Ischemia Modified Albumin as a Novel Biochemical Indicator in Peripheral Artery Patients

Mustafa Etli 1, Hasan Basri Savas 2*

1 Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Cardiovascular Surgery, Alanya, Antalya, Turkey
2 Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Medical Biochemistry, Antalya, Turkey

ABSTRACT

Objective: Peripheral artery disease (PAD) is the name given to blocked blood flow in major arteries other than cerebral and coronary circulation. The diagnosis and treatment of PAD is very important because of the high frequency of PAD. In this study, it was aimed to investigate the possible change in next generation oxidative stress-antioxidant status indicators as a biochemical parameter that may be useful in the diagnosis and follow-up of PAD.

Method: Forty healthy controls and 40 male and female patients with peripheral artery disease who applied to the Alanya Alaaddin Keykubat University Research and Training Hospital were enrolled in the study on a voluntary basis. Total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), ischemia modified albumin (IMA) and Thiol balance were studied with the colorimetric method.

Results: The TOS (p <0.001), OSI (p <0.001), IMA (p = 0.027), total thiol (0.004) levels were higher in the peripheral artery patients compared to the control group, while the TAS (p <0.001) and native thiol / total thiol ratio (index 3) (p = 0.018) values were significantly lower.

Conclusion: The increase in TOS and IMA levels may be used as an indicator of peripheral artery disease. There is an increase in inflammation in the ischemia and atherosclerosis processes. An ischemia-specific protein called ischemia-modified albumin may be used as a rapid and practical laboratory marker in common peripheral artery disease.

Keywords: peripheral arterial disease, oxidative stress, antioxidants, atherosclerosis, biomarkers

INTRODUCTION

Peripheral artery disease (PAD) is the name given to the blocking of blood flow in major arteries other than cerebral and coronary circulation. PAD may occur for many reasons such as thromboembolism, vasculitis, and degeneration. However, the most common condition that causes PAD is atherosclerosis. PAD usually occurs in the lower extremities and leads to increased mortality. PAD often exhibits silent picture in its clinical course. Since PAD has silent clinical course, possible laboratory parameters that can provide early diagnosis of the disease are very valuable. PAD follows a clinical course ranging from a simple leg pain to limb loss. Patients often apply to the cardiovascular surgery clinic after repeated polyclinic admissions. Patients may apply to the clinic with severe ischemia findings from simple claudication. In order to diagnose PAD, firstly, Doppler Ultrasonography (USG), contrast-enhanced Computed Tomography (CT), Magnetic Resonance (MR) imaging, angiography, definitive diagnosis are performed by conventional angiography. The frequency of PAD in the population defined as adults reaches 32%. Over the age of 80, the frequency of PAD exceeds 40%. PAD is more common in men. The diagnosis and treatment of PAD are very important due to the high frequency of PAD, leading to increased morbidity and mortality [1-3]. Oxidative stress and antioxidant status are in equilibrium in organisms. Disruption of this balance towards oxidative stress accelerates atherosclerosis. Therefore, increased oxidative stress is likely to cause an increase in the frequency of PAD. Biochemically,
many new generation indicators of oxidative stress and antioxidant status have begun to be measured. When the increase in oxidative stress exceeds the antioxidant capacity, the amount of free oxygen radicals begins to increase. Free oxygen radicals cause various damages. They adversely affect lipid structures, membranes and genetic material. They disrupt the structure of proteins. On the other hand, there are many enzymes showing antioxidant activity. These include superoxide dismutase, glutathione peroxidase, catalase and many similar enzymes. Additionally, antioxidant components that are not in enzyme structure also affect this balance. The total antioxidant status (TAS) and total oxidant status (TOS) are general laboratory parameters that show the overall balance of oxidative stress and antioxidant status very well. Thanks to the oxidative stress index (OSI) calculated on these two parameters, it may be possible to maintain a general idea instead of measuring all the effects of oxidative damage and all components of antioxidant capacity separately [4-15]. The thiol structure contains a sulphydryl group. Thanks to sulphydryl, thiol is very effective in preventing oxidative stress. If there is a significant increase in oxidative stress, thiol is oxidized, and the disulfide bond is formed. If oxidative stress decreases, and antioxidant capacity increases, disulfide bonds can turn back into the thiol structure. This cycle is called the dynamic thiol balance. Dynamic thiol balance is disrupted at the end of the increase in oxidative stress. For this reason, the incidence of many diseases, including atherosclerosis and PAD, increases. Oxidation enhances ischemia. The albumin structure is modified due to oxidative stress and accompanying ischemia. The N-terminal modified albumin structure is called ischemia modified albumin (IMA) [16]. Therefore, TAS, TOS, OSI, dynamic thiol balance and IMA levels, which are next generation oxidative stress and antioxidant status indicators, are likely to be a significant laboratory marker in the diagnosis, treatment, and monitoring of PAD. The present study aimed to investigate the possible change in next generation oxidative stress-antioxidant status indicators as a biochemical parameter that could be useful in the diagnosis and follow-up of PAD.

**MATERIALS AND METHODS**

**Design**

Forty healthy controls and 40 male and female patients with peripheral artery disease who applied to the Alanya Alaaddin Keykubat University Research and Training Hospital were enrolled in the study with their voluntary consent.

**Ethical Situation**

The research was carried out with the approval of the Alanya Alaaddin Keykubat University Clinical Research Ethics Committee, dated 01-08-2019 and numbered 10354421-2019 / 7. During the clinical trial, the Declaration of Helsinki was followed in accordance with the ethical principles.

**Measured Parameters**

TAS, TOS, OSI, IMA and Thiol balance were studied by the colorimetric method after all blood was collected. The data obtained were subjected to statistical analysis, and the existence of a significant variation between the groups was checked. P <0.05 was determined as the level of statistical significance.

**Total Antioxidant Status, Total Oxidant Status and Oxidative Stress Index Analysis**

Serum total antioxidant statuses (TAS), total oxidant statuses (TOS) measurement and Oxidative Stress Index (OSI) calculation was made according to the standard colorimetric method, which has been used in many studies before [9].

**Ischemia Modified Albumin Measurement**

Serum IMA values were measured with Albumin cobalt binding analysis. For determination of the amount of cobalt not bound to albumin, 25 μl of dithiothreitol (final concentration 1.67 mmol / l) was introduced to the measuring cuvette following incubation and mixed so that dithiothreitol did not bind to albumin and formed a colored complex with cobalt. The colored complex that was formed was analyzed by spectrophotometry at a wavelength of 500 nm [16].

**Thiol Balance Measurement**

Thiol measurement was made according to the standard colorimetric method, which has been used in many studies before [17].

**RESULTS**

Forty patients with peripheral artery disease and 40 healthy volunteers were enrolled in this study. Thirty patients were male, and 10 were female. The mean age of the patients was 68 ± 8 years. The characteristics of the patient and control groups are presented in Table 1. There was a difference between the TAS, TOS, OSI, IMA and Total thiol levels of the participants in the patient group in comparison to the healthy adults. The TAS, OSI, IMA and total thiol levels of the patients were greater than those of the healthy adults, and their TAS levels were lower than those of the healthy adults. On the other hand, there was no significant difference between the groups regarding the Native thiol and Disulfide values (Table 2).

When the ROC analysis of the laboratory results was performed, the area under the curve was found to be the highest in OSI, TOS, total thiol and IMA, respectively. While the cut-off value for OSI was determined as 2.85, the sensitivity was 75%, specificity was 88%, PPV was 86%, and NPV was 77%. While the cut-off value for TOS was determined as 3.63, the sensitivity was 73%, specificity was 80%, PPV was 78%, and NPV was 74%. While the cut-off value for total thiol was determined as 380.92, the sensitivity was 55%, specificity was 83%, PPV was 76%, and NPV was 65%. While the cut-off value for IMA was determined as
0.82, the sensitivity was 35%, specificity was 85%, PPV was 70%, and NPV was 57% (Table 3, Figure 1).

**DISCUSSION**

Peripheral artery disease is an important vascular disorder that mostly develops based on atherosclerosis and mostly involves the lower extremities. It is one of the four main topics of cardiovascular diseases. Despite the high frequency and clinical importance of the disease, angiography used in the diagnosis may only be useful in the late period [1-3]. There is a need for a practical, inexpensive and non-invasive biochemical test that can be useful in diagnosis for early PAD. It is thought that increased oxidative stress may trigger atherosclerosis and peripheral artery disease. In our study, as the mean ± standard deviation in control and peripheral artery groups, respectively, TAS was found as 1.35 ± 0.22 and 1.06 ± 0.2, TOS was found as 2.99 ± 0.63 and 4.20 ± 1.29, and OSI was found to be 2.27 ± 0.57 and 4.17 ± 1.60. There was a significant and mutually supportive change regarding all three parameters between the groups (p<0.001). For this reason, according to our results, the deterioration of the balance between oxidant-antioxidant status towards the increase of oxidative stress may be evaluated in relation to peripheral artery disease.

The new generation oxidative stress indicator IMA levels known to increase in the case of ischemia were found respectively as 0.68 ± 0.17 and 0.82 ± 0.34 (p = 0.027). There was a significant change regarding the IMA levels between the groups. Therefore, the increase in the IMA levels may be used as an indicator of peripheral artery disease.

**Table 1.** The characteristics of the patient and control groups

| Healthy Control (Mean±SD) | Peripheral Arterial Disease (n=40) |
|---------------------------|-----------------------------------|
| Age (years)               | 66.4±7.8                          | 68.4±8.6                          |
| Sex                       | Male: 28 (70%)                    | Male: 30 (75%)                    |
|                           | Female: 12 (30%)                  | Female: 10 (25%)                  |
| Smoking                   | 14 (35%)                          | 14 (35%)                          |
| Diabetes Mellitus         | 16 (40%)                          | 17 (42.5%)                        |

**Table 2.** Comparison of Laboratory Results of Patients and Healthy Adults

| Group       | Healthy Control (Mean±SD) | Peripheral artery disease (Mean±SD) | p   |
|-------------|---------------------------|------------------------------------|-----|
| TAS         | 1.35±0.22                 | 1.06±0.20                          | 0.001|
| TOS         | 3.00±0.63                 | 4.20±1.29                          | 0.001|
| OSI         | 2.27±0.56                 | 4.16±1.60                          | 0.001|
| IMA         | 0.68±0.16                 | 0.82±0.34                          | 0.035|
| Native Thiol| 274.80±71.82              | 294.89±83.29                       | 0.133|
| Total thiol | 306.85±77.86              | 364.01±95.62                       | 0.004|
| Disulfide   | 34.85±14.06               | 37.45±17.31                        | 0.751|

**Table 3.** ROC Analysis of Laboratory Results

| Test Results | AUC | P     | Asymptotic 95% Confidence Interval |
|--------------|-----|-------|-----------------------------------|
|              |     |       | Lower Bound | Upper Bound |
| TAS          | 0.174 | 0.001 | 0.084  | 0.264  |
| TOS          | 0.769 | 0.001 | 0.657  | 0.881  |
| OSI          | 0.849 | 0.001 | 0.757  | 0.940  |
| IMA          | 0.637 | 0.036 | 0.513  | 0.760  |
| Native Thiol | 0.598 | 0.133 | 0.471  | 0.724  |
| Total Thiol  | 0.693 | 0.003 | 0.576  | 0.811  |
| Disulfide    | 0.521 | 0.751 | 0.390  | 0.651  |

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, IMA: Ischemia-modified albumin

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, IMA: Ischemia-modified albumin, AUC: area under the curve
There is an increase in inflammation in the ischemia and atherosclerosis processes. Ischemia-specific protein called ischemia-modified albumin may be used as a rapid and practical laboratory marker in common peripheral artery disease.

IMA also occurs due to high oxidative stress in different models of ischemia that affect other organs other than the myocardium [18]. Serum IMA level may vary in end stage renal disease, cerebrovascular ischemia, non-cardiac ischemic diseases, cardiopulmonary resuscitation, acute mesenteric ischemia, pulmonary embolism, as well as peripheral vascular diseases [18-21]. In this study, the values of OSI, TOS, Total thiol and IMA increased in accordance with the literature. IMA does not rise in the case of immune system disorders, gastrointestinal diseases and non-ischemic heart diseases [18]. IMA was determined as significantly greater in patients with acute ischemic stroke, and there was a positive correlation between thiobarbituric acid-reactive substance (TBARS) and IMA levels [22]. Oxidative stress biomarkers are increased in patients with scleroderma. IMA was identified as significantly greater in patients with systemic sclerosis [23].

Plasma IMA values were greater in the acute period of rat models with mesenteric ischemia in comparison to controls [24]. Ischemia leads to proinflammatory reaction cascades causing creation of reactive oxygen species (ROS) [21-27].

In oxidative stress and tissue inflammation, the increase in blood disulfide values and disulfide / native thiol, disulfide / total thiol ratios, indicating thiol / disulfide imbalance in vitiligo, were significant [28]. As shown in this study, the rise in oxidative stress and tissue inflammation was found to be significant. In our study, OSI, TOS, total thiol, IMA supported the result because of the increase in inflammation due to oxidative stress and atherosclerosis due to ischemia in peripheral artery patients.

It was determined that the oxidant-antioxidant balance was towards oxidants in acute ischemia which was observed in patients with acute cerebral infarction (ACI) and acute intracerebral hemorrhage (AIH). The absence of a difference in albumin, IMA, TAS, TOS, IMA / albumin ratio (IMAR) and OSI values shows that comparable processes have a function in the two conditions. It was ascertained that the oxidant-antioxidant balance was already disrupted in the early hours of stroke, much before detection of ischemia on Cranial CT (CCT) images. Ischemic modified albumin / albumin ratio, TOS and OSI were found to be useful in the early diagnosis of not only ischemic but also hemorrhagic strokes. Additionally, the change between patients with acute stroke and healthy adults indicates that these biomarkers may be utilized to make a distinction between actual stroke and healthy adults mimicking stroke [29]. The authors claimed that biomarkers emerging from the deterioration of the oxidant-antioxidant balance due to ischemia could be useful in the early identification of ischemia in an acute stroke study. In our study, we think that we can detect the effects of ischemia occurring in PA patients at an early stage with oxidant-antioxidant balance parameters. Their study supported the results of our study.
IMA is useful in cardiac diseases in evaluating early ischemia before the onset of non-transient cardiac damage, preventing progression to infarction, and preventing later complications. Clinical use of IMA in cardiac ischemia is useful, sensitive, cheap, and easy in terms of early diagnosis [30]. There are similar causes such as atherosclerosis and ischemia in the pathogenesis of cardiac and peripheral events. In the study conducted in cardiac ischemia, measuring the IMA level in the early period supports our study by claiming that cardiac complications will decrease.

It is known that increased oxidative stress can lead to many serious diseases [31,32]. Our research results show that there is a possible relationship between increased oxidative stress levels and peripheral artery disease.

LIMITATIONS

This study was conducted as a clinical study on patients who were diagnosed with peripheral artery disease and had consent. It is a single center research. The number of patients included in the study was limited to 40 due to its single center. More significance levels can be reached in new studies to be conducted with larger patient groups. In addition, there was a lack of research in the literature examining serum thiol disulfide balance in peripheral artery patients. Although the results of our research fill this gap, the discussion section of our article was not as extensive as we would like due to the lack of similar research to compare.

CONCLUSION

Oxidant-antioxidant markers will contribute to prevention of a process from claudication to amputation in the extremity with early evaluation of ischemia before the onset of vascular damage in peripheral artery disease. Although oxidant-antioxidant markers were used at acute stages in most studies, and although there was slow progressing ischemia and atherosclerosis in PAD, the markers were found to be significantly higher. In patients with PAD, the use of thiol-disulfide balance ischemia-modified albumin levels, which is an indicator of oxidative stress and antioxidant status, may provide early diagnosis and treatment. In addition, after the clinical use of the parameters we investigated, the mortality, morbidity, unnecessary radiation exposure and patient costs of the patients can be significantly reduced. The clinical use of OSI, TOS, total thiol and IMA in peripheral ischemia is beneficial, sensitive, cheap, and easy for early diagnosis. There is a need for studies on oxidant-antioxidant markers in patients with peripheral artery disease and diseases related to ischemia.

HIGHLIGHTS

According to our research results:

- The increase in total oxidant status (TOS) levels may be used as an indicator of peripheral artery disease.
- The increase in ischemia modified albumin (IMA) levels may be used as an indicator of peripheral artery disease.

- There is an increase in inflammation in the ischemia and atherosclerosis processes. An ischemia-specific protein called ischemia-modified albumin may be used as a rapid and practical laboratory marker in common peripheral artery disease.

Author contributions: All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Funding: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Declaration of interest: No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, shareholding and similar situations in any firm.

Protection of humans: The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the 2013 Helsinki Declaration of the World Medical Association.

Data confidentiality: The authors declare having followed the protocols in use at their working center regarding patients’ data publication.

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