

**OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN PATIENTS WITH BELL'S PALSY**

## OKSIDATIVNI STRES I ANTIOKSIDANTNI STATUS KOD OBOLELIH OD BELOVE PARALIZE

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**Summary**

**Background:** Bell's palsy (BP) is the most common acute mononeuropathy of unilateral facial paralysis. Immune, infective and ischaemic mechanisms are potential contributors to the development of BP, but the precise cause remains unclear. Recently, oxidative stress has been proposed as a risk factor of various idiopathic diseases. The aim of this study was to investigate the possible role of oxidative stress in patients with BP.

**Methods:** Thirty-two patients with BP and 30 healthy controls were included in this study. Serum total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were measured by the Erel method.

**Results:** Serum TOS activities and OSI values were significantly higher in patients with BP compared with the control group ( $P < 0.003$  for all comparisons), whereas there was no significant difference between the groups in terms of TAS levels ( $P > 0.05$ ).

**Conclusions:** The data suggest that oxidative stress is increased in BP. These results of high oxidative stress in patients with BP may be helpful to clarify the etiopathogenesis of BP and contribute to improvement in the management or prevention of the disease.

**Keywords:** Bell's palsy, total oxidant status, total antioxidant status, oxidative stress

**Kratak sadržaj**

**Uvod:** Belova paraliza (BP) najčešća je akutna mononeuropatija unilateralne facijalne paralize. Razvoju BP potencijalno doprinose imunski, infektivni i ishemijski mehanizmi, ali precizni uzrok ostaje nepoznat. Oksidativni stres nedavno je predložen kao faktor rizika za razna idiopatska oboljenja. Cilj ove studije bio je da se istraži potencijalna uloga oksidativnog stresa kod obolelih od BP.

**Metode:** U ovu studiju uključeno je 32 obolelih od BP i 30 zdravih kontrolnih ispitanika. Ukupni oksidantni status (TOS) u serumu, ukupni antioksidantni status (TAS) i indeks oksidativnog stresa (OSI) mereni su Erelovom metodom.

**Rezultati:** Aktivnosti TOS u serumu i vrednosti OSI bile su značajno povišene kod obolelih od BP u poređenju s kontrolnom grupom ( $P < 0,003$  za sva poređenja), dok u pogledu nivoa TAS nisu uočene značajne razlike ( $P > 0,05$ ) između grupa.

**Zaključak:** Podaci pokazuju da je oksidativni stres povišen u BP. Dobijeni rezultati o povišenom oksidativnom stresu kod obolelih od BP mogu pomoći da se rasvetli etiopatogeneza BP i doprineti boljem menadžmentu ili prevenciji ove bolesti.

**Ključne reči:** Belova paraliza, ukupan oksidantni status, ukupan antioksidantni status, oksidativni stres

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## Introduction

Bell's Palsy (BP), named after the Scottish anatomist and surgeon Sir Charles Bell who first described it, is a disease that occurs as a result of seventh cranial nerve (facial nerve) dysfunction. It is characterized by facial paralysis, leading to an inability to control the facial muscles on the affected side without a known cause (1), and is the most common acute mononeuropathy, accounting for approximately 60%–75% of all cases of unilateral facial paralysis (2, 3). The incidence of BP ranges from 11.5 to 40.2 in 100,000 cases. The exact aetiology and pathogenesis of the disease is still debated, although it is thought to occur as a result of vascular ischemia, autoimmunity or a viral infection of the nerve sheath (3, 4). Among these, the most commonly accepted aetiology is that of viral infection. Although direct proof has not yet been obtained, increasing evidence suggests that latent herpes virus (herpes simplex virus (HSV) type 1 and herpes zoster virus) reactivation in the geniculate ganglion causes this paralysis. Additionally, isolation of herpes virus DNA from the facial nerve through sensitive polymerase chain reaction techniques during acute paralysis supports this hypothesis (5–7). Nevertheless, the reasons for the activation of latent viruses remain to be elucidated (3, 8).

Oxidative stress (OS) is a condition associated with excess production of reactive oxygen species arising through various oxidation pathways and a disruption of the oxidant-antioxidant balance due to a decrease in total antioxidant capacity. Further, it is thought to play an important role in several inflammatory diseases and the antioxidant system is considered to be related to immunity (9–12).

To date, there has been no report investigating OS in BP. Therefore, the present study aims to identify the role of OS in patients with BP by measuring the levels of total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI).

## Materials and Methods

### *Patient selection*

A total of 32 patients diagnosed with acute facial palsy (15 males and 17 females) between January 2014 and March 2016 were included in the study. Patients were subjected to a detailed ear nose throat, neurological and systemic examination. Radiological imaging was performed when necessary in order to identify the possible aetiology of facial paralysis; patients identified with a cause were excluded. Additionally, patients with chronic diseases or malignancies, those administered long-term medication, and smokers were excluded from the study.

Age- and sex-matched healthy subjects (n=30) who showed no signs of any systemic disease were

included in the study as the control group. The subjects in the control group were asymptomatic with an unremarkable medical history and a normal physical examination. None of the control subjects were taking antioxidant vitamin supplements.

The study was approved by the local ethics committee of the authors' institution (Protocol No: 2016-50) and was performed according to the principles of the Declaration of Helsinki. Written consent was obtained from all patients before inclusion in the study.

### *Blood sample collection*

After overnight fasting, peripheral venous blood samples were taken from patients and controls. Following coagulation, samples were immediately separated from the cells by centrifugation at 3000 g for 10 minutes, and then stored at 80 °C until determination of oxidative status via measurement of TOS and TAS levels through photometric methods (Architect C1600, Abbott Laboratories, Abbott Park, Chicago, Illinois, USA).

### *Analysis of blood samples*

*Measurement of TOS.* The TOS of serum was determined by using a novel automated method (13). Oxidants present in the sample oxidize the ferrous iono-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. Ferric ion reacts with xylenol orange in an acidic medium to produce a coloured complex. The intensity of the colour, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) equivalents per litre (µmol H<sub>2</sub>O<sub>2</sub> equiv/L).

*Measurement of TAS.* The TAS of serum was determined using an automated analyser (Architect C1600) (14). Briefly, potent free-radical reactions were initiated through the production of a hydroxyl radical via the Fenton reaction, and the reaction rates were monitored by following the absorbance of coloured dianisidyl radicals. Using this method, the antioxidative effect of the sample against potent free-radical reactions, which were initiated by the synthesized hydroxyl radical, was measured. Both intra- and inter-assay coefficients of variation were lower than 3%. Data were expressed as mmol trolox equiv/L.

### *Measurement of OSI*

OSI was calculated by dividing the TOS by the TAS. Data were expressed in »arbitrary units« (AU) and calculated using the following formula (13):

OSI (AU) = TOS ( $\mu\text{mol H}_2\text{O}_2$  equiv/L)/TAS (mmol trolox equiv/L).

### Statistical analysis

Data were analysed with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Distributions of the groups were analysed using the one-sample Kolmogorov–Smirnov test. Additionally, the parametric variables were compared using the Student *t*-test, whereas the non-parametric continuous variables were compared with the Mann–Whitney U test. A *P* value of less than 0.05 was regarded as statistically significant.

### Results

Thirty-two patients with BP and 30 healthy subjects were included in the study. There were 15 males (46.8%) and 17 females (53.2%) in the patient group and 15 females (50%) and 15 males (50%) in the control group. Mean age was  $42 \pm 8$  years (14–68 years) in the patient group and  $43 \pm 7$  (15–70 years) in the control group. The groups were statistically similar in terms of age and sex ( $P > 0.05$ ).

TOS and OSI levels were detected to be significantly higher in the patient group compared to the control group ( $P < 0.05$ ). However, there was no significant difference for TAS ( $P > 0.05$ ). A comparison of TAS, TOS and OSI data of the groups is presented in *Table I*.

### Discussion

The present study evaluates the relationship between OS and BP. The most common form of facial palsy is idiopathic facial palsy, also known as BP. The precise cause of BP remains unclear (2, 3), although autoimmunity, viral infective and ischaemic mechanisms are believed to play an important role in this disease (4, 5). Of these, the viral theory has been the most widely accepted. McCormick (6), who firstly claimed the viral aetiology theory for BP, argued that there was an acute inflammatory neuropathy at the

facial nerve in the presence of HSV in the geniculate ganglion. Later, in 1996, Murakami et al. (5) isolated the HSV genome from the endoneurial fluid of the facial nerve in BP, supporting the theory of a viral aetiology. The activation of the latent herpes virus under certain circumstances in the geniculate ganglion was considered to cause paralysis. However, the reason for the reactivation of the virus in the latent state remains to be elucidated.

OS represents an imbalance between reactive oxygen species and the antioxidant defence capacity of the body. Excess levels of free radicals or decreased antioxidant capacity may lead to OS. Previous studies have shown that OS leads to DNA and endothelial damage, and it has been reported as the underlying reason for various diseases (6, 7).

The various oxidant and antioxidant molecules in blood serum can be detected through different methods using several laboratory kits, with their individual measurement being impractical, time consuming and labour intensive, and requiring complicated techniques. Herein, instead of measuring the levels of the various oxidant and antioxidant molecules separately, TOS and TAS were measured through a method recently developed by Erel (13, 14).

To the best of our knowledge, this is the first study evaluating OS in BP. The results showed that OSI and TOS levels were higher in patients with BP compared with healthy subjects. However, there was no significant difference in terms of TAS levels between the groups. OSI represents the ratio of TOS to TAS, leading to a more objective evaluation than the measurement of OS. The widely accepted theory regarding the etiopathogenesis of BP is that compression of the facial nerve occurs due to oedema in the facial nerve canal as a result of an acute inflammatory neuropathy. This neuropathy is suspected to occur due to activation of latent HVS viral infection or an autoimmune reaction. However, the exact events leading to this inflammation remain unknown (3, 5, 8, 15). It is likely that the increased OS levels observed herein may play a role in the development of BP. However, since there are no previous studies assessing OS in BP the results could not be com-

**Table I** Total antioxidant status (TAS), total oxidant status (TOS), and the oxidative stress index (OSI) of patients with BP and control subjects.

	Patient Group (n=32)	Control Group (n=30)	<i>P</i>
TAS ( $\mu\text{mol Trolox Eq/L}$ )	$1.4 \pm 0.2$ (1.1–1.8)	$1.8 \pm 0.2$ (1–2.3)	0.713
TOS ( $\mu\text{mol H}_2\text{O}_2$ Eq/L)	$17.2 \pm 14.2$ (3.2–52.6)	$8.4 \pm 5.9$ (2.3–24)	0.003*
OSI (arbitrary unit)	$12.2 \pm 9.4$	$4.6 \pm 3.2$	0.002**

\* Statistically significant

pared. Nevertheless, it is well known that BP is more common in patients with diabetes mellitus and pregnant women, especially those in the third trimester (16, 17). Increased TOS and OSI levels and defects of antioxidant protection have been shown in studies investigating the OS status in patients with diabetes mellitus (18, 19). Similarly, increased OS and decreased TAS were reported during pregnancy in studies evaluating OSI (20). Additionally, Capaccio et al. (10) assessed OSI in patients with idiopathic sudden sensorineural hearing loss, which is a similar disease to BP in terms of etiopathogenesis and treatment, and reported a significantly higher OSI in cases compared with controls. Considering these results, the higher incidence of both OS and BP in diabetes mellitus patients and pregnant women supports the link between high OS status and BP determined in the patient group herein.

There are some limitations to the present study. Firstly, since it was the first study evaluating OS levels in cases with BP, it was not possible to compare the

present results with those of previous reports. Additionally, it would be better to group patients according to the stage of disease, but this could not be done due to the lack of a sufficiently high number of patients. Finally, the OS status of patients before and after treatment and the correlation between treatment responses were not analysed.

## Conclusion

The data presented herein suggests that OS is increased in BP and may thus help to clarify the etiopathogenesis of the disease. Future studies investigating the origin of OS in BP patients with a larger patient population are warranted.

## Conflict of interest statement

The authors stated that they have no conflicts of interest.

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