	-0,722
	P	**0,000*****	**0,000*****
Disulfide/ Native Thiol	R	-0,101	0,399
	P	0,562	0,082
Disulfide/ Total Thiol	R	-0,107	0,398
	aP	0,542	0,082
Native Thiol/ Total Thiol	R	0,107	-0,398
	P	0,542	0,082
Selenium	R	0,135	0,207
	P	0,448	0,381

**:p<0,05 ***:p<0,001*

Pearson's correlation analysis showed no significant linear relationship between age and disulfide/native thiol, disulfide/total thiol, native thiol/ total thiol, selenium (p>0.05), however there is moderate negative relationship between age and native thiol (r:-0.532; p<0.001) and the total thiol (r: -0.568; p<0.001) (Table 2). Pearson correlation analysis in the PTC patients group showed no significant linear relationship between age and disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, selenium (p>0.05). However, there is a significant linear relationship between the age and the native thiol (r: -0.726; p<0.001) and the total thiol (r: -0.722; p<0.001).

DISCUSSION

The inflammation shown in the PTC pathophysiology is a free radical source. If the formation of free radicals exceeds the antioxidant capacity, many metabolic and functional disorders occur. It is known that free radicals play a significant role in the pathogenesis of many diseases such as atherosclerosis, cancer, neurodegenerative diseases, drug toxicity and infection.[18,19]

Enzymatic or non-enzymatic antioxidant mechanisms enters the cycle to protect the organism against harmful effects of oxidative stress. Thiol is an organic compound containing a group of sulfhydryl (-SH) that plays a critical role in preventing the formation of any oxidative stress state in cells and oxidizes to reversible disulfide bonds.[16] The resulting disulfide bond structures can be reduced to thiol groups again, thus maintaining the thiol-disulfide balance. Selenium (Se) is a trace element that plays an important biological role against oxidative damage through antioxidant enzymes glutathione peroxidases (GPx1 and Gpx4) and selenoproteins (SelP).[20]

In our study there was no significant difference between patient and control group in native thiol (-SH), disulphide (-S-S), total thiol ((-SH)+(-S-S)), disulphide/native thiol (-S-S/-SH), disulfide/total thiol (-S-S)/(-SH+-S-S-), native thiol/ total thiol (-SH/ -SH+-S-S-) and selenium statistically (p>0,05).

However, there was a moderate negative correlation between age, native thiol (r:-0,567; p<0.001) and total thiol (r: -0,601; p <0.001) levels in the normal group. In the PTC patient group, there was a strong linear relationship between age and native thiol (r: -0.726; p <0.001) and total thiol (r: -0.722; p<0.001) levels. These results have shown that antioxidant capacity decreases with increasing age, especially in PTC patients.

Recently, new colorimetric methods for the determination of thiol/disulfide homeostasis have been used to study neurological diseases such as parkinson, alzheimer, migraine, stroke, epilepsy, as well as many diseases thought to play a role in immunological and inflammatory mechanisms. Abnormal thiol/disulfide balance is associated with many diseases and indicates that antioxidant systems are affected in inflammatory diseases, especially during the active period.[21,22]

This study is the first to assess dynamic thiol-disulphide homeostasis in patients with pseudotumor cerebri in the literature. In this respect, this study is important. In our study, there was no significant difference in oxidative stress levels between the PTC patient group and the normal group. Selenium levels were evaluated in addition to thiol/disulfide homeostasis to evaluate oxidative stres, similar results were obtained. We found that antioxidant balance did not change in patients with

pseudotumor cerebri. In the further studies that will evaluate this balance in advanced stage of disease in larger samples may contribute to understanding disease pathology.

However, we found that antioxidant capacity decreased significantly with age in both groups, more prominently in PTC patients. Oxidative substances play an important role both in the pathogenesis of senescence-related degenerative diseases (such as Alzheimer's disease and atherosclerosis), and in conditions resulting from the senescence process such as tissue atrophy.[23,24] This has shown the amount of oxidants increases with aging and the antioxidant systems that try to prevent damage are insufficient in PTC patients.

The main restrictive factor of our work is the low number of subjects. Another restrictive factor is the short follow-up periods of the patients to detect the changes in thiol/disulfide homeostasis.

CONCLUSION

Thiol chemistry is a rapidly developing area in basic and practical molecular life sciences. The measurement of Thiol/Disulfide homeostasis may be critical to revealing the effects of oxidative stress and assessing disease activation. Randomized controlled studies with larger patient populations are needed to elucidate oxidative stress, which is very important in pathogenesis of PTC patients.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Writing, Discussion, Data Collection, Idea, and Design of the Manuscriptl: *Gülhan Sarıçam;* ***Idea, Design, Analysis:*** *Hatice Ferhan Kömürcü;* ***Statistical Analyses:*** *Ömer Kaplan.*

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