

Thiol/disulphide homeostasis and oxidative stress in patients with peripheral facial paralysis

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ABSTRACT

The main pathophysiological mechanism responsible from the peripheral facial paralysis (PFP) is the ischemia, inflammation and swelling of the facial nerve. The purpose of the present study was to assess the oxidative stress parameters including the thiol/disulphide homeostasis in patients with peripheral facial paralysis.

A total of 32 patients with PFP and 32 healthy controls were recruited in this cross-sectional study. Serum samples were compared for thiol/disulphide homeostasis (TDH), total oxidant status (TOS), total antioxidant capacity (TAC), paraoxonase (PON), stimulated paraoxonase (SPON), arylesterase (ARES), ceruloplasmin (CLP), myeloperoxidase (MPO), and catalase (CAT) levels.

There were no significant differences between PFP patients and the control group regarding age and gender distribution. Remarkably, TOS ($p=0.034$), CAT ($p<0.001$), ARES ($p<0.001$), native thiol ($p<0.001$), total thiol ($p<0.001$), and native thiol/total thiol ratio ($p<0.001$) were significantly higher in the control group. In contrast, serum ceruloplasmin level ($p=0.005$) as well as disulphide/native thiol (%) ($p=0.001$) and disulphide/total thiol (%) ($p<0.001$) ratios were found to be higher in PFP patients compared to the control group.

Thiol/disulphide homeostasis that was suggested as a useful indicator of oxidant/antioxidant imbalance may be a practical marker in diagnosis and follow-up of PFP. Further studies are warranted to determine the effects of nutritional and therapeutic approaches for normalization of oxidative stress in treatment and prevention of PFP.

Key Words: Homeostasis, oxidative stress, peripheral facial paralysis, thiol/disulphide

Introduction

Facial paralysis is a widespread clinical disorder of the facial nerve that can cause distress (1). Peripheral facial paralysis (PFP) is the acute onset lower motor neuron palsy which is associated with remarkable functional and cosmetic morbidities (2, 3). The underlying etiology of this benign neurological disorder which presents as a mononeuritis is vague (4). Idiopathic PFP is the most common presentation of facial paralysis that constitutes for 60-75% of cases with acute, unilateral facial paralysis (5).

The main pathophysiological mechanism responsible from the PFP is the ischemia, inflammation and swelling of the facial nerve (1, 6). It was shown that edema leading to the disturbance of microcirculation and the resultant ischemic process was associated with arteriolar spasm and thrombosis of the facial nerve within the facial canal (7).

Although usually self-limited, PFP may lead to significant temporary oral incompetence and potential eye injuries due to the failure of closure of the eyelid. Additional long-term poor outcomes may be devastating for the patient. In that aspect; prompt treatment to improve facial functions and to enhance recovery is essential (8).

Oxidative stress is defined as the imbalance between pro-oxidant and antioxidant molecules; and consequently reactive oxygen species (ROS) may accumulate as a result of ischemic events. The reactive oxygen species are associated with the impaired cellular metabolism causing lipid peroxidation, nucleic acid damage and modification of proteins (9, 10). Oxidation of-SH (sulfhydryl) groups in amino acids is the initial sign of protein oxidation. Thiols are functional sulfhydryl groups and oxidation of thiol groups leads to the formation of disulphide bonds. These disulphide bonds are reduced to thiol groups when oxidative stress is diminished. In recent

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Table 1. Comparison of results in peripheral facial paralysis patients and control group. Data is expressed either as mean±standard deviation (range: minimum-maximum) or median-interquartile range (range: minimum-maximum)

	Peripheral facial paralysis	Control	P value
Age (years)	46.40 ± 14.49 (22-78)	47.90 ± 14.43 (23-72)	0.680
Gender distribution (M/F)	17/15	16/16	0.802
Total antioxidant capacity	1.50 ± 0.25 (1.17 -2.32)	1.58 ± 0.16 (1.94-1.27)	0.117
Total oxidant status	6.92 ± 4.33 (2.86 - 31.60)	5.52 ± 5.61 (0 - 20.39)	0.034*
Paraoxanase-1	143.06 ± 106.58 (5.2 - 422.3)	194.98 ± 152.08 (50.0 - 685.1)	0.119
Stimulated paraoxanase	490.01 ± 394.64 (2.1 - 1546.9)	652.26 ± 532.74 (152.2- 2443.9)	0.174
Arylesterase	228.26 ± 65.06 (53.4 - 370.7)	320.72 ± 52.32 (219.3 - 447.8)	<0.001*
Ceruloplasmin	85.25 ± 36.55 (34.30 - 214.12)	66.22 ± 41.60 (28.66 - 259.27)	0.005*
Catalase	107.87 ± 63.84 (8.3 - 289.5)	180.70 ± 102.29 (75.3 - 482.9)	<0.001*
Myeloperoxidase	114.75 ± 41.00 (7.63 - 167.05)	113.16 ± 47.25 (4.38 - 159.50)	0.886
Native thiol (SH)	294.64 ± 82.62 (80.8 - 479.1)	428.08 ± 49.68 (340.0 - 554.3)	<0.001*
Total thiol (SH+SS)	331.93 ± 84.56 (126.8 - 528.4)	461.46 ± 50.14 (370.9 - 574.1)	<0.001*
Disulphide (SS)	18.43 ± 5.02 (8.0 - 27.0)	17.31 ± 5.23 (7.0 - 33.0)	0.388
SS/SH (%)	7.02 ± 4.44 (2.97 - 28.46)	4.12 ± 1.38 (1.48 - 8.62)	0.001*
SS/Total SH (%)	5.94 ± 2.74 (2.80 - 18.14)	3.80 ± 1.22 (1.44 - 7.88)	<0.001*
SH/Total SH (%)	87.97 ± 60 (63.72 - 94.21)	92.72 ± 1.80 (88.74 - 97.20)	<0.001*

Hint: *: statistically significant; F: female; M: male; SH: thiol; SS: disulphide

publications, thiol/disulphide balance was determined as a novel marker of oxidative stress (7, 10).

Understanding the pathophysiology more clearly may support the development of novel diagnostic and therapeutic modalities in PFP. The purpose of the present study was to assess the balance of oxidant-antioxidant molecules including the thiol/disulphide homeostasis (TDH) together with paraoxanase (PON), stimulated paraoxanase (SPON), arylesterase (ARES), ceruloplasmin (CLP), myeloperoxidase (MPO), and catalase (CAT) levels in patients with PFP. To the best of our knowledge, this is one of the first studies in literature investigating the role of thiol derivatives in PFP.

Materials and Methods

Study Design: This prospective cohort study was performed in the Plastic and Reconstructive

Surgery and Otorhinolaryngology departments in Yuzuncu Yil University, Turkey. The study was approved by local Institutional Review Board and written informed consent was obtained from all participants (Date: 23 May 2017, Decision Number: 03).

Totally, 32 patients with PFP (15 women, 17 men) and 32 healthy controls (16 women, 16 men) were recruited in the study. The average ages for PFP and control groups were 46.40±14.49 and 47.90±14.43 years, respectively.

Exclusion criteria were as follows; presence of identified causes of acute PFP (such as temporal bone fracture, trauma, Ramsay Hunt syndrome, schwannoma of the facial nerve), acute and chronic ear infections diagnosed clinically and/or by imaging methods and systemic diseases including diabetes mellitus, autoimmunity, and acute or chronic infectious diseases. The control group was selected among healthy hospital staffs without any chronic diseases having normal

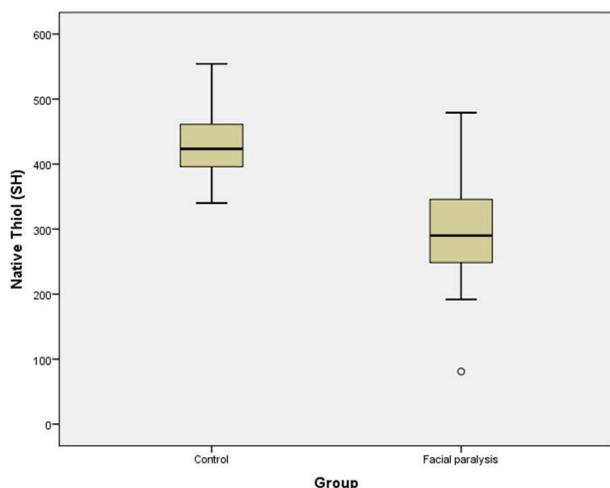


Fig. 1. Native thiol levels in peripheral facial paralysis (PFP) patients and control group

physical examination and laboratory findings. All investigators strictly adhered to the ethical standards announced in the Declaration of Helsinki.

Diagnosis of Peripheral Facial Paralysis: Diagnosis and grading of PFP were performed according to House-Brackmann (HB) facial nerve grading system (11). All patients underwent magnetic resonance imaging of temporal bone and the images were assessed for contrast enhancement of the facial nerve on T1-weighted views. After collection of the peripheral venous blood samples, all patients received prednisone treatment initiated at a dose of 1 mg/kg/day and tapered within 2 weeks (2).

Biochemical Analysis: Venous blood samples were obtained from participants after an overnight fasting period. These samples were centrifuged at 2,300 rpm (Hettich Lab Technology, Tuttlingen, Germany) for 10 minutes and serum samples were stored at -80°C . Laboratory staff that carried out the biochemical analyses were blind for the clinical information and outcomes of patients. Furthermore, the results were not accessible for the treating physicians, study staff, or investigators during the study.

Thiol/Disulphide Homeostasis: Serum thiol/disulphide homeostasis (TDH) was determined with a recently developed novel and automatic measurement method (12) by using an automated clinical chemistry analyzer (Roche, Cobas 501, Mannheim, Germany). Native thiol (-SH) and total thiol (-SH+S-S-) levels were measured directly, and disulphide (-S-S-) level, disulphide/total thiol ratio (-S-S-/-SH+S-S-) and disulphide/native thiol ratio (-S-S-/-SH) were obtained with calculation (7).

Catalase (CAT) Activity: Measurement of serum levels of CAT was performed using Aebi kinetic method (13, 14). At the beginning, the serum sample was diluted with Tris/HCl buffer at a pH of 7.4. 2.5 ml of substrate was mixed with 50 mM Tris/HCl buffer at a pH of 7.4. After 10 seconds, absorbance was measured at 240 nm and the kinetic changes of absorbance were marked every 30 seconds during 2 minutes for determination of CAT level.

Total Oxidant Status (TOS) and Total Antioxidant Capacity (TAC) Levels: Plasma TOS and TAC levels were assessed using the novel automated measurement method of Erel (15, 16).

Paraoxonase-1 (PON), Stimulated Paraoxonase (SPON) and Arylesterase (ARES) Levels: Paraoxonase (PON), stimulated paraoxonase (SPON) and ARES levels were evaluated using previously described methods (17, 18).

Ceruloplasmin (CLP) and Myeloperoxidase (MPO) Levels: Serum levels of CLP and MPO were measured as described by Tufan et al (10).

Statistical Analysis: The data was analyzed with the IBM Statistical Package for Social Sciences (SPSS) Statistics 20 software program (*SPSS Inc., Chicago, IL, USA*). Normal distribution of variables was tested with Kolmogorov-Smirnov test. Parametric tests were performed for variables with normal distribution, whereas variables without a normal distribution were assessed with non-parametric tests. Pearson Chi-Square test was performed to compare the categorical variables. The variables with or without normal distribution were compared with Independent-Samples T-test and Mann-Whitney U test, respectively. Quantitative data was expressed as either mean and standard deviation, or median and interquartile range. The confidence interval was 95%, and p values less than 0.05 were considered as statistically significant.

Results

An overview of comparison of descriptive and biochemical variables is summarized in Table 1. Accordingly, there were no significant differences between PFP patients and the control group regarding baseline age and gender distribution (46.40 ± 14.49 vs 47.90 ± 14.43 years, $p=0.680$ and 17 vs 16 males $p=0.802$; respectively). Although total antioxidant capacity, antioxidant enzymes paraoxonase-1, and stimulated

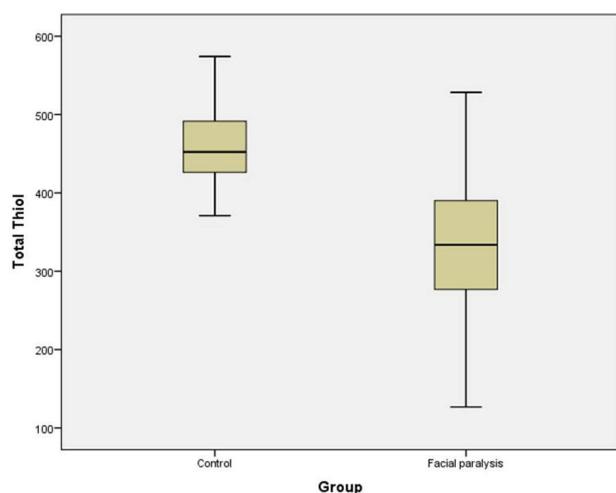


Fig. 2. Total thiol levels in PFP patients and control group

paraoxanase levels were not statistically significantly different between groups (Table 1); other markers of antioxidant system including CAT ($p < 0.001$), ARES ($p < 0.001$), native thiol (SH) ($p < 0.001$) (Figure 1), total thiol (SH+SS) ($p < 0.001$) (Figure 2) levels, and native thiol/total thiol ratio ($p < 0.001$) (Figure 3) were significantly higher in the control group (Table 1).

In contrast, serum ceruloplasmin level ($p = 0.005$) as well as the markers of augmented oxidative stress including disulphide (SS)/native thiol (SH) % ($p = 0.001$) (Figure 4) and SS/total thiol % ($p < 0.001$) (Figure 5) ratios were determined to be higher in PFP patients compared with the control cases. TOS levels were higher in the PFP group ($p = 0.034$) (Table 1).

Discussion

In this study through the aim to assess the serum levels of oxidative stress indicators in patients with PFP; we determined that the serum levels of oxidative stress indicators and TDH markers were remarkably altered in PFP. Levels of CLP and disulphide derivatives were higher; while thiol derivatives, CAT and ARES levels were lower in PFP patients compared with the control group. In this aspect disturbance of balance between oxidant-antioxidant molecules may have an important role in the pathogenesis of PFP and indicators of thiol/disulphide homeostasis may serve as markers in the diagnosis and follow-up of PFP.

Peripheral facial paralysis is defined as the acute, idiopathic, unilateral facial nerve paresis or paralysis that develops in less than 3 days (1). The most frequent cause is the edema of the facial

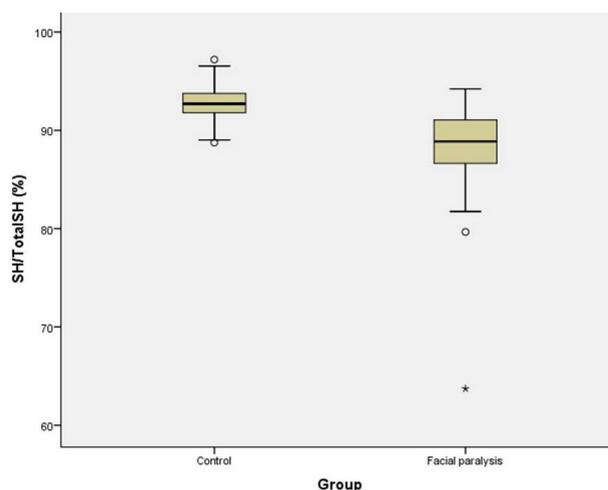


Fig. 3. Native thiol/total thiol ratio (%) in PFP patients and control group

nerve attributed to herpes or varicella-zoster virus infections (1). Other possible etiologic factors include ischemia, autoimmunity, inflammation and hereditary causes (1).

Even though the underlying etiological factors of PFP have not been well understood, the weakness of the facial nerve may be associated with the inflammation and edema. This inflammatory cascade may be aggravated by oxidative stress which develops as a result of an impaired balance between antioxidant defense mechanisms and ROS.

A spreading inflammatory reaction accompanied by oxidative stress is prevalent in some neurological disorders. The oxidative stress in neurons may lead to the disruption of actin filament organization which consequently causes a progressive degeneration of neurons; moreover free oxygen radicals may also impair the neuronal dynamics and physiological redox signaling (19). On the other hand; anti-oxidant molecules preserve neural growth and morphology by counteracting deleterious effects of free oxygen radicals. In a very recent study, Terzi et al (20) reported that serum total oxidant status, and oxidative stress index were significantly higher in patients with Bell's palsy compared with the control group that may be suggested to play a role in etiopathogenesis. We also determined serum CAT and ARES levels were remarkably lower and TOS levels were higher in PFP group compared with the control cases defining an imbalance between oxidant-antioxidant molecules. Supporting our findings; inhibition of neuronal nitric oxide synthase that is an oxidative stress-related biomarker was shown to facilitate the axonal regeneration in a rat model (21). Moreover, in an experimental study vitamin E, an anti-

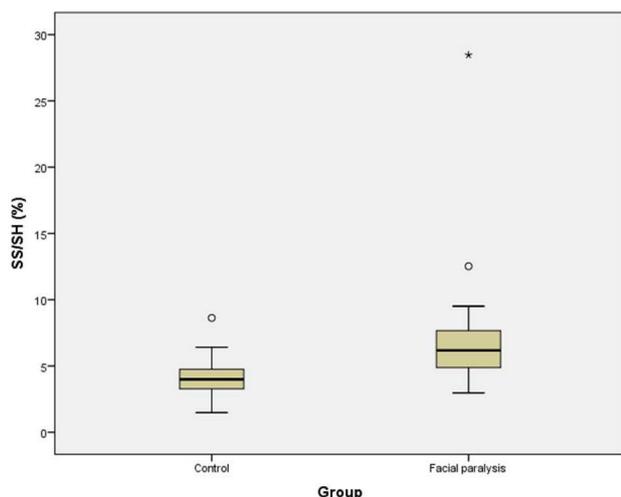


Fig. 4. Disulphide/native thiol ratio (%) in PFP patients and control group

oxidant, was reported to have a positive effect on nerve healing in rats with traumatic facial palsy (22). Similarly, Serefican et al also reported that Thymoquinone which has antioxidant and anti-inflammatory effects was slightly better than methylprednisolone for functional nerve recovery in a traumatic facial nerve paralysis animal model (23). All these studies were also supporting our findings about the role of augmented oxidative stress in PFP.

The thiol redox state plays a vital role in various cell responses including receptor modification, signal transduction, immune regulation, cell proliferation, apoptosis, antioxidant defenses, xenobiotic metabolism, protein structure and activity (24). Our data indicated that a prominent degree of oxidative stress was evident in patients with PFP that was reflected as an increase in disulphide/thiol ratio and ceruloplasmin levels. Conversely, thiol levels were found to be higher in the control group. Although ceruloplasmin is regarded as a metalloenzyme having antioxidant properties, an ambivalence on its effects regarding the augmentation of oxidative stress especially on vascular tissues is also known (25). Moreover, a synergy between ceruloplasmin and inflammatory markers and total oxidant status was also shown before (26). We have determined an increase in ceruloplasmin levels in parallel with an increase in oxidative stress markers in patients with PFP which warrants further investigations to define the exact role of ceruloplasmin in PFP.

It has been postulated that TDH could be a contributing factor in the pathogenesis of disorders such as cardiovascular diseases, diabetes mellitus, and rheumatoid arthritis. The etiology of the shift of thiol/disulphide balance may be linked

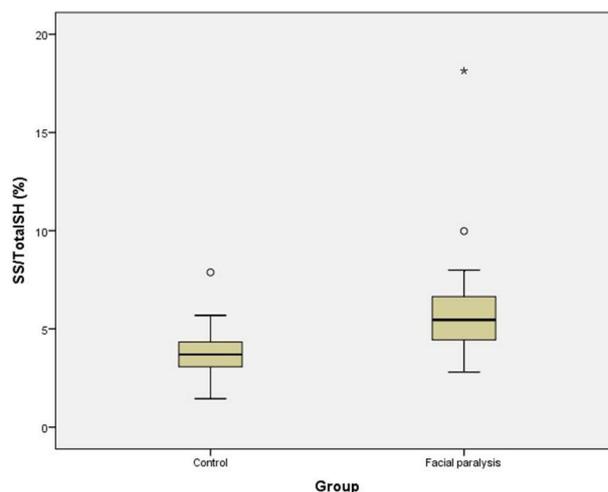


Fig. 5. Disulphide/total thiol ratio (%) in PFP patients and control group

to a higher reactive oxygen and/or nitrogen species burden in the hematic compartment or a decreased dietary supply of the reduced forms (27). Very recently, Babademez et al (7) investigated the relationship between Bell's palsy and thiol/disulphide homeostasis on 77 patients and 38 healthy controls and reported that; the mean native thiol and total thiol levels were significantly lower and disulphide levels were higher in the Bell's palsy. Similarly we also determined lower native thiol and total thiol levels in patients with PFP.

Thus, we believe that thiol metabolism may have a role in pathophysiology of Bell's paralysis and may be important in development of new diagnostic and therapeutic strategies. Prospective trials investigating the efficacy of antioxidant treatments may delay the extension of oxidative stress injury, and may yield more favorable therapeutic outcomes. This approach may not only improve the treatment outcomes, but may aid to avoid unnecessary surgical interventions.

Owing to the complexity of oxidative stress and anti-oxidant mechanisms, demonstration of a causal relationship between markers and disease is difficult. The roles of confounding factors mandate a multidisciplinary and collaborative approach to the medical care of patients with PFP. In this purpose, the use of other parameters such as TOS, ARES, CLP and CAT in addition to the thiol/disulphide homeostasis indicators can provide important clues and clinical implications.

On the other hand, our study possesses certain limitations including relatively small sample size and restriction of data with the experience of a single institution. Moreover, the degree of PFP was not uniform in all patients. For these reasons,

multi-centered and larger studies are warranted to define the role of thiol/disulphide homeostasis in PFP.

In conclusion, we suggest that augmented oxidative stress and impairment of anti-oxidant mechanisms are involved in the pathogenesis of PFP. Thiol/disulphide homeostasis was defined as useful indicator of oxidant/antioxidant imbalance and may be used as a practical marker in diagnosis of PFP patients. Nutritional and therapeutic approaches for normalization of redox state can be beneficial against PFP. Further, prospective clinical trials on large series are warranted to determine the effects of nutritional and therapeutic approaches for normalization of oxidative stress in treatment and prevention of PFP.

Abbreviations: PFP: Peripheral facial paralysis; TDH: Thiol/disulphide homeostasis; TOS: Total oxidant status; TAC: Total antioxidant capacity; PON: Paraoxonase; SPON: Stimulated paraoxonase; ARES: Arylesterase; CLP: Ceruloplasmin; MPO: Myeloperoxidase; CAT: Catalase; ROS: Reactive oxygen species; HB: House-Brackmann; -SH: Native thiol; -SH+-S-S-: Total thiol; -S-S: Disulphide; *: statistically significant; F: Female; M: Male.

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