Thiol/disulphide homeostasis in Bell's palsy as a novel pathogenetic marker

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Objectives: The aim of this study was to investigate the relationship between Bell's palsy and a novel oxidative stress parameter, thiol/disulphide homeostasis.
Design: A prospective study evaluating oxidative stress in Bell's palsy.
Setting: This research took place in the department of Otorhinolaryngology, Ataturk Training and Research Hospital.
Participants: Totally, 77 patients with Bell's palsy and 38 healthy controls were included in this study.
Main outcome measures: The blood levels of total and native thiol and disulphide activity were assessed, and their levels were compared in the patients and controls.
Results: There were statistically significant differences between the patients and controls regarding thiol/disulphide parameters. The mean native thiol and total thiol were significantly lower and disulphide levels were higher in the Bell's palsy than controls. On binary logistic regression analysis, the created model showed 45.3% variation. The cut-off value was 18.95 for disulphides.
Conclusion: Native and total thiol levels were low in the Bell's palsy. This metabolic disturbance may have a role in the pathogenesis of Bell's palsy.

Bell's palsy is the appellation commonly used to describe an acute peripheral facial palsy of unknown cause. The precise pathophysiology of Bell's palsy remains an area of debate. Active herpes simplex virus (HSV) sequences have been identified in the endoneurial fluid of the facial nerve during facial nerve decompression surgery in patients with Bell's palsy.[1] A theory proposes that oedema and ischaemia result in compression of the facial nerve within the facial bony canal. However, the cause of the oedema and ischaemia has not been understood clearly. In addition, this oedema can be seen in MRI scans with facial nerve enhancement. The primary ischaemic theory of pathogenesis is to produce arteriolar spasm and thrombosis in the vessels supplying the nerve within the rigid bony fallopian canal. Later a secondary ischaemic theory is an inflammatory, viral or immunologic oedema causing disturbances of the microcirculation, leading to loss of nerve conductivity. Both concepts suggest that these processes lead to anoxia followed by compensatory dilation of the vessels and transudation.[2]

The imbalance between pro-oxidants and antioxidants, if not deactivated by the cellular antioxidant system, results in oxidative stress. Reactive oxygen species (ROS) derived from ischaemic events can generate products leading to cellular deregulation. Excess of these species can react with cellular macromolecules and results in lipid peroxidation, nucleic acid damages and protein modifications.[3] Protein oxidation by the reactive derivatives leads to nitration of aromatic amino acids, oxidation of thiol groups and formation of advanced oxidation protein products and transformation of some amino acid residues to the carbonyl derivatives. It is known that free radicals cause oxidation of -SH groups in sulphur-containing amino acids of proteins and these are the earliest observable signs of protein oxidation.[4] Thiols, which consist of a sulphur atom and a hydrogen atom bound to a carbon atom, are functional sulphhydryl groups.[5] Thiol groups of proteins like albumin are oxidised by oxygen molecules and are reversibly

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converted to disulphide bonds. These formed disulphide bonds can be reduced to thiol groups in a condition of decreased oxidative stress. Thus, the thiol/disulphide balance is maintained.[6] Thiol/disulphide levels are measured one by one and cumulatively with a novel and automated method.[7]

In this study, we investigated the relationship between thiol/disulphide homeostasis and idiopathic facial nerve paralysis, that is Bell's palsy.

Materials and methods

Totally, 124 patients with acute peripheral facial paralysis admitted to the otorhinolaryngology clinic between June 2014 and September 2015. The patients who had a known aetiology for acute facial paralysis such as Ramsay Hunt syndrome and facial schwannoma, or who had an underlying systemic disease such as diabetes mellitus, autoimmune and cardiovascular diseases, were excluded from the study. Thus, 77 patients with idiopathic acute peripheral facial paralysis or Bell's palsy were included in the study. In addition, 38 healthy age- and gender-matched subjects were included and comprised the control group. The healthy control group was selected among hospital staff who had no known history of chronic illness or drug use according to history and physical examination. A written informed consent was taken from the patients and controls. The local ethical committee of the hospital approved the study. All investigators confirm to ethical standards as described in the Declaration of Helsinki.

The facial functions were graded according to House-Brackmann (HB) facial nerve grading system on admission to the clinic. All patients were evaluated with temporal magnetic resonance imaging on the day of admission. The patients were subdivided into two groups according to contrast enhancement of the facial nerve on T1-weighted MRI: those with facial paralysis with oedema (FPWE) and those with facial paralysis without oedema (FPWOE). All patients were treated with prednisone 1 mg/kg/day tapered in 2 weeks.

Biochemical analysis

Venous blood samples were collected from the subjects before the steroid administration and MRI. All samples centrifuged in the cold at 2300 ***g*** for 10 min. Serum samples were separated and stored at −80°C. Serum thiol/disulphide homeostasis was determined with a recently developed novel and automatic measurement method[7] by using an automated clinical chemistry analyser (Roche, Cobas 501, Mannheim, Germany). Native thiol (-SH) and total thiol (-SH+-S-S-) 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.

Statistical analyses

SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Macintosh, Version 22.0. Armonk, NY: IBM Corp.) statistical software was used to analyse the data. The descriptive data were given as mean ± standard deviation. The distribution of data was calculated by using the Kolmogorov–Smirnov test. Continuous variables with normal distribution were determined to have mean standard deviation, while continuous variables without normal distribution were determined as median and interquartile range. Categorical variables are shown as numbers and percentages. While continuous variables with normal distribution were compared with an independent sample *t*-test, those with non-normal distribution were compared with the Mann–Whitney *U*-test when applicable. The relationship between variables was analysed by Pearson's or Spearman's correlation analysis according to the distribution type of the parameters. The receiver operating characteristic (ROC) curve was used to show the sensitivity and specificity and optimal cut-off value of disulphide level for predicting Bell's palsy. The significance was set at $P < 0.05$.

Results

The demographic and clinical characteristics and laboratory findings of the participants are shown in Table 1. Body mass indexes (BMI), mean age and gender distribution were similar between the study and control groups ($P > 0.05$).

The mean native thiol and total thiol levels and native thiol/total thiol ratios were lower in the study group when compared to the control group ($P < 0.05$, $P > 0.05$ and $P < 0.05$, respectively). However, the mean disulphide level, the disulphide/native thiol and the disulphide/total thiol ratios were higher in the study group when compared to the control group ($P < 0.05$). There was no correlation between the HB grades and thiol profiles ($P > 0.05$; Fig. 1).

The mean native thiol levels in FPWE group were significantly higher than in FPWOE group ($P < 0.05$; Table 2). The other thiol/disulphide parameters were not significantly different between FPWE and FPWOE groups ($P > 0.05$).

In order to investigate the predictive values of native and total thiol variables that showed a significant difference between the controls and patients, binary logistic regression analysis was performed. Accordingly, the created model was

Table 1. Demographic and clinical characteristics of the study and control groups

	Patients (n = 77)	Controls (n = 38)	P value*
Age	38.48 ± 10.31	37.37 ± 10.75	0.593
Gender (n, %)			
Female	38 (33)	18 (15.7)	
Male	39 (33.9)	20 (17.4)	
BMI (kg/m^2)	23.4 ± 2.1	23.8 ± 1.8	
House-Brackmann grading (n, %)			
Grade 1	0		
Grade 2	12 (10.4)		
Grade 3	28 (24.3)		
Grade 4	22 (19.1)		
Grade 5	10 (8.7)		
Grade 6	5 (4.3)		
Thiol/disulphide homeostasis			
-SH, μmol/L	386.24 ± 54.84	415.19 ± 42.54	**0.005**
-SH+-S-S-, μmol/L	433.41 ± 53.92	446.63 ± 43.3	0.191
-S-S-, μmol/L	23.58 ± 7.99	15.72 ± 5.7	**0.000**
-S-S-/-SH, %	6.29 ± 2.61	3.83 ± 1.49	**0.000**
-S-S-/-SH+-S-S-, %	5.5 ± 1.92	3.52 ± 1.27	**0.000**
-SH/-SH+-S-S-, %	88.99 ± 3.85	92.94 ± 2.55	**0.000**

-SH, native thiol; -SH+-S-S-, total thiol; -S-S-, disulphide.
Results were given as mean ± SD.
*P value < 0.05 considered significant. Bolds are statistically significant.

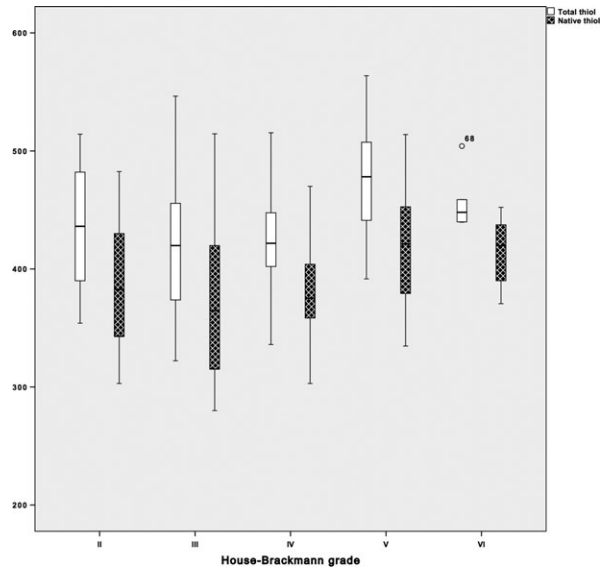

Fig. 1. The correlation of House-Brackmann grade and thiol profiles in Bell's palsy patients.

significant and explained 45.3% of the variation (Table 3). Receiver operating characteristic analysis was performed to determine the cut-off value of disulphide level to predict Bell's palsy patients. The cut-off value of disulphide level was 18.95, with a sensitivity of 75.3% and a specificity of 71.1% (area under the curve 0.80; Fig. 2).

Discussion

Bell's palsy is an acute unilateral facial nerve paresis or paralysis that appears in less than 72 h without any identifiable cause.[8] The most likely cause of Bell's palsy is oedema of the facial nerve induced by the herpes virus and varicella zoster virus.[9] Other suggested causes include ischaemia, autoimmune inflammatory disorders and heredity.[2,10,11] Although the aetiology of Bells palsy is not clear, the facial weakness is thought to result from facial nerve inflammation and oedema. An inflammation triggered by oxidative stress is the cause of vascular diseases like thrombosis,[12] metabolic syndrome[13] and neurodegenerative diseases.[14] Oxidative stress starts as result of an impaired balance between antioxidant defence and reactive oxygen species, and can be identified in most of the key steps in the pathophysiology of vascular diseases. The labyrinthine segment of the facial nerve contains a few and small intrinsic blood vessels compare to the mastoid and tympanic segments. This may indicate that the labyrinthine segment of the facial nerve may be more vulnerable to oxidative stress.

Dynamic thiol/disulphide homeostasis has a critical role in the organism. Changes in the thiol/disulphide balance serve as components for antioxidant protection, detoxification, regulation of enzymatic activity and cellular signalling mechanisms.[15,16] Thiol groups have an important role in cells by minimising the toxic effects of oxygen-activated

Table 2. Thiol/disulphide profiles of subjects in correlation with the facial nerve enhancement on contrasted MRI

n = 115	FPWOE (n = 34) Mean ± SD	FPWE (n = 23) Mean ± SD	P value*
-SH, µmol/L	375.25 ± 48.4	404.03 ± 54.37	**0.047**
-SH+-S-S-, µmol/L	422.73 ± 47.95	447.97 ± 53.19	0.074
-S-S-, µmol/L	23.73 ± 10.09	21.96 ± 6.36	0.459
-S-S-/-SH, %	6.51 ± 3.29	5.58 ± 1.9	0.226
-S-S-/-SH+-S-S-, %	5.63 ± 2.34	4.97 ± 1.55	0.24
-SH/-SH+-S-S-, %	88.73 ± 4.68	90.05 ± 3.1	0.205

-SH, native thiol; -SH+-S-S-, total thiol; -S-S-, disulphide; FPWOE, facial paralysis without oedema; FPWE, facial paralysis with oedema
Results were given as mean ± SD.
*P value < 0.05 considered significant. Bolds are statistically significant.

Table 3. Predictive value of native thiol and total thiol with binary logistic regression analysis

	P value	Wald	Odds ratio	95% Confidence interval Lower	Upper
-SH, µmol/L	0.000*	19.315	1.088	1.048	1.13
-SH+-S-S-, µmol/L	0.000*	22.306	0.911	0.876	0.947
		Nagelkerke R^2 = 0.453			

-SH, native thiol; -SH+-S-S-, total thiol; -S-S-, disulphide.
*P < 0.05 is considered significant for statistical analyses.

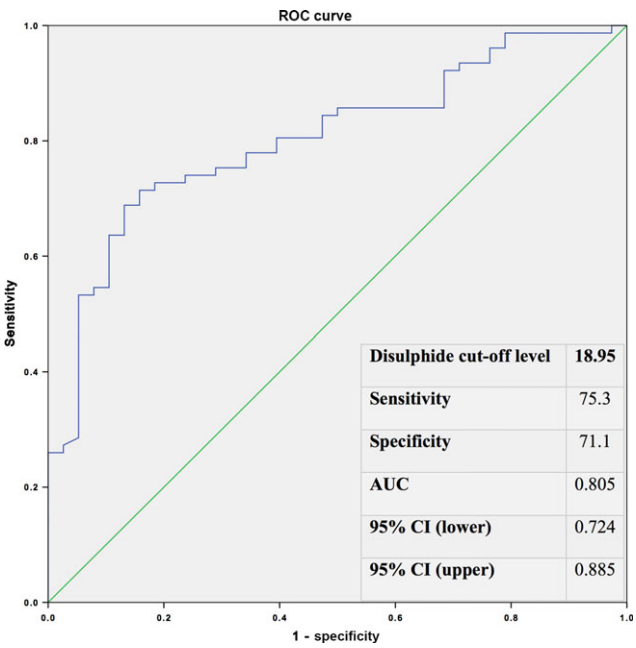

Fig. 2. Receiver operating characteristic (ROC) analysis of disulphide level in patients with Bell's palsy.

processes. The sulphhydryl groups are associated with proteins, and when thiol level decreases in serum, its antioxidant power will also decrease. Changes in thiol/disulphide homeostasis have been associated with various diseases, such as diabetes mellitus, cancer, chronic kidney disease and liver disorders.[17,18] A coagulation disorder or the dysfunction in the autonomic nervous system can cause emboli or vasospasm in vasa nervorum of the facial nerve with resultant Bell's palsy.[19,20] Omori *et al.*[19] found high thrombin–antithrombin-3 and α-plasmin inhibitor–plasmin complex levels in patients with Bell's palsy and suggested that this could be an evidence for the coagulation disorder and circulatory impairment in the etiopathogenesis of the disease. Recently, Kum *et al.*[21] investigated the relationship between neutrophil-to-lymphocyte ratio (NLR) in Bell's palsy and found NLR to be a significant marker supporting the presence of inflammation. Similarly, Bucak *et al.*[22] found NLR to be a useful and novel serum marker for predicting the patient prognosis. In our study, as seen in Table 1, the serum native and total thiol levels were significantly lower and the disulphide levels were higher in patients with Bell's palsy than those of healthy controls. This situation indicates that the balance has shifted to the oxidative side. The increase in the disulphide/total thiol and disulphide/native thiol ratios (-S-S-/(-SH+-S-S-) and (-S-S-/-SH)) and the decrease in the native thiol/total thiol ratio (SH/(−-SH+-S-S-)) show that the thiol/disulphide redox balance system shifted to the side of disulphide bond formation. These results may support a role of thiol/disulphide homeostasis in inflammation of facial nerve in Bell's palsy patients.

MRI with gadolinium enhancement may be obtained in patients with Bell's palsy, although neural enhancement is not pathognomonic for the disease since facial neural enhancement can also be seen in healthy individuals.[23] The normal enhancement of facial nerve in Bell's palsy can be explained by breakdown of the blood–nerve barrier. However, marked enhancement of the facial nerve in the facial canal on contrast-enhanced MR images is characteristic of peripheral facial nerve palsy.[24] According to Sartoretti-Schefer *et al.*,[25] the intensity of contrast enhancement did not correspond to the severity, duration or course of the facial nerve palsy. In our study, we did not find a significant role of thiol/disulphide homeostasis between FPWOE and FPWE. These findings may indicate that the intensity of the enhancement was obviously independent of the severity of nerve damage. Therefore, no relationship could be established between the intensity of the enhancement and oxidative stress.

House-Brackmann grading is frequently used to determine the severity and the prognosis of Bell's palsy.[26] Some authors found a positive correlation between NLR values and the grade.[21,22,27] In our study, HB grading did not show any significant correlation with any of the parameters studied (Fig. 1). Our result suggests that the severity of symptoms did not have a relationship with oxidative stress.

Our study has several limitations, which should be addressed in the further studies. Although the thiol/disulphide homeostasis seems altered in Bell's palsy, it is still unclear whether there is a causal relationship in between. There is also need for a longitudinal follow-up to determine whether alterations of thiol/disulphide homeostasis may have predictive value in Bell's palsy.

Our results are in accordance with the previous studies regarding impact of oxidative damage in Bell's palsy.[22,27] According to our results, native thiol, total thiol and disulphide levels are specific markers of Bell's palsy when used separately. However, the increase in total and native thiol levels may be protective from Bell's palsy, and the increase in disulphide levels increases the risk of Bell's palsy. In addition, according to binary regression analysis, the model considers total thiol and native thiol as significant variables in Bell's palsy ($P = 0.000$ for both). In our study, it was found that the cut-off value of disulphide level was 18.95, with a sensitivity and specificity of 75.3% and 71.1%, respectively. This finding may be critical as it may indicate that Bell's palsy is induced by an oxidative stress which is associated with disulphide levels. That is, an aggressive therapeutic approach with antioxidants may be needed in the treatment of Bell's palsy. This contention is supported by a previous study that showed the role of nitric oxide in HSV-1-related facial nerve paralysis in mice as well as prevention of facial paralysis by edaravone, a free radical scavenger.[28] The thiol/disulphide homeostasis seems impaired and may be used as an oxidative metabolism marker in Bell's palsy.

Conclusions

Thiol concentrations were independently associated with major thiol/disulphide homeostasis, suggesting that thiol/disulphide homeostasis may be involved in inflammation and oxidative stress. The thiol/disulphide balance has shifted to the oxidative stress in the patients with Bell's palsy.

Keypoints

- The labyrinthine segment of the facial nerve may be more vulnerable to oxidative stress.
- Thiol/disulphide homeostasis may play a critical role in inflammation of facial nerve in Bell's palsy.
- The intensity of contrast enhancement did not correspond to the severity of oxidative stress.

Conflict of interest

None of the authors has any conflict of interest, financial or otherwise.

References

1 Murakami S., Mizobuchi M., Nakashiro Y. *et al.* (1996) Bell palsy and herpes simplex virus: identification of viral DNA in endoneurial fluid and muscle. *Ann. Intern. Med.* **124**, 27–30
2 Iritani H., Nishimura Y. & Minatogawa T. (1991) Neurophysiology of ischemic facial nerve paralysis in an animal model. *Acta Otolaryngol.* **111**, 934–942
3 Halliwell B. & Gutteridge J.M.C. (1984) Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet* **1**, 1396–1397
4 Kayalı R. & Çakatay U. (2004) Protein oksidasyonunun ana mekanizmaları. *Cerrahpaşa J. Med.* **35**, 83–89
5 Sen C.K. & Packer L. (2000) Thiol homeostasis and supplements in physical exercise. *Am. J. Clin. Nutr.* **72**(Suppl. 2), 653–669
6 Jones D.P. & Liang Y. (2009) Measuring the poise of thiol/disulfide couples in vivo. *Free Radic. Biol. Med.* **47**, 1329–1338
7 Erel O. & Neselioglu S. (2014) A novel and automated assay for thiol/disulphide homeostasis. *Clin. Biochem.* **47**, 326–332
8 Baugh R.F., Basura G.J., Ishii L.E. *et al.* (2013) Clinical practice guideline: Bell's palsy. *Otolaryngol. Head Neck Surg.* **149**, 1–27
9 Stjernquist-Desatnik A., Skoog E. & Aurelius E. (2006) Detection of herpes simplex and varicella-zoster viruses in patients with Bell's palsy by the polymerase chain reaction technique. *Ann. Otol. Rhinol. Laryngol.* **115**, 306–311
10 Burgess L.P., Yim D.W. & Lepore M.L. (1984) Bell's palsy: the steroid controversy revisited. *Laryngoscope* **94**, 1472–1476
11 Adour K.K. (1982) Current concepts in neurology: diagnosis and management of facial paralysis. *N. Engl. J. Med.* **307**, 348–351

12 Xu J., Lupu F. & Esmon C.T. (2010) Inflammation, innate immunity and blood coagulation. *Hamostaseologie* **30**, 5
13 Kaneto H., Katakami N., Matsuhisa M. *et al.* (2010) Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. *Mediators Inflamm.* **2010**, 453892
14 Candore G., Bulati M., Caruso C. *et al.* (2010) Inflammation, cytokines, immune response, apolipoprotein E, cholesterol, and oxidative stress in Alzheimer disease: therapeutic implications. *Rejuvenation Res.* **13**, 301–313
15 Circu M.L. & Aw T.Y. (2010) Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic. Biol. Med.* **48**, 749–762
16 Biswas S., Chida A.S. & Rahman I. (2006) Redox modifications of protein–thiols: emerging roles in cell signaling. *Biochem. Pharmacol.* **71**, 551–564
17 Prabhu A., Sarcar B., Kahali S. *et al.* (2014) Cysteine catabolism: a novel metabolic pathway contributing to glioblastoma growth. *Cancer Res.* **4**, 787–796
18 Rodrigues S.D., Batista G.B., Ingberman M. *et al.* (2012) Plasma cysteine/cystine reduction potential correlates with plasma creatinine levels in chronic kidney disease. *Blood Purif.* **34**, 231–237
19 Omori H. & Ikeda M. (1994) Intravascular hypercoagulability in patients with recent Bell's palsy. *Eur. Arch. Otorhinolaryngol.* **251**, 278–282
20 Fisch U. & Esslen E. (1972) Total intratemporal exposure of the facial nerve. Pathologic findings in Bell's palsy. *Arch. Otolaryngol.* **95**, 335–341
21 Kum R.O., Yurtsever Kum N., Ozcan M. *et al.* (2015) Elevated neutrophil to lymphocyte ratio in Bell's palsy and its correlation with facial nerve enhancement on MRI. *Otolaryngol. Head Neck Surg.* **152**, 130–135
22 Bucak A., Ulu S., Oruc S. *et al.* (2014) Neutrophil-to-lymphocyte ratio as a novel-potential marker for predicting prognosis of Bell palsy. *Laryngoscope* **124**, 1678–1681
23 Hong H.S., Yi B.H., Cha J.G. *et al.* (2010) Enhancement pattern of the normal facial nerve at 3.0 T temporal MRI. *Br. J. Radiol.* **83**, 118–121
24 Kinoshita T., Ishii K., Okitsu T. *et al.* (2001) Facial nerve palsy: evaluation by contrast-enhanced MR imaging. *Clin. Radiol.* **56**, 926–932
25 Sartoretti-Schefer S., Brändle P., Wichmann W. *et al.* (1996) Intensity of MR contrast enhancement does not correspond to clinical and electroneurographic findings in acute inflammatory facial nerve palsy. *AJNR Am. J. Neuroradiol.* **17**, 1229–1236
26 Kang T.S., Vrabec J.T., Giddings N. *et al.* (2002) Facial nerve grading systems (1985–2002): beyond the House-Brackmann scale. *Otol. Neurotol.* **23**, 767–771
27 Ozler G.S. & Gunak G. (2014) Neutrophil-lymphocyte ratio: a new predictive and prognostic factor in patients with Bell palsy. *J. Craniofac. Surg.* **25**, 944–945
28 Hato N., Kohno H., Yamada H. *et al.* (2013) Role of nitric oxide in the onset of facial nerve palsy by HSV-1 infection. *JAMA Otolaryngol. Head Neck Surg.* **139**, 1339–1342