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Serum Pro-Oxidant-Antioxidant Balance, Advanced Oxidized Protein Products (AOPP) and Protein Carbonyl in Patients With Stroke

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Abstract

Background: Stroke is the second cause of death and disability in the world, and oxidative stress which could be considered as a prognostic factor in patients for prevention, follow up and determination of stroke's severity has a conceivable role in neural damage.
Objectives: The aim of our study was to explain the association between oxidant/antioxidant imbalance as a prognostic factor in patients with stroke.
Patients and Methods: Pro-oxidant-antioxidant balance (PAB), advanced oxidized protein products (AOPP) and protein carbonyl were measured in patients with ischemic stroke and hemorrhagic stroke as well as healthy group. Sera were collected from 18 ischemic and 23 hemorrhagic stroke patients, and 34 healthy volunteers. The PAB assay, routine biochemical parameters, lipid profile and total protein concentration were assessed in patients and healthy group.
Results: The level of AOPP ($P < 0.007$), protein carbonyl ($P < 0.001$) and PAB ($P < 0.01$) were significantly higher in stroke patients than the healthy group.
Conclusions: There are increased level of protein carbonyl, AOPP and PAB in patients with stroke. Measuring these factors may be beneficial as prognostic factors in treatment and follow up of the patients with both hemorrhagic and ischemic strokes. More investigations with more cases and longer follow up are needed to approve that these factors play an important role in prognosis of stroke.

Keywords: Hemorrhagic Strokes, Ischemic Strokes, Oxidative Stress, Protein Carbonyl, Prognostic Factor, PAB Assay, Biochemical Parameters, Lipid Profile, Total Protein Concentration

1. Background

Stroke is the fourth leading cause of death in the US, that is a person dies every four minutes (1, 2). Stroke is a medical emergency in which the leg, arm and facial paralysis along with trouble in walking, speaking and understanding are evident as the major symptoms (3, 4). There are two main kinds of stroke with different causes. Ischemic strokes are the most common type (85% of cases) that occurs when the brain circulation is reduced by blocked arteries or plaques (5). The hemorrhagic stroke happens when a blood vessel in the middle or near surface of the brain leaks blood leading to a pressure on a special part of the brain (6, 7). The pressure of aggregated blood in the space between brain and skull (8) and oxidative stress (5, 9) may be other reason for hemorrhagic stroke (10). Recently researchers have focused on determination of oxidative biomarkers in stroke patients during acute phase and recovery (11, 12).

Studies in the field of oxidative stress and clinical implications revealed that generation of free radicals prompting oxidative stress plays a role in pathogenesis of the brain injuries (13). In normal condition, oxidants and antioxidants are in a balance status (14, 15). Several sources of free radicals generation are respiratory chain, inflammatory cells, xanthine oxidase, cyclooxygenase, and mitochondria (16). Oxidative stress is an imbalance between pro-oxidants and antioxidants in favor of pro-oxidants (15, 17, 18). Oxidative stress damages cellular compartments such as endoplasmic reticulum (19), mitochondria (20) and also cellular components including: proteins, lipids and DNA, initiating the signaling path-

ways to start the cellular apoptosis (18, 21). Cancer (17, 22), sickle cell anemia (23), diabetes mellitus (24), atherosclerosis (25), Alzheimer's disease (26), heart failure (HF) (27), cardiovascular disease (CVD) (28), autism (29) and stroke (10) are some diseases that oxidative stress is involved in their pathogenesis and development (30).

Reactive oxygen species (ROS) during normal and/or abnormal conditions modify the amino acids, peptides and proteins. They produce substances that are known as advanced oxidized protein products (AOPP) (31). Oxidants produce the α-ketoacyl derivative by the cleavage of proteins and/or peptides and blocking the N-terminal amino acid (31, 32). The existence of protein carbonyl can be considered as a marker of protein oxidation, and several methods have been developed to measure it (33). Several studies show that oxidative stress and aging are in connection with protein oxidation (12, 19, 24).

2. Objectives

In this study, the serum protein carbonyl, PAB and AOPP in patients with stroke (hemorrhagic and ischemic) were measured to substantiate the potency of these parameters as stroke prognostic factors.

3. Patients and Methods

3.1. Subject

This study was conducted on two groups of patients and a healthy group. The patient groups included 18 ischemic and 23 hemorrhagic stroke patients who had been admitted within the first 24 hours after their attack. The patients were hospitalized after the neurologist made diagnosis based on the brain CT scan and clinical examination. Excluding criteria were the consumption of iron, antioxidants like vitamin E and C, immune-suppressive or anti-inflammatory drugs in at least 3 months before the attack. The previous history of any inflammatory disease, cancer, autoimmune disorder, hematological disorder, renal or hepatic disease or cerebrovascular diseases that was confirmed by CT scan was also considered as the exclusion criteria. The healthy group consisted of 34 healthy volunteers who were similar to the patients regarding their age, sex and weight.

3.2. Blood Sampling

Blood samples were taken from all the participants. After clotting, samples were centrifuged for 15 minutes at 2500 rpm at room temperature to obtain the serum. In addition, to measure the oxidative stress parameters before starting treatment, blood samples were collected and the serum was obtained and stored at -80°C until analysis. Hemolytic samples were excluded from the test. This study was confirmed by the ethical committee of Mashhad University of Medical Sciences. Patients declared their informed consent in writing before participating in the project.

3.3. Chemicals

TMB powder (3, 3', 5, 5' - Tetramethylbenzidine, AppliChem), peroxidase from horse radish practical grade (Applichem), chloramine T (Applichem: A4331), advanced oxidation protein products (AOPP) ELISA kit (Applichem), DMSO (Applichem), Uric acid (Applichem) and protein carbonyl kit (Sigma-Aldrich) were utilized. All the reagents were prepared in double distilled water.

3.4. Routine Biochemical Analysis

For each patient, fully fasted lipid profile including triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and total cholesterol, urea and creatinine along with total protein were determined.

3.5. Pro Oxidant-Antioxidant Balance (PAB) Assay

The PAB assay was done according to the previous method (34). For preparation of standard solutions, different proportion (0 -100%) of 250 μM hydrogen peroxide and 3 mM uric acid (in 10 mM NaOH) were mixed. TMB cation was made by mixing 60 mg TMB powder in 10 mL DMSO. Then 400 μL of TMB/DMSO was added to 20 mL of acetate buffer [0.05 M, pH 4.5] and 70 μL of fresh chloramine T (100 mM) solution was added into this 20 mL. In a dark place, the solution was mixed for two minutes and was incubated for two hours in room temperature. Then 25 U of peroxidase enzyme solution was added into 20 mL TMB cation, distributed in 1 mL and was stored at -20°C.

For making TMB solution, 10 mL of acetate buffer [0.05 M, pH 5.8] was mixed by 200 μL of TMB/DMSO. In order to prepare the working solution, 1 mL TMB cation was mixed well with 10 mL of TMB solution and incubated in dark place at room temperature for 2 minutes. This solution had to be used immediately. 200 μL of working solution was added to each well of an ELISA plate. Then 10 μL of serum, blank (distilled water) or standard solution were mixed and incubated for 12 minutes in a dark place at 37°C. After incubation, 50 μL of 2 N HCl was added to each well. The measurement was done by an ELISA reader at 450 nm wavelength. Based on the standard samples' data, the standard curve was prepared. According to the values obtained from the standard curve, the unknown samples were calculated.

3.6. Protein Carbonyl Measurement

In this study, the protein carbonyl concentration was measured using the protein carbonyl kit (Sigma-Aldrich Co, Cat No: MAK094). After the testing process, the absorption was measured for each sample at 375 nm wavelength.

3.7. AOPP Measurement

AOPP assay is based on the method developed by the witch-Sarsat (35). PBS was used to dilute 40 μL sera up to 200 μL. Then, 10 μL potassium iodide reagent and 20 μL acetic acid added to each sample. The absorbance was measured at 340 nm wavelength instantly.

3.8. Biochemical and Clinical Parameters

All biochemical and clinical parameters were tested and analyzed by the normal procedure and routine clinical tests.

3.9. Statistical Analysis

SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The unpaired t-test was used to define group comparisons. Parametric and non-parametric correlations were estimated by using Pearson and Spearman correlation coefficients, respectively. The statistical significance level was set to $P < 0.05$.

4. Results

4.1. Evaluation of PAB Values

Although PAB value in the healthy group was 127.30 (114.83 - 146.14) (HK unit), the corresponding value in the ischemic stroke patients was 158.28(117.5 - 228.44) (HK unit) and in the hemorrhagic stroke patients was 149.12 (103.68 - 195.90) (HK unit). Our results showed a significant increase in PAB values in both hemorrhagic and ischemic stroke patients compared to the controls (P value = 0.01) (Table 1).

4.2. Evaluation of Protein Carbonyl

The protein carbonyl concentration of the healthy group was 128.3 $\pm$ 39.3 (nmol/mg protein) whereas the respective values were 4.9744 $\pm$ 5.26911 (nmol/mg protein) for ischemic patients and 7.9562 $\pm$ 5.97083 (nmol/mg protein) for hemorrhagic patients. These numbers reflected a significant extreme difference between the two groups (P < 0.001).

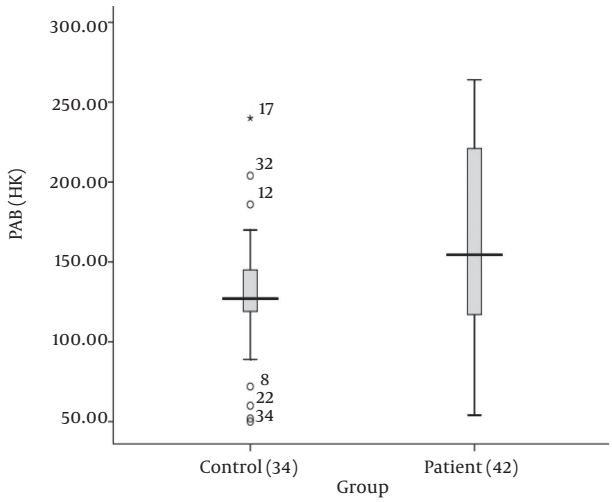

Figure 1. The Comparison of PAB Between Patients and Controls, * Significant

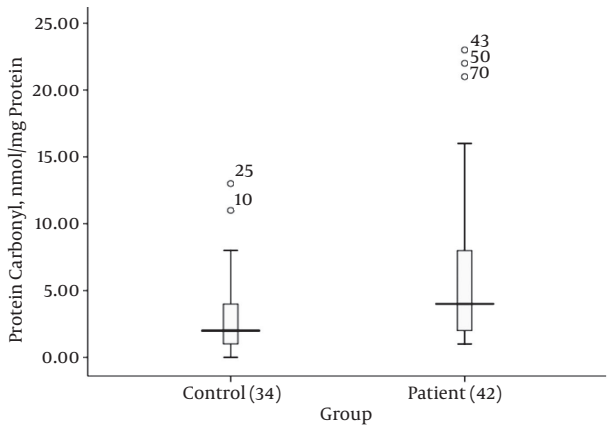

Figure 2. The Comparison of Protein Carbonyl Between Patients and Controls

4.3. Evaluation of AOPP Values

The average amount of AOPP obtained from the healthy group was 15.78 $\pm$ 6.51 (μmol/lit) and it was 20.87 $\pm$ 9.02 (μmol/lit) for the stroke patients. Along with two other oxidative stress biomarkers, AOPP amounts were significantly different between the stroke patients and healthy controls (P value = 0.007).

4.4. Biochemical Factor

Among biochemical factors, urea and blood sugar showed significant difference between patients and healthy group. The result of the high-density lipoprotein cholesterol (HDL-C) (P = 0.792), low-density lipoprotein cholesterol (LDL-C) (P = 0.355) and total cholesterol (P =

Table 1. The Comparison of Oxidative Stress Biomarker

Oxidative Stress Biomarker	Control Group (N = 34)[a]	Patient Group[a]		P Value[b]
		Ischemic, (N = 18)	Hemorrhagic, (N = 23)	
PAB, HK	127.30 (114.83 - 146.14)	158.28 (117.5 - 228.44)	149.12 (103.68 - 195.90)	0.01
Protein carbonyl , nmol/mg protein	128.3 ± 39.3	4.9744 ± 5.26911	7.9562 ± 5.97083	0.001
AOPP, μM	15.78 ± 6.5	22.5889 ± 6.82646	19.9180 ± 10.44	0.007

[a]Values are presented as median (first quarter ± third quarter) or mean ± SD.
[b]P values calculated in (both ischemic and hemorrhagic) patients and controls.

0.147) showed no effective difference between patients and controls (Figure 3).

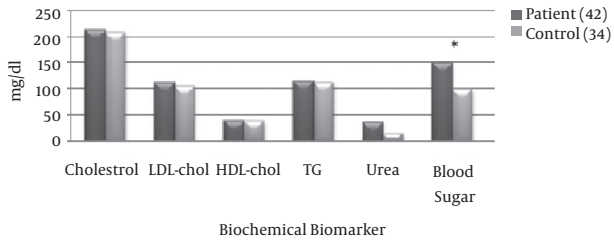

Figure 3. The Comparison of Biochemical Markers in Patient and Controls, * Significant

4.5. Evaluation of the Oxidative Stress Biomarkers and Biochemical Parameters

Although cholesterol showed a positive correlation with AOPP, it had an inverse correlation with PAB and protein carbonyl. Along with cholesterol, the results from triglyceride concentrations revealed an inverse correlation with PAB, AOPP and protein carbonyl. The creatinine level and urea also showed inverse correlations with PAB and AOPP.

5. Discussion

Explanation of oxidative stress profile was the aim of the present study. The previous studies approved that oxidative damage is recognized as a neuronal destructive mechanism in different stages after stroke (36).

When the oxygen uptake of the brain cells is reduced, the stroke occurs. In this situation which is defined as hypoxia, the brain cells loss their normal functionalities and metabolism (37-39). The presence of abundant polyunsaturated fatty acids (40) and lower levels of antioxidant enzymes such as catalase and glutathione peroxidase in brain cells, makes them vulnerable and sensitive to free radicals. The superoxide anion and hydroxyl radicals are generated in the brain and interact with unsaturated fatty acids to produce lipid radicals, lipid peroxides and malondialdehyde (41). Increased oxidative stress products can speed up the clinical complications in patients with stroke (42). Increased production of free radicals changes the oxidant-antioxidant balance in favor of the former. Incomplete removing of free radicals causes the oxidation of lipids, sugars, proteins and nucleic acids which can extend the pathological consequences of stroke (43).

Arslan et al. showed that protein carbonyl and ox-LDL increase thromboangitis obliterans (TAO) in patients in comparison with healthy people (44). Numerous studies have demonstrated that the assessment of lipid peroxidation items can be utilized for the determination of stroke patient status (45, 46). Increased levels of these markers together with MDA are frequently reported in stroke patients (47, 48). This is consistent with the results reported in this study in two groups of patients with stroke, including ischemic and hemorrhagic, AOPP significantly increased compared to the healthy group. Similar to our results, Lehmann et al. reported that AOPP was significantly increased in patients with acute ischemic stroke (42). Pena-Sanchez et al. compared the activities of AOPP in 25 patients suffering from Alzheimer's disease and 30 healthy controls. They observed a significant difference in AOPP levels between control and patient groups (49). Recent investigations on the levels of AOPP in plasma and spinal liquid in amyotrophic sclerosis patients indicated the association of AOPP level with premature sclerosis and nervous tissue damages (50). Kaneda et al. observed that patients with myocardial perfusion problems have higher amounts of AOPP like dialysis patients. AOPP concentration in both groups was significantly different from the values of this product in healthy volunteers (51-52). Cichon et al. demonstrated the increment of protein carbonyl in stroke patients compared to the control group (53). Moon et al. showed in 99 patients with atherosclerotic stroke (71 acute and 28 chronic patients), the amount of protein carbonyl in patients with acute stroke is more than chronic

ones (54).

In this study, three biomarkers of oxidative stress including AOPP, PAB and protein carbonyl were evaluated in the serum of stroke patients and healthy controls. A significant difference between these parameters in patients and healthy controls demonstrated the importance of oxidative stress factors in brain injuries after stroke. The reduction of antioxidant defense mechanisms in stroke is effective in increasing the damages caused by free radicals and other oxidants.

Therefore, the consumption of more antioxidant drugs might be effective in reducing the tissue destructive processes, which more studies are needed. Moreover, these markers may be used as prognostic factors for stroke.

5.1. Conclusion

These studies along with several previous studies showed that the oxidative stress and its metabolites have an important role in tissue damages and brain injuries. Understanding these mechanisms will greatly contribute to control their damages and prevent the tissue destruction. Therefore, there is a need of more studies to understand the details of this disease.

Footnote

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