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Research / Araştırma

# Thiol/Disulphide Homeostasis in Men with Heroin Addiction

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**ABSTRACT**

## Thiol/disulphide homeostasis in men with heroin addiction

**Objective:** Heroin addicts have increased oxidative stress which can disturb thiol/disulfide (SH/SS) homeostasis, causing disulfide formation. No study has determined the serum thiol amount and blood disulfide amount in heroin addicts. The aim of the study was to investigate dynamic SH/SS homeostasis in heroin addicts.

**Methods:** Serum SH/SS statuses of 31 heroin addicts and 31 healthy controls were compared to determine the changes in SH/SS homeostasis in heroin addicts. Blood serum native thiol and total thiol (ToSH) levels were measured and the disulfide bond amount was calculated as the half value of the difference between native thiol and ToSH levels. For comparison t-test was used.

**Results:** SH and ToSH levels were significantly lower ($p<0.001$ for both) in heroin addicts than in the healthy group whereas disulfide levels were significantly higher ($p<0.001$). Heroin addicts had significantly higher SS/ToSH and SS/SH ratios and significantly lower SH/ToSH ratios than healthy individuals.

**Conclusion:** The results showed that SH and ToSH levels were decreased in heroin addicts and SH/SS homeostasis was also disturbed with a shift to the disulfide bond formation side. Results of this study could contribute to the knowledge about pathogenesis of heroin addiction and also to its management. We suggest that replacement of the thiol gap and reduction of excess SS might have positive effects in treatment results.

**Keywords:** Addiction, disulfide, heroin, oxidative stress, thiol

**ÖZET**

## Eroin bağımlısı erkeklerde tiol/disülfit homeostazi

**Amaç:** Eroin bağımlılığının oksidatif stres artışı ile ilişkili olduğu bilinmektedir. Oksidatif stres disülfit oluşumuna yol açarak tiol/disülfit (SH/SS) homeostazını bozabilir ve böylece protein işlevlerinde değişimlere neden olabilir. Eroin bağımlılarında serum tiol miktarı ve kan disülfit miktarının araştırıldığı bir çalışma mevcut değildir. Bu çalışmanın amacı eroin bağımlılığında dinamik SH/SS homeostazını incelemektir.

**Yöntem:** Otuz bir eroin bağımlısı erkek ve otuz bir sağlıklı erkekte natif tiol-disülfit değişimlerini içeren kan SH/SS homeostazı incelendi. Serum natif tiol ve total tiol (ToSH) düzeyleri ölçüldü; natif tiol ve ToSH düzeyleri farkının yarı değeri olarak disülfit bağ düzeyi hesaplandı. Karşılaştırmalar t testi ile yapıldı.

**Bulgular:** Eroin bağımlısı erkeklerde SH ve ToSH konsantrasyonları kontrollere göre daha düşük (her ikisi için $p<0.001$) ve SS düzeyleri daha yüksek ($p=0.001$) saptandı. Eroin bağımlılarında SS/ToSH ve SS/SH oranları sağlıklı erkeklerden daha yüksek (her ikisi için $p<0.001$) ve SH/ToSH oranı daha düşük ($p<0.001$) saptandı.

**Sonuç:** Çalışmanın sonuçları serum SH ve ToSH düzeylerinin eroin bağımlısı erkeklerde azalmış olduğu ve SH/SS homeostazının disülfit bağı oluşumu yönünde bozulduğunu göstermektedir. Bu sonuçlar eroin bağımlılığı patogenezi ve eroin bağımlılığına yaklaşım açısından katkı sağlayabilir. Eroin bağımlılığının tedavi sürecinde tiol eksikliğinin yerine konması ve aşırı disülfit miktarının azaltılması tedavi yanıtı açısından olumlu etki gösterebilir.

**Anahtar kelimeler:** Bağımlılık, disülfit, eroin, oksidatif stres, tiol

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## INTRODUCTION

Heroin is an illegal drug with a high potential for addiction and was reported as "the most widely used illicit opiate in the world" in the 2010 World Drug Report (1). Many proteins in the human body involve cysteine residues that contain a functional sulfhydryl group in the side chain. These sulfhydryl groups could be oxidized and form disulfide bonds under oxidizing conditions (2). Proteins that include cysteine residues are therefore more sensitive to oxidative stress, and function could be lost due to their modifications (3). Dynamic thiol-disulfide homeostasis is defined as the determination of reversible disulfide bonds that could be reduced to thiol groups (4) and provide information about the overall redox state. Recently, a novel automated method for determining thiol/disulfide homeostasis was developed (4).

Previous studies have reported that heroin addicts have increased oxidative stress (5-7). Oxidative stress could disturb thiol/disulfide homeostasis, causing disulfide formation and subsequently leading to changes in protein functions. Eating patterns in heroin addicts have been reported to vary according to many factors, but these addicts tend to eat cheap and quick food and often suffer from loss of appetite during active heroin use (8). So heroin addicts tend to have nutritional deficiencies. However, no study has reported the serum thiol status of heroin addicts.

The aim of the study was to determine thiol/disulfide homeostasis in heroin addicts. To our knowledge, this is the first report to determine thiol/disulfide homeostasis in addiction.

## METHOD

The study group consisted of 62 men, 31 heroin addicts (mean age±SD 26.4±6.4; mean body mass index±SD 20.7±1.9), and 31 healthy controls (mean age±SD 26.4±6.8; mean body mass index±SD 20.5±2.0). Control group consisted of age and body mass index (BMI) matched healthy volunteers. All patients were interviewed by two independent clinicians in Research, Treatment and Training Center for Alcohol and Substance Dependence of Ankara Numune Training and Research Hospital. The subjects were selected based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV-TR). To be included in the study, the participants had to meet the following criteria: (1) being aged 18 or above, (2) heroin being the predominant drug of abuse and continuous consumption of sole heroin lasting for more than 3 months, (3) being currently on only buprenorphine-naloxone maintenance treatment, and (4) no existing withdrawal signs or symptoms. The exclusion criteria were as follows: (1) concurrent use of multiple drugs and indefinable predominant drug; (2) history of alcohol abuse; (3) comorbid psychiatric disorder (e.g., psychotic disorder and affective disorder); (4) mental retardation; and (5) physical illness (e.g., hepatic disease, renal disease, and infectious disease). The control group participants were recruited from the community, who met the following criteria: (1) being aged 18 or above, (2) no physical or mental disorder, and (3) no history of alcohol or any illicit drug abuse.

### Ethics Statement

The study was conducted in accordance with the Helsinki Declaration and the study protocol was approved by the Local Clinical Research Ethics Committee. After complete description of the study to the patients, written informed consent was obtained from all participants.

### Sampling Technique

Fasting blood samples were obtained from the addicts and the controls in plain tubes. Sera were separated by centrifugation at 1300g for 10 min and stored at -80°C until the analysis was conducted. Thiol/disulfide homeostasis tests were performed as described previously (4). Briefly, reducible disulfide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups, including reduced and native thiol

groups, were determined after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total thiols and the native thiols gave the dynamic disulfide amount. After the native and total thiols were determined, disulfide amounts, disulfide/total thiol ratios (SS/SH+SS), native thiol/total thiol ratios (SH/SH+SS), and disulfide/native thiol ratios (SS/SH) were calculated.

### Statistical Analysis

The evaluation of normal distribution of continuous variables were done using the Kolmogorov-Smirnov tests. The presence of a statistically significant difference between the groups were examined with the t-tests for normally distrubuted variables. The proportions of patients who had a history of smoking were presented with cross tabulations. The chi-square or Fisher's exact test, where appropriate, was used to compare this proportion between groups. Associations between heroin dose, exposure time, and the homeostatic parameters were determined by using Pearson's correlation test. A p value of less than 0.05 was considered statistically significant. The findings of the study were analyzed with the Statistical Package for Social Sciences for Windows ver. 18 (SPSS Inc., Chicago, USA, 2009) software.

**Table 1: Sociodemographic and clinical features of the heroin addicts and the controls**

| | Addicts (n=31) | | Controls (n=31) | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| **Age** | 26.4 | 6.4 | 26.4 | 6.8 |
| **Body mass index** (kg/m$^2$) | 20.7 | 1.9 | 20.5 | 2.0 |
| | **n** | **%** | **n** | **%** |
| **Smoking rate** | 24 | 77.2 | 23 | 74.2 |
| | **Mean** | **SD** | **Mean** | **SD** |
| **Smoking** (package-year) | 8.4 | 8.2 | 8.5 | 8.4 |
| | **n** | **%** | **n** | **%** |
| **Education** | | | | |
| Primary | 22 | 71.0 | 19 | 61.3 |
| High school | 6 | 19.4 | 7 | 22.6 |
| University/college | 3 | 9.6 | 5 | 16.1 |
| **Marital status** | | | | |
| Single | 21 | 67.7 | 22 | 71.0 |
| Married | 7 | 22.6 | 7 | 22.6 |
| Divorced | 3 | 9.7 | 2 | 6.4 |
| **Occupation** | | | | |
| Employee | 17 | 54.8 | 19 | 61.3 |
| Unemployed | 12 | 38.7 | 9 | 29.0 |
| Student | 2 | 6.5 | 3 | 9.7 |
| **Monthly income** (Turkish liras) | | | | |
| Less than 800 | 13 | 41.9 | 10 | 32.3 |
| Between 800 and 1500 | 10 | 32.3 | 11 | 35.5 |
| More than 1500 | 8 | 25.8 | 10 | 32.3 |
| | **Mean** | **SD** | | |
| **Amount of daily heroin use** (grams) | 2.4 | 1.5 | - | - |
| **Duration of heroin addiction** (months) | 5.8 | 3.1 | - | - |
| | **n** | **%** | | |
| **Method of heroin use** | | | | |
| Kit (foil) | 24 | 77.4 | - | - |
| Intravenous | 3 | 9.7 | - | - |
| Both foil and intravenous | 4 | 12.9 | - | - |
| | **Mean** | **SD** | | |
| **Buprenorphine-naloxone treatment** | | | | |
| Daily dosage of buprenorphine (milligrams) | 7.8 | 2.5 | - | - |
| Duration (days) | 6.2 | 3.8 | - | - |

## RESULTS

Sociodemographic and clinical features of heroin addicts and the controls were given in Table 1. The difference between smoking rates in the addict (n=24, 77.2%) and the control group (n=23, 74.2%) was not significant (p>0.05). The smoking amount calculated as package-year was also similar (8.4±8.2 for heroin addicts and 8.5±8.4 for controls) (p>0.05). Sociodemographic features of the two groups were also similar (Table 1). There were no significant correlations among the homeostatic variables and the dose and the duration of heroin use.

Native thiol and total thiol levels were significantly lower in the heroin addicts than the control group whereas disulfide levels were significantly higher (Table 2 and Figures 1–2). The heroin addicts had significantly higher disulfide/total thiol and disulfide/native thiol ratios and significantly lower native thiol/total thiol ratios than the controls (Table 2).

## DISCUSSION

The results showed two main abnormalities related to thiol/disulfide homeostasis in heroin addicts. The thiol/disulfide homeostatic status was weakened, and the balance shifted to the disulfide formation side. The thiol/disulfide homeostasis status has been reported to have a role in detoxification, signal transmission, apoptosis, signaling mechanisms, and enzyme regulation (3,9). Disturbance of the thiol/disulfide homeostatic status might cause changes in these processes. This might also have a role in the pathogenesis of the disturbance of neurotransmission processes related to symptoms observed in heroin addicts.

Heroin has been reported to cause hypoxia (6,10), and this is claimed as a reason for creating the oxidative stress condition (7). Increased oxidative stress seems to be a reason for the shift of the balance to oxidizing conditions. Glutathione is the most abundant thiol containing intracellular molecule (11) and is converted

**Table 2: Thiol/disulfide homeostatic parameters in the addicts and the controls**

| | Addicts (n=31) | | Controls (n=31) | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | p |
| **Native thiol, µmol/L** | 280.40 | 54.22 | 428.34 | 39.63 | <0.001 |
| **Total thiol, µmol/L** | 329.30 | 55.11 | 464.88 | 39.87 | <0.001 |
| **Disulfide, µmol/L** | 24.45 | 8.48 | 18.27 | 4.44 | <0.001 |
| **Disulfide/native thiol, %** | 9.14 | 3.76 | 4.31 | 1.19 | <0.001 |
| **Disulfide/total thiol, %** | 7.57 | 2.54 | 3.95 | 1.00 | <0.001 |
| **Native thiol/total thiol, %** | 84.84 | 5.09 | 92.09 | 2.01 | <0.001 |

The p value is significant.

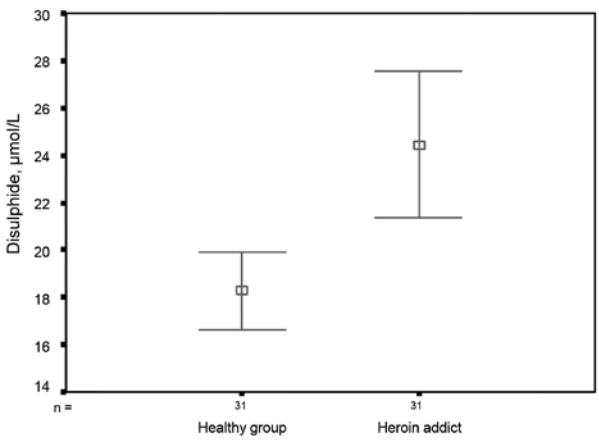


**Figure 1: Disulfide amounts in the addicts and the controls**

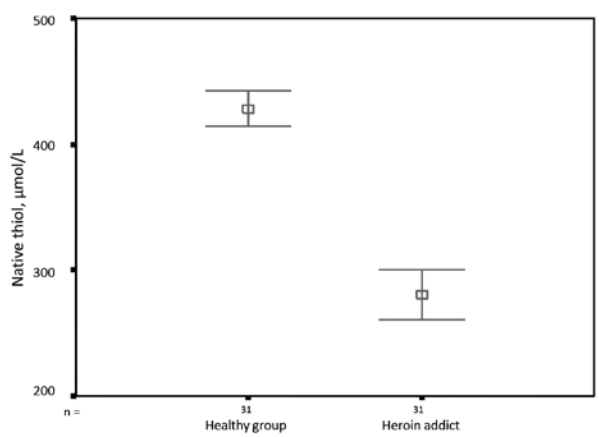


**Figure 2: Native thiol amounts in the addicts and the control groups**

to GSSG when oxidized. It has been reported that reduced glutathione is decreased and oxidized glutathione is increased in the saliva of the heroin addicts (12). Reduced glutathione is dominant in the intracellular environment. The reported status in saliva, which is an extracellular environment, might reflect the oxidative and antioxidative balance of the organism. However, the total thiol concentration we determined in our study is about 100-fold when compared with saliva glutathione concentrations (13) and therefore reflects the status of the organism with a stronger power.

Heroin addicts are reported to have a 3.4-fold risk of being underweight (14). The tendency to nutritional deficiency may be a predisposing factor for thiol deficiency, by affecting intake of amino acids that contain sulfur, methionine, and cysteine. However, no study has determined the serum thiol amount in heroin addicts, and heroin metabolism does not include thiol consumption (15); therefore, the decrease in the total thiol levels is thought to be because of a nutritional deficiency. The replacement of amino acids that contain sulfur might help close this gap.

Most of the drugs of abuse are reported to be related to glutamatergic abnormalities (16). N-Methyl-D-aspartate (NMDA) receptors for glutamate are reported to play an important role in opiate tolerance, dependence, and withdrawal (17-20). In addition to endogenous substances such as glycine, $Mg^{2+}$, and $Zn^{2+}$, agents that modify the redox state of cysteinyl side chains in the receptor such as alcohols, tricyclic antidepressants, and $H^{+}$ ions are also reported to affect the functions of NMDA receptors (21,22). Moreover, it was reported that the reduction of the sulfhydryl groups in the NMDA receptor using dithiothreitol increases the activation (23). Our findings in this study show a tendency to disulfide formation, and this might affect the neurotransmission process within the brain through these mechanisms.

There are limitations of the study. First of all, this is a cross-sectional study including only the patients who applied to ASATRC for addiction treatment. As another limitation, the relationship between thiol/disulfide homeostasis and buprenorphine-naloxone treatment is not exactly known. Mean duration of the buprenorphine-naloxone treatment of the patients was 6.2±3.8 days, and oxidative stress parameters are known to show the changes in longer durations like 2-4 weeks (24,25). So the changes in thiol/disulfide turnover were probably affected mainly by heroin use. The small number of participants and absence of female heroin addicts in the study are also limitations. Exclusion of patients using any psychotropic other than buprenorphine-naloxone and any drug other than heroin in last three months was the main reason for this small sample size. Most of our patients with heroin addiction in ASATRC are men. There were only two female patients who met the inclusion criteria, and we excluded them in order to eliminate possible effects of gender difference. Considering the central nervous system, serum thiol/disulfide levels are not as powerful as the levels in cerebrospinal fluid; but it is much more practical to examine.

In conclusion, thiol/disulfide homeostasis was disrupted in heroin addicts. The novel test we used in this study provides accurate and precise data for determining and monitoring the in vivo efficiency of thiol donor drugs such as N-acetylcysteine. Supplementing with a whey-protein-rich diet, which accelerates glutathione synthesis, may also be beneficial for addicts.

| Contribution Categories | Name of Author |
|---|---|
| Development of study idea | V.O.K. |
| Methodological design of the study | F.M.Y., O.E. |
| Data acquisition and process | S.N., S.K., A.B., I.T.O. |
| Data analysis and interpretation | V.O.K., F.M.Y., S.N., I.T.O. |
| Literature review | V.O.K., F.M.Y., O.E., E.G. |
| Manuscript writing | V.O.K., F.M.Y. |
| Manuscript review and revisation | V.O.K., F.M.Y., O.E., I.T.O., E.G. |

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