Oxidative Stress Balance as a diagnostic marker in Stroke

Mina Rahmati[1], Naser Mobarra[1, 2]✱, Hossein Ghannadan[3]

1. Metabolic Disorders Research Center, Department of Biochemistry, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
2. Department of Clinical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3 Department of Neurology, Golestan University of Medical Sciences, Gorgan, Iran.

Abstract

Background and objectives: Ischemic stroke (IS) is a debilitating disorder with no reliable prognostic or diagnostic biomarker. Here, we evaluated the serum level of Oxidative Stress Balance and assessed their diagnostic and prognostic value for IS.

Methods: Sera from 52 patients with IS and 52 age- and sex-matched healthy subjects were collected. All patients were subjected to sample collection at the time of admission, 24 and 48 hours later, at the time of discharge and three months later .Oxidative Stress Balance expression was assessed by ELISA. Statistical measures for diagnostic accuracy of quantitative measures were conducted.

Results: We demonstrated Oxidative Stress Balance was elevated at the time of admission in comparison to normal subjects. ROC curve analyse revealed that Oxidative Stress Balance (AUC = 0.7337; P<0.0001) was acceptable diagnostic value to discriminate IS patients from normal subjects. Kaplan-Meier survival analyse shown that Oxidative Stress Balance(P=0.8584) had no prognostic value.

Conclusion: Oxidative Stress Balance could be introduced as suggested biomarker to segregate IS patients from normal subjects.

Keywords: Biomarker; Ischemic; Oxidative Stress Balance; Stroke

Introduction

Stroke is one of the leading causes of adult disability [1].Among all strokes, 87% are ischemic and 13% are hemorrhagic strokes [2]. Ischemic stroke is the result of interrupted blood flow within the area of an occluded blood vessel and hemorrhagic stroke is either a brain burst or a leak of weakened blood vessels [3]. Blood biomarkers have been increasingly paid attention as a complementary diagnostic tool for the neuroimaging study of the stroke. Neuronal, astroglial, inflammatory, and hemostatic markers as unique marker are investigated [4].Reactive oxygen species (ROS) play a major role in damaging the brain after traumatic brain injury, such as stroke. Oxidants also interfere with production of mitochondrial signals and action of enzymes involved in DNA repair [5]. Findings have shown in the normal state 2 to 5% of the electron transfer chain present in mitochondria produce, anionic free radicals (O2–) and hydrogen peroxide (H2O2), but this dangerous compounds removed by antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. In injuries such as stroke, these antioxidant defense enzymes are unable to function properly due to the oxidants overproduction by cytosolic and mitochondrial enzymes. Finally, brain cells greatly damaged due to impaired antioxidant protective action and the inability to regenerate antioxidants [6]. Oxidative stress results from an imbalance between the presence of oxidants (free radicals, RNS, and ROS) and antioxidants which potentially leading to injury such as stroke [7]. Consequently, given the importance of oxidative stress balance, it can be assess as a risk factor for stroke. In this study we want to find these effects in the stroke patients.

Material and methods

The study was carried out on 52 patients with documented Ischemic Stroke (IS) events in the Sayyad Shirazi Hospital in Gorgan, Iran. Furthermore, the patients who had cancer, unstable angina, recent or old myocardial infarction (MI), acute infection or any acute illness were also excluded. Demographic and clinical information's were obtained by direct interviewing with patients or their family. Each subject gave their informed written consent to participate in the study, which had been approved by the Gorgan University of Medical Science Ethics Committee (code of ethics: IR.GOUMS.REC.1395.23).Sex- and age-matched healthy control subjects (52 people) who lived in Gorgan, Iran were recruited as the control group.

Blood Sampling

Blood samples were collected in 5 steps, (0-12) hours after stroke, 24 and 48 hours after stroke, discharge time and 3 months later. After being allowed to clot, the blood was centrifuged at 2500 rpm for 15 minutes at room temperature to obtain serum. Afterwards serum was stored at -70°C prior to analysis.

The method of measuring the PAB was mentioned in previous studies (alamdari ,parizadeh). The Standard Solutions were prepared by mixing various proportions (0 - 100%) of 250 uM hydrogen peroxide (30%) (Merck, Darmstadt, Germany)with 3 mM uric acid (in 10 mM NaOH). TMB powder (3,3,5,5'-Tetramethylbenzidine, Fluka, Buchs, Switzerland) (60 mg) was dissolved in 10 mL DMSO. For the preparation of the TMB

cation, 400 uL of the TMB/DMSO Solution was added to 20 mL of acetate buffer(pH 4.5), and then 70 uL of fresh chloramine T trihydrate (Applichem: A 433 l, Darmstadt, Germany) (l00 mM) solution was added. The solution was mixed well and incubated for 2h at room temperature in a dark place. Then 25 U of peroxidase enzyme solution(Applichem: 230 U/mg, A379l,0005, Darmstadt, Germany) was added to 20 mL of TMB cation solution, dispensed in 1 mL, and stored at -20°C. In order to prepare the TMB solution, 200 mL of TMB/DMSO was added to 10 mL of acetate buffer (pH5.8) and the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB Solution. This working solution was incubated for 2 min at room temperature in a dark place and immediately used. Ten micro liters of each sample, standard and blank (distilled water), added to each well in a 96 well plate and then added 200 mL of working solution and incubated at 37 °C for 12 min in a dark place. At the end of the incubation time, 100 mL of 2NHCl was added to each well, and the optical density (OD) was measured in an ELISA reader at 450 nm with a reference wavelength of 570 or 620 nm. A standard curve was provided from the values of the standard samples. The values of the PAB are expressed in arbitrary unit (AU), which showed the percentage of hydrogen peroxide in the standard solution. Afterwards, the values of the unknown samples were calculated based on the Values obtained from the Standard curve.

Statistical analyses

ROC curve analysis was conducted to evaluate the diagnostic utility of each variable. Pearson and Spearman correlation studies were applied to correlate quantitative variables. Linear regression was used to showing relationship between dependent variable and independent variables. To compare the means between more than two groups we used Two-way ANOVA or Kruskal Wallis and post hoc tests. For evaluate the prognostic value for different time after stroke after following the outcome of the disease (death or survival), Kaplen-Meier survival test was used. We used Graph Pad and SPSS software for Windows, Version 16.0, Chicago, USA) for statically analysis.

RESULT

The serum level of Oxidative Stress Balance

Serum Oxidative Stress Balance in the IS patients collected at the time of admission was higher than the normal controls ($P < 0.0001$) (Figure 1). Serum levels of Oxidative Stress Balance has not significantly different between the time of admission and 3 months after the incident stroke (Figure 2).
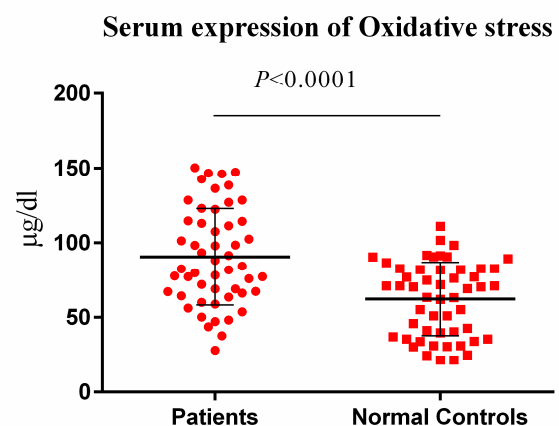
Figure1: The serum levels of Oxidative Stress Balance at the time of admission; Oxidative Stress Balance is up regulated in IS patient's .To compare the means between two groups, students' T-test was used (Patients: 52, Healthy subjects: 52). Statistics on each scattered plot demonstrates

Mean±SD. Level of significant P-values were 0.05.

Serum expression of Oxidative stress
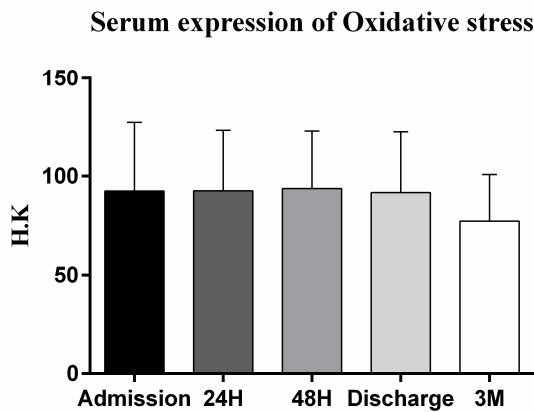
Figure 2: The serum levels of Oxidative Stress Balance in 5 different time points; Oxidative Stress Balance serum levels did not change significantly in all 5 time-points among IS patients. One-Way ANOVA and Tukey's post-test or Kruskal-Wallis and Dunn-Bonferroni post-test were used to evaluate the differences between the means of various groups. (Patients: 52, Healthy subjects: 52). Bar charts show Mean±SD for each value. Level of significant P-values were 0.05.

Diagnostic value of Oxidative Stress Balance

The area under the curve (AUC) for serum Oxidative Stress Balance was 0.73(P=<0.0001) with 67.31% Sensitivity (95% CI: 52.89 to 79.67%) and 63.46% Specificity (95%CI: 48.96% to 76.38%).The cut-off point was set at the fold change level of 76.97 and likelihood ratio (LR) of 1.842 (Figure 3).

ROC of Oxidative stress
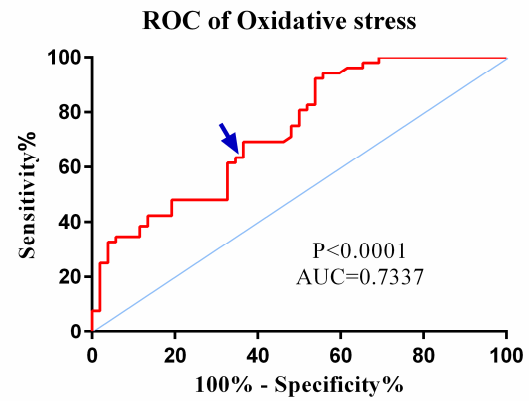
Figure 3: ROC curve analyses; area under the curve (AUC) for Oxidative Stress Balance was 0.73(P= <0.0001) with 67.31% Sensitivity (95% CI: 52.89 to 79.67%) and 63.46% Specificity (95%CI: 48.96% to 76.38%).The cut-off point was set at the fold change level of 76.97 and likelihood ratio (LR) of 1.842. All values are the expression levels at the time of zero (initial referring to the hospital).

The prognostic value of Oxidative Stress Balance

Subjects were divided into groups based on the serum concentration of biomarker, in to two categories of high and low levels based on the optimum cut-off points derived from ROC curve analyses. Log-rank test result revealed that Oxidative Stress Balance (P = 0.85) were not capable of predicting the outcomes of IS (Figure 4).

Survival of oxidative stress
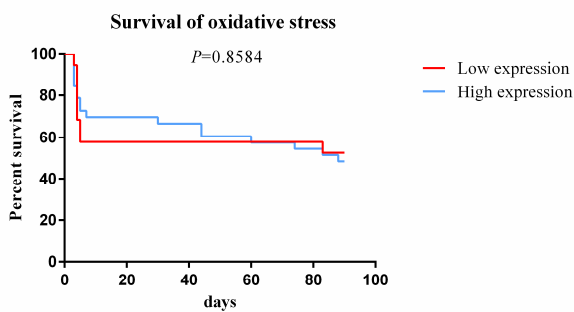
Figure 4: Survival analyses; IS patients were subdivided into two categories of high and low expression on the basis of the optimum cut-off points of suggested biomarkers. Log-rank test results showed that Oxidative Stress Balance was not able to predict the outcome (survival) of the disease after 90 days of follow up.

Discussion

ROS played a role in brain injuries such as ischemic stroke, and the occurrence and outcome of vessel occlusion initiate a defective cycle that results in excessive ROS production and neuronal injury [8]. Serum Oxidative Stress Balance was higher in stroke patients than in the control group and we also did not found significant relationship between the time of admission and 3 months after stroke. According to the ROC curve analyses and calculated AUC, Oxidative Stress Balance would be a poor diagnostic marker. The result of Kaplan-Meier analysis was not significant and serum Oxidative Stress Balance could not be used as a prognostic marker. There have been previous reports that Oxidative Stress Balance is increased in the blood of stroke patients compared to controls [9, 10]. Previous studies have identified that

Oxidative Stress cannot be a prognostic marker [7], just like our findings.

CONCLUSION

The serum levels of Oxidative Stress Balance were higher in stroke patients. ROC analysis revealed that serum Oxidative Stress Balance was a poor biomarker for IS. According to the importance of Oxidative Stress Balance and consideration to previous works, our study showed that Oxidative Stress Balance may not be suitable diagnostic and prognostic markers for IS.

Declarations

Conflict of interest

None

Authors' contributions

All authors contributed equally to this work.

References

1. He J, Klag MJ, Wu Z, Whelton PK (1995) Stroke in the People's Republic of China. I. Geographic variations in incidence and risk factors. Stroke 26 (12):2222-2227

2. Shirley R, Ord EN, Work LM (2014) Oxidative Stress and the Use of Antioxidants in Stroke. Antioxidants (Basel, Switzerland) 3 (3):472-501. doi:10.3390/antiox3030472

3. Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, Levine SR, Mosca L, Sacco RL, Sherman DG, Wolf PA, del Zoppo GJ (2001) Primary prevention of ischemic stroke: A statement for healthcare professionals from the Stroke Council of the American Heart Association. Stroke 32 (1):280-299

4. Vijayan M, Reddy PH (2016) Peripheral biomarkers of stroke: Focus on circulatory microRNAs. Biochimica et biophysica acta 1862 (10):1984-1993. doi:10.1016/j.bbadis.2016.08.003

5. Cherubini A, Polidori MC, Bregnocchi M, Pezzuto S, Cecchetti R, Ingegni T, di Iorio A, Senin U, Mecocci P (2000) Antioxidant profile and early outcome in stroke patients. Stroke 31 (10):2295-2300

6. Aygul R, Demircan B, Erdem F, Ulvi H, Yildirim A, Demirbas F (2005) Plasma values of oxidants and antioxidants in acute brain hemorrhage: role of free radicals in the development of brain injury. Biological trace element research 108 (1-3):43-52. doi:10.1385/bter:108:1-3:043

7. Parizadeh MR, Azarpazhooh MR, Mobarra N, Nematy M, Alamdari DH, Tavalaie S, Sahebkar A, Hassankhani B, Ferns G, Ghayour-Mobarhan M (2011) Prooxidant-antioxidant balance in stroke patients and 6-month prognosis. Clinical laboratory 57 (3-4):183-191

8. Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, Feuerhake W (2013) Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS & neurological disorders drug targets 12 (5):698-714. doi:10.2174/1871527311312050015

9. Jena I, Nayak SR, Behera S, Singh B, Ray S, Jena D, Singh S, Sahoo SK (2017) Evaluation of ischemia-modified albumin, oxidative stress, and antioxidant status in acute ischemic stroke patients. Journal of natural science, biology, and medicine 8 (1):110-113. doi:10.4103/0976-9668.198346

10. Shoeibi A, Razmi N, Ghabeli Juibary A, Hashemy SI (2017) The Evaluation and Comparison of Oxidative Stress in Hemorrhagic and Ischemic Stroke %J Caspian Journal of Neurological Sciences. 3 (11):206-213. doi:10.29252/nirp.cjns.3.11.206