Oxidative stress in healthy individuals; circadian rhythm of thiol-disulfide balance

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Abstract
Aim: The dynamic thiol-disulfide balance plays a critical role in the healthy functioning of the organism. The most important role of this system is participation in the cell’s antioxidant defense. The hormone melatonin hormone has a circadian rhythm. It is not known whether the balance of thiol-disulfide shows secretion like melatonin throughout the day. This study examined the circadian rhythm of thiol-disulfide balance and variability with daily living activities in a healthy young population.

Material and Methods: Venous blood samples were taken every 2 hours from 8 healthy volunteers and the levels of melatonin, thiol disulfide and peripheral blood elements (lymphocytes and monocytes) were investigated. During the study, the daily activities of the volunteers were not restricted and the starvation-satiety variables were also studied.

Result: No significant time-dependent statistical change was observed in the 24-hour follow-up of native thiol, total thiol and disulfide levels, and measurements of blood lymphocyte and monocyte. The starvation and satiety levels were compared, and no significant difference was observed in thiol-disulfide balance.

Discussion: In this study, thiol-disulfide balance did not follow a circadian rhythm like melatonin, and was not affected by starvation and satiety.

Keywords
Thiol; Disulfide; Antioxidant; Oxidative stress; Melatonin; Circadian rhythm

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Introduction

In the organism, under the influence of free radicals, various defense systems arise that protect from the harmful effects of these radicals. These are defense systems, protective mechanisms, recovery mechanisms, physical defense and antioxidant defense systems [1]. Antioxidants are substances that directly inactivate oxidants [2]. Thiols (RSH) are organic compounds containing a sulfhydryl group (-SH) and are a member of both enzymatic and non-enzymatic antioxidant system. The plasma thiol pool consists of albumin thiols, protein thiols, and less commonly, low molecular weight thiols [3]. Albumin with its high concentration in the circulation is the main component that carries protein-bound thiols [4]. As a result of the oxidation reaction of thiols (RSH), covalently bonded disulfide bonds (RSSR) are formed. As a result of the activation of antioxidant systems in a normally functioning organism, disulfide bonds are reduced back to thiols groups [5]. Thus, the dynamic thiol-disulfide balance is also preserved. The dynamic thiol-disulfide balance takes part in antioxidant defense of the cell, detoxification, signal transduction, apoptosis, regulation of enzymatic activities, transcription factors, and cellular signal transduction mechanisms [6, 7]. In addition, the dynamic thiol-disulfide balance is involved in the etiopathogenesis of many diseases. Examples of diseases in which abnormal thiol-disulfide balance is seen, are fibromyalgia, nephrotic syndrome, pneumonia [8, 9, 10]. Melatonin (MLT) is a hormone synthesized mainly by the pineal gland and released into the circulation [11]. Studies of melatonin, which main function is to regulate the circadian rhythm of the organism, have also shown its anti-inflammatory function [12, 13, 14]. The MLT oscillation shows a circadian rhythm. Synthesis and release that increase in darkness are suppressed with light and decrease to basal level [15].

Previous studies examining the balance between thiol-disulfide balance and circadian rhythm have used low molecular weight thiols (cysteine, glutathione) other than albumin [16, 17]. With a new method developed by Erel and Neşelioglu in 2014, the dynamic thiol-disulfide balance depending on albumin can now be measured. In studies conducted with this newly developed automated method, plasma disulfide levels were found to be high in degenerative diseases and low in proliferative diseases [18].

Although the upper and lower limits reflecting normal values for healthy and young adult population were determined, variability during the day has not been investigated in previous studies. It is not known whether there will be a change in thiol-disulfide balance, especially during daily activities (eating, sleeping, miction and defecation). The aim of this study was to demonstrate the relationship between the circadian rhythm of thiol-disulfide balance, changes that may occur in the levels of daily living activities, and the MLT and peripheral blood elements in healthy young population.

Material and Methods

Study population

The study was conducted between February 1, 2016 and March 15, 2016 in the Internal Medicine Clinic of Ankara Ataturk Training and Research Hospital. A total of 5 females and 3 males aged between 26 and 36 years were included in the cross-sectional study. All parameters used in the study were studied specifically for the study group, and the study was initiated after obtaining the written consent from each volunteer participating in the study. Also, this study was approved by the local ethics committee of Ankara Ataturk Training and Research Hospital. The body mass index (BMI) of the study participants ranged from 20 to 25. The inclusion criteria for healthy sleeping volunteers were as follows: no cigarette smoking, no known chronic disease, no pregnancy status or suspected pregnancy, no psychiatric problem, no more than 1 cup of caffeine daily caffeine, no chronic drug use including oral contraceptive (OKS) and non-steroidal anti-inflammatory (NSAID). Those with active malignant disease or chronic illness, those with a BMI above 25, and those who refused to participate in the study were excluded.

Study Design

Venous blood samples were taken every 2 hours, starting at 08:00 in the morning (fasting) from each volunteer participating in the study, and the levels of thiol/disulfide, MLT and peripheral blood elements (lymphocytes and monocytes) were studied in the blood samples. Blood samples were taken at 08:00, 12:00, 16:00 to show fasting values and at 10:00, 14:00 and 18:00 to show satiety values. One night prior to enrolment in the study, volunteers’ meals were cut at 24:00 to standardize baseline fasting values. Each volunteer was provided with breakfast at 08:30, lunch at 12:30, dinner at 16:30, and snack at 21:00, according to the standard nutrition program. During the study, the volunteers were not restricted in their daily activities and were allowed to continue their basal activities. The volunteers’ blood samples were taken at 22:00, and then the eyelids were connected to sleep in a dark and silent room. At 24:00, 02:00, 04:00 and 06:00 blood samples were taken with heparinized vascular access; without interrupting the sleep cycles of the volunteers. After taking blood samples at 06:00, the volunteers were awakened and the study was terminated.

Biochemical Parameters

After the samples were centrifuged at 3600 rpm for 10 minutes in the biochemistry laboratory, the parameters of thiol disulfide in the blood were studied on the Roche Hitachi Cobas c501 automatic analyzer in the Biochemistry Laboratory of the Ankara Ataturk Training and Research Hospital using the automatic measurement method developed by Erel and Neselioglu. MLT samples were studied with an ELISA kit in Ankara Ataturk Training and Research Hospital Biochemistry Laboratory.

Statistical Analysis

The analysis of the data was made using the IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) package program. Descriptive statistics were shown as mean, standard deviation, minimum and maximum for continuous numerical variables, and categorical variables as the number of cases and (%). The daily follow-up of the cases, it was investigated, using variance analysis in repeated measurements, whether there was a statistically significant change in melatonin, native thiol, total thiol, disulfide, lymphocyte and monocyte measurements performed at two-hour intervals. Using the Bonferroni corrected multiple comparison test, the follow-up times that showed...
statistically significant differences between each other were determined. At p <0.05, the results were considered statistically significant.

**Results**

**The characteristics of the research group**
The demographic characteristics of 8 volunteers are shown in Table 1: 63% of the research population were female, 37% were male, and the mean age was 28.6 ± 3.6 years. The mean BMI of healthy volunteers with a body mass index range of 21.1-23.5 was 22.1 ± 1.1.

| Variables                        | n=8   |
|----------------------------------|-------|
| Age (year)                       | 28.6±3.6 |
| Age range (year)                 | 26-36 |
| Gender                           |       |
| Female                           | 5 (%62.5) |
| Male                             | 3 (%37.5) |
| Body mass index (kg / m2)        | 22.1±1.1 |
| Body mass index range (kg / m2)  | 21.1-23.5 |

**Changes in Plasma Melatonin measurements during the day**
During follow-up, the average MLT values remained stable between 08:00 and 18:00, and the daily average minimum MLT value (885.92 ng / L) was recorded at 08:00. Average MLT measurements started to increase from 20:00 and reached its maximum value during the day (10601.39 ng / L) at 02:00. A decrease was observed in the average measurements after this time period, but the study ended at 06:00 without a decrease to the minimum value. The course of the average MLT values during the day is shown graphically in Figure 1.

**Changes in Plasma Native and Total Thiol measurements during the day**
During the 24-hour follow-up period, no significant time-dependent statistical changes were observed in the mean native and total thiol values of the cases. In the course of both native and total thiol levels, the maximum value was recorded at 10:00, and the minimum value was recorded at 02:00. No statistically significant difference was observed between the levels of native and total thiols during fasting and satiety at 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00 (p>0.05).

**Changes in Plasma Disulfide measurements during the day**
During the 24-hour follow-up, no significant time-dependent statistical changes were observed in the mean disulfide values of the patients. No statistically significant difference was observed between the levels of native and total thiols during fasting and satiety at 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00 (p>0.05).

**Peripheral blood immune markers Lymphocyte and Monocyte measurements during the day**
There was no statistically significant difference in blood lymphocyte and monocyte measurements during the 24-hour follow-up (p>0.05). The mean blood lymphocyte and monocyte levels increased from 14:00 in the measurements following a constant course until 14:00, and the maximum level was reached at 22:00 for lymphocytes and at 00:00 for monocytes.

**Discussion**
Our study is the first study investigating the circadian rhythm of thiol/disulfide balance using this new method developed by Erel and Neşelioğlu. In addition, when the literature was reviewed, no study examining melatonin, peripheral blood lymphocyte, monocyte levels and activities of daily living and thiol/disulfide relationship was found.

Circadian rhythms control numerous activities, including the production and elimination of oxygen radicals, the activation and inhibition of the appropriate genes [19]. Antioxidant enzymes and low molecular weight antioxidants such as glutathione also have circadian rhythm and daily rhythmic changes as melatonin [20]. A study by Blanco et al. with 63 healthy volunteers demonstrated a circadian rhythm of the balance of glutathione and cysteine [17]. However, it is known that low molecular weight thiols such as glutathione and cysteine represent a small portion of the plasma thiol pool [21]. Albumin thiols are the main component carrying protein-bound thiols with their high concentration in the circulation [22]. In our study, no significant time-dependent statistical change was observed in the 24-hour follow-up of native thiol, total thiol and disulfide levels. We think that the circadian change may not have been observed due to the long half-life of albumin (21 days) and the fact that most of the thiol groups measured in our study originated from albumin.
One of the aims of our study was to examine the relationship between fasting and satiety and thiol disulfide. In our study, when the hunger and satiety levels after each 3 meals were compared, no significant difference was observed in thiol-disulfide balance, and this was attributed to the fact that albumin was not acutely affected by dietary changes during the day. In the study conducted by Blanco et al., a significant increase was observed in glutathione and cysteine levels after meals [17]. However, it should be kept in mind that these low molecular weight proteins represent a limited part of the plasma thiol pool.

As it is known, many laboratory parameters (such as blood glucose and lipid levels) are affected by the fasting state of the individual. These data are available; this new automated method is important in terms of showing that there will not be any limitation for hunger and satiety variables in the working schedule.

Melatonin levels in plasma regularly fluctuate during a 24-hour period. This circadian rhythm is controlled by the hypothalamus, and the main regulator of the rhythm is the light-dark cycle in the external environment [23]. In our study, similar results were obtained with the data supported in the literature. The MLT levels of healthy volunteers in our study followed a constant course between 08:00 and 18:00 and then increased from 20:00 and reached the daily maximum level at 02:00 at night. In our study, the circadian rhythms of peripheral blood lymphocyte and monocyte levels and the relationship with thiol-disulfide balance was also examined. The circadian rhythm of lymphocyte and monocyte levels followed a similar course as the results of the study by Sennels et al. [24]. These changes in peripheral blood lymphocyte and monocyte levels may be associated with an increase in cortisol and MLT levels.

**Conclusion**

Our study is important in that it is the first study evaluating the circadian rhythm of thiol-disulfide balance. In conclusion, measurement of thiol-disulfide level with this new automated method should be used to monitor the degenerative and inflammatory picture, which is longer than the half-life of albumin and to show the presence of oxidative stress, without being affected by the hunger and satiety status of the individual. Our study has some limitations. The small number of volunteers participating in the study (n = 8) and its relationship with micturition defecation could not be examined. Newer and larger studies are needed to investigate the thiol-disulfide balance.

**Scientific Responsibility Statement**

The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

**Animal and human rights statement**

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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**Conflict of interest**

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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