Experimental and Applied Medical Science, 3, 2: 345-353, 2022. DOI 10.46871/eams.2022.39

ANALYSIS OF THIOLE/DISULFIDE HOMEOSTASIS IN PATIENTS WITH DECUBITUS ULCER

Hamit YILDIZ [1], Deniz YILDIZ PEHLİVAN [2]

[1]Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Gaziantep, Turkey.
[2]İzmir Kâtip Çelebi University, Faculty of Medicine, Department of Physiology, İzmir, Turkey.

ABSTRACT

Aim: *There is a growing body of literature that recognises the importance of decubitus ulcer. Decubitus ulcer is tissue necrosis and ulceration that inevitably occurs in tissues exposed to pressure for a long time. Reoxygenation of ischemic tissue results in the formation of reactive oxygen metabolites, during ischemia/reperfusion. Thiols are antioxidant substances belonging to the mercaptan group. This study aims to contribute to this growing area of research by exploring thiol/disulfide levels, a new oxidative stress parameter, in patients with decubitus ulcer.*

Materials and Method: *This study consists of two groups, one is the control group and the other is the patient group. While the control group consists of 50 healthy individuals, the patient group consists of 50 patients with decubitus ulcer. Native thiol and total thiol levels were measured by spectrophotometric method. Data management and analysis were performed using SPSS software (version 22). Statistical significance level was accepted as $p<0.05$.*

Results: *While the average native thiol, total thiol level, reduced thiol ratio, and thiol oxidation reduction ratio levels in the patient group were lower than the control group; disulfide and oxidized thiol levels were higher than the control group ($p<0.05$).*

Conclusion: *Total thiols, native (free) thiols and disulfide levels were significantly changed in the patient group compared to healthy individuals. The most striking result to emerge from the data is that plasma dynamic thiol/disulfide levels alter and shift to the disulfide side in patients with decubitus ulcers.*

Keywords: *Thiol/disulfide homeostasis, decubitus ulcer, oxidative stress, tissue damage*

***Corresponding Author:** Hamit Yıldız, Tel: +90 544 908 9090, E-mail: drhyildiz@hotmail.com, ORCID ID: 0000-0001-7858-5123.

Introduction

Since Jean Martin Charcot described it in 19th century, a considerable amount of research has grown up around the theme of pressure ulcers. Decubitus ulcers, also called pressure sores, are tissue necrosis and ulceration of the skin and/or soft tissue due to prolonged or strong pressure (1). Blood flow stops in the area of tissue exposed to a pressure above the capillary pressure (more than 30 mmHg). This results tissue hypoxia, necrosis and ulceration (2). Decubitus ulcers usually occur in areas where there are bony prominences. The severity of these injuries is evaluated in four stages. Stage I is the mildest of the decubitus ulcers. The epidermis is intact but there is non-blanchable hyperemia. The dermis is exposed in Stage II. However subcutaneous fat tissues is not affected. In Stage III, there is a full-thickness skin loss, but fascia, muscle, tendon, ligaments, cartilages and bony tissues are intact. In stage IV, complete loss of skin and serious damage occurs in subcutaneous structures such as skin, muscles and tendons (2), (3), (4).

Oxidative stress is defined as the deterioration in molecular and cellular functions as a result of the disruption of the balance between the formation of free radicals or reactive oxygen species and the antioxidant system. Reactive oxygen species are constantly formed in the human body and are removed by antioxidant defense systems. Antioxidants can act by scavenging reactive oxygen species, preventing their formation or repairing the damage they cause (5). In severe lesions such as skin ulcers or burn wounds, lipid peroxides are markedly increased. Lipid peroxidation is caused by the deformation of cell membrane phospholipids by oxidizing radicals. It has been reported that free radicals have a role in ischemic and inflammatory diseases and make wound healing difficult (6).

Thiols are a class of organic sulfur derivatives (mercaptans) containing a sulfhydryl group (-SH) in their active site (7) and are very important buffers that can interact with almost all physiological oxidants (8). Thiols, which are important antioxidants, have critical roles in the non-enzymatic destruction of reactive oxygen molecules and in preventing the formation of oxidative stress (9). It has been reported that thiols in plasma play physiological roles as free radical scavengers and act as antioxidants (10).

While oxidative products such as reactive oxygen species are reduced by donating their unpaired electrons to thiol-containing compounds; thiol groups are oxidized (11), (12). Thiols enter into oxidation reactions and

form disulfide bonds with oxidant molecules (9), (11). This bond is called an SS-bond or disulfide bridge. The disulfide bonds formed can be reduced back to thiol groups. Thus, dynamic thiol/disulfide homeostasis is achieved (12) (Figure 1). Thiol/disulfide homeostasis is required for detoxification (9). Thiol/disulfide homeostasis parameters are native thiol, total thiol, native thiol/total thiol ratios (antioxidant parameters) and disulfide, disulfide/native thiol, and disulfide/total thiol ratios (oxidant parameters) (9), (10). Native thiol contains only reduced thiols; total thiol includes both reduced and oxidized thiols (9).

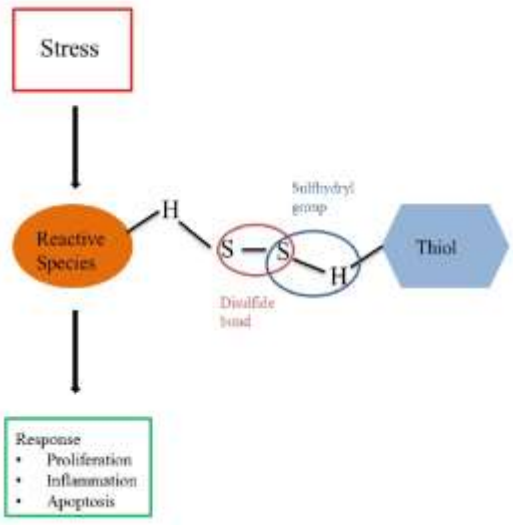

Figure 1. Thiol-disulfide homeostasis (13).

Measurement of plasma total thiols and assessment of thiol/disulfide homeostasis have been reported to be good indicators of excess free radical formation in humans (10). Systemically, oxidative stress can be assessed by measuring thiols (R-SH, sulfhydryl compounds) (13). In this study, it was aimed to investigate the blood levels of thiol and disulfide, which is a marker of total oxidant status, and the thiol/disulfide ratio in patients with decubitus ulcer hospitalized in the intensive care unit.

Material and Method

50 patients with decubitus ulcer and 50 healthy control cases hospitalized in Gaziantep University Medical Faculty Internal Diseases Intensive Care Unit in 2019 were included in the study. Gaziantep University Faculty of Medicine Ethics Committee approval was obtained for this study. For the evaluation of thiol/disulfide homeostasis, 10 ml venous blood samples were taken from each individual in the patient and control groups. The collected blood was transported to the laboratory in the first 30 minutes and centrifuged at 1500 rpm for 10 minutes and the plasma was separated. The obtained samples were stored in capped eppendorf tubes at -80°C until biochemical analysis.

Laboratory Method

Thiol/disulfide homeostasis parameters (Thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol),

which is one of the oxidative stress markers, were measured with a method developed by Erel and Neşelioğlu (12). Native thiol level (NTL) and total thiol level (TTL) were determined and their levels were measured. Half of the difference between the results obtained by subtracting the native thiol (-SH) amount from the total thiol (-SH+- S-S-) content shows the disulfide (-S-S-) level. In addition, thiol oxidation reduction ratio (-SH) × 100/ (-SS-), oxidized thiol ratio ((-S-S) x 100/ (-SH+-S-S-)) and reduced thiol ratio [-SH × 100/ (-SH+) -S-S-)] was calculated using these parameters.

Statistical analysis

Excel 2019 and SPSS 22.0 (Statistical Package for Social Sciences) statistics software were used to evaluate all the data obtained from the study. Normality analysis of variable and continuous data was evaluated with the Kolmogrov Smirnov test. The Student's T test was used to compare normally distributed data, while the Mann Whitney U test was used to compare data that did not show normal distribution. Normally distributed data were given as mean ± standard deviation. Data that did not show normal distribution were given as median (minimum/maximum). Statistical significance level was defined as $p < 0.05$.

Results

In this study, we compared thiol and disulfide levels and thiol/disulfide ratios which are indicators of total oxidant status, in patients with decubitus ulcer in the intensive care unit and healthy volunteers. Fifty people, 16 (32%) male and 34 (68%) female, were included in the healthy control group. Fifty patients, 21 (42%) male and 29 (58%) female, were included in the decubitus ulcer developed patient group. The mean age of the control group was 58.22 ± 9.10; the mean age of patients with decubitus ulcer was 55.96 ± 11.63 (Table 1). There was no statistical difference between the groups in terms of age and gender variables ($p>0.05$).

The oxidant and antioxidant parameters we examined in our study are given in Table 2. We observed that the levels of total thiol level, native thiol level, reduced thiol ratio, and thiol oxidation reduction ratio were significantly lower in patients with decubitus ulcer compared to the control group ($p<0.05$). On the other hand, disulfide and oxidized thiol levels were higher in the decubitus ulcer group compared to the control group ($p<0.05$).

Table 1. Demographic characteristics of the decubitus ulcer patient group and the healthy control group.

	AGE (years)	**GENDER n (%)**	**P value**
Decubitus ulcer patient group	55.96 ± 11.63	Male 21 (%42) Female 29 (%58)	>0.05
Healthy control group	58.22 ± 9.10	Male 16 (%32) Female 34 (%68)	>0.05

Table 2. Oxidant and antioxidant levels of the groups.

	Decubitus ulcer patient group (n=50)	**Healthy control group (n=50)**	**P value**
TTL (µmol/L)	214.97 ± 75.95	423.62 ± 70.31	<0.05
NTL (µmol/L)	115.16 ± 54.11	307.14 ± 57.74	<0.05
DISULPHIDE (µmol/L)	59.9(2.10/130.60)*	48.15 (7.70/129.80)*	<0.05
RTR (%)	53.49 ± 16.75	72.88 ± 11.28	<0.05
OTR (µmol/L)	25.65 (12.80/99.20)*	13.45(2.90/31.50)*	<0.05
TORR (%)	297.08 ± 197.13	477.48 ± 197.40	<0.05

TTL: total thiol level, NTL: native thiol level, RTR: reduced thiol ratio, OTR: oxidized thiol level, and TORR: thiol oxidation reduction ratio. *Values are presented as median (minimum/maximum).

Discussion

In this study, we investigated the thiol/disulfide homeostasis parameters, which are among the oxidant-antioxidant parameters, in patients with decubitus ulcer hospitalized in the intensive care unit. Ischemia develops when prolonged and/or strong mechanical pressure is applied to the body surface. As a result, cellular changes that can lead to tissue necrosis and ulceration occur (14). Prolonged pressure causes occlusion of blood vessels, ischemia, decline of nutrients and accumulation of metabolites. All these conditions cause tissue damage. Ischemia/reperfusion injury has an important place in the pathogenesis of decubitus ulcer. The duration of ischemia and the frequency of ischemia/reperfusion injury cycles increase tissue damage. Changes in the patient's body position eliminate the mechanical force on the skin. This causes tissue reperfusion (15). During ischemia/reperfusion, reoxygenation of ischemic tissue triggers the formation of reactive oxygen metabolites that have deleterious effects on cellular functions (14), (16) and may exacerbate the damage caused by ischemia (15).

Oxidative stress occurs as a result of the imbalance between reactive oxygen species and antioxidant molecules (the imbalance in favor of prooxidants). Overproduction of reactive oxidative species causes deterioration in the structure of proteins and lipids. Breakage in DNA structures and oxidation in cell membrane proteins and lipids occur (11). There is evidence that oxidative stress leads to harmful biochemical reactions and contributes to many diseases (17). Studies have shown that oxidative stress develops in patients with pressure ulcers (18), increased malondialdehyde levels and myeloperoxidase activity and decreased glutathione level (14), (16).

In physiological conditions, proteins targeted by reactive oxygen species are thiols. Thiols are mercaptans containing sulfhydryl residues and are the main molecules that coordinate antioxidant protective mechanisms (9). Thiol synthesis can be performed in all eukaryotic living cells. As the synthesized thiol groups are used for antioxidant purposes in the cell, their levels in the blood decrease (19). Measurement of total thiol in plasma and evaluation of thiol/disulfide homeostasis is an important indicator of free radical formation (10), (12). The deterioration of thiol-disulfide homeostasis as a result of the increase in oxidative molecules can be considered as a harbinger of possible diseases (11). Thiol/disulfide homeostasis has important

roles in antioxidant defense, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and cellular signal transduction mechanisms. It has been reported that thiol/disulfide homeostasis is disrupted (the balance shifts towards disulfide) in the pathogenesis of various diseases such as cancer, diabetes mellitus, cardiovascular diseases, chronic renal failure, and rheumatoid arthritis. Systemic oxidative stress can be demonstrated by a decrease in free thiol (native thiols) and total thiol levels and an increase in disulfide levels (13). Erel and Neşelioğlu (12) showed that the reduced thiol concentration increased, the native thiol concentration decreased, and the disulfide values increased under conditions of increasing oxidant concentrations.

In this study, we drew attention for the first time to thiol/disulfide homeostasis in patients with decubitus ulcer in the intensive care unit and showed that this balance was impaired in patients with decubitus ulcer. Thiol is an antioxidant molecule that is abundant in the organism. Thiol-disulfide balance is an important parameter that shows the disorder in the oxidant-antioxidant balance. Our results showed that thiol levels were lower and disulfide levels were higher in patients with decubitus ulcer. This situation reveals the presence of oxidative process in patients with decubitus ulcer. It can be said that disulfide bond formation from the thiol group increases due to the reactive oxygen species formed and the thiol-disulfide balance shifts to the disulfide side. Dynamic thiol/disulfide homeostasis is only one of many oxidant-antioxidant systems in our body and does not show the total antioxidant level of the body (19). The inability to evaluate other inflammatory markers and oxidative stress parameters and the fact that they were not compared with thiol/disulfide balance parameters are among the limitedness of our study.

Conclusion

We showed that thiol/disulfide homeostasis, which is one of the oxidative stress parameters, is altered in patients with decubitus ulcer. With this study, he drew attention to this subject for the first time and gave information about the subject. However, more in-depth and comprehensive research is needed.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

No financial support was received from any institution for this research. All researchers contributed equally to the study.

REFERENCES

1. Liao X, Ju Y, Liu G, Zhao X, Wang Y, Wang Y. Risk factors for pressure sores in hospitalized acute ischemic stroke patients. Journal of stroke and cerebrovascular diseases. 2019;28(7):2026–30.
2. Kumar N, Rao S. Ayurvedic management of dusta vrana w.s.r. decubitus ulcer: a case study. Journal of ayurveda and integrated medical sciences. 2020;5(4):434–7.
3. Yarkony GM. Pressure ulcers: a review. Arch phys bled rehabil. 1994;75:908–17.
4. Salcido R, Popescu A, Ahn C. Animal models in pressure ulcer research. J spinal cord med. 2007;30:107–16.
5. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. The american journal of medicine. 1991;91:14–22.
6. Latha B, Babu M. The involvement of free radicals in burn injury: a review. Burns. 2001;27:309–17.
7. Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. American journal of clinical nutrition. 2000;72:653–69.
8. Tanrıverdi F, Yüzbasioglu Y, Ercan Haydar FG, Gökhan S, Özhasenekler A, Yıldırım Ç, et al. Evaluation of thiol disulphide homeostasis and neutrophile lymphocyte ratio in carbon monoxide intoxication. Ankara medical journal. 2020;20(1):47–56.
9. Elmas B, Karacan M, Dervişoğlu P, Kösecik M, İşgüven ŞP, Bal C. Dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in obese children and its relationship with inflammatory-cardiovascular markers. Anatolian journal of cardiology. 2017;18:361–9.
10. Elmas B, Yıldız T, Yazar H, İlçe Z, Bal C, Özbek B, et al. New oxidative stress markers useful in the diagnosis of acute appendicitis in children: Thiol/disulfide homeostasis and the asymmetric dimethylarginine level. Pediatric emergency care. 2020;36(8):362–7.
11. Yıldız H. Thiol/disulphide homeostasis in intensive care unit patients with sepsis and septic shock. Turkish journal of medical sciences. 2020;50:811–6.
12. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clinical biochemistry. 2014;47(18):326–32.
13. Leimkuhler M, Bourgonje A, van Goor H, van Leeuwen B, Bock de G. Systemic oxidative stress and antioxidant capacity in cancer patients. Journal of translational science. 2020;6:1–3.
14. Şener G, Sert G, Şehirli AÖ, Arbak S, Uslu B, Gedik N, et al. Pressure ulcer-induced oxidative organ injury is ameliorated by B-glucan treatment in rats. International immunopharmacology. 2006;6:724–32.
15. Kumar S, Theis T, Tschang M, Nagaraj V, Berthiaume F. Reactive oxygen species and pressure ulcer formation after traumatic injury to spinal cord and brain. Antioxidants. 2021;10:1–15.
16. Şener G, Sert G, Şehirli AÖ, Arbak S, Gedik N, Ayanoğlu-Dülger G. Melatonin protects

against pressure ulcer-induced oxidative injury of the skin and remote organs in rats. J pineal res. 2006;40:280–7.

17. Castro L, Freeman BA. Reactive oxygen species in human health and disease. Nutrition. 2001;17:161–5.
18. Khlifi L, Graiet H, Sahli S, Ben-hadj-mohamed M, Bouzidi N, Miled AE. Evidence of metabolic imbalance and oxidative stress among patients suffering from pressure ulcers. Journal of dermatologıcal treatment. 2019;30(4):414–21.
19. Apaydın ZP, Yıldız H, Samim FS, Alaşehirli B. Investigation of dynamic thiol disulfide homeostasis in acute respiratory failure patients in intensive care unit. Experimental and applied medical science. 2021;2(2):164–75.