

Oxidative stress in osteomyelitis and burns: Specific features of its role in development of pathology

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ABSTRACT

Introduction: The purpose of this study was to evaluate and compare the features of the initiation and development of oxidative stress in patients with osteomyelitis and burns. **Methods:** We studied the oxidative metabolism of blood of 20 healthy subjects (controls), 15 patients with burns, and 18 patients with chronic osteomyelitis. All patients included in the second group had thermal burns of the I-II-III degree in trunk and limbs on an area of 31 - 80% of the body surface without thermal inhalation trauma. After standard sample preparation, a wide range of parameters of oxidative metabolism was determined in the blood. The intensity of free radical processes in blood plasma and red blood cells, and the total antioxidant activity was evaluated by Fe-induced biochemiluminescence. The concentration of malonic dialdehyde in blood plasma and red blood cells was determined. The level of diene and triene conjugates and Schiff bases was determined spectrophotometrically using reagent kits. The catalase and superoxide dismutase activities in the red blood cells of patients from each of the groups was also determined. **Results:** We showed that in osteomyelitis, which is a long-lasting process, changes in the balance of free radical generation and activity of the antioxidant system were compensatory and mostly related to changes in blood plasma. On the contrary, in burn victims, oxidative stress signs had a maladaptive character. They were seen in blood plasma and red blood cells, and accompanied by a pronounced depletion of enzyme antioxidant system reserves. **Conclusion:** Our study demonstrate the role of oxidative stress in patients with burns and chronic osteomyelitis, and demonstrate some specific features leading to formation of disease pathology. Such features of oxidative stress may be useful in future design of new approaches to correct the pathology of diseases.

Key words: oxidative stress, burns, osteomyelitis, free radical processes, antioxidant system

INTRODUCTION

It is known that the reactions of free radicals and the opposing activity of a wide range of antioxidant systems are the fundamental basis for various aspects of the functioning of cells and tissues¹⁻⁴. In particular, they contribute to the renewal of cellular structures and processes, including biological membranes, energy metabolism, and phagocytic reactions^{1,4-7}. All of these processes, combined with oxidative metabolism, can be utilized to disrupt underlying conditions of various pathologies^{4,8-11}. This disruptive condition is called oxidative stress, and can be regarded as a complex universal syndrome^{8,12-15}. In the laboratory, signs of oxidative stress include a pronounced activation of free radical processes, accompanied by a significant inhibition of the corresponding blood and tissue systems^{5,13,15-17}. Indeed, despite confirmation in literature of the significant role of oxidative stress in the pathogenesis of various diseases, the specificity of its implementation in a particular pathology is still poorly understood. To

a greater extent, this applies to surgical diseases, including traumatological and orthopedic pathologies. A few studies have been recently devoted to this issue. Thus, there is evidence of the involvement of oxidative stress in the pathogenesis of thermal trauma¹⁸⁻²¹ and chronic post-traumatic inflammatory processes, such as osteomyelitis²²⁻²⁴. However, there is no information about the formation and progression of oxidative stress in these diseases. The purpose of the study herein was to conduct a comparative evaluation and analysis of the features of the formation and development of oxidative stress in patients with osteomyelitis and burns.

MATERIAL - METHODS

Patient and control groups

We studied the oxidative metabolism of blood of 20 healthy subjects (control), 15 patients with burns, and 18 patients with chronic osteomyelitis. All patients included in the second group had thermal burns of the I-II-III degree in the trunk and/or limbs (on an area

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ranging from 31 - 80% of the body surface); these patients were absent of thermal inhalation trauma. After receiving the study participants' voluntary informed consent, blood samples from patients of the second and third groups were obtained at admission to the hospital. In patients with burns, blood samples were taken up to 4 days after the moment of injury.

Laboratory assays

After standard sample preparation, a wide range of parameters of oxidative metabolism was determined in the blood. In particular, the intensity of free radical processes in blood plasma and red blood cells, as well as the total antioxidant activity, were evaluated by Fe-induced biochemiluminescence. In addition, the concentration of malonic dialdehyde in blood plasma and red blood cells was determined. The level of diene and triene conjugates, and of Schiff bases, was determined spectrophotometrically using reagent kits. Catalase and superoxide dismutase activities in the red blood cells of patients from each of the designated groups were also determined.

Statistics

The results were processed using the Statistica 6.0 program¹³. All the data were processed using standard algorithms of descriptive statistics and were presented as Mean±SD. The Student's t-test was used for the detection of statistical differences ($p < 0.05$ was set as the statistical significance level).

RESULTS

It was discovered that the integral indicator of the intensity of free radical processes in blood plasma and red blood cells (the light sum of Fe-induced biochemiluminescence) showed intergroup differences (Figure 1).

Thus, there is a stimulation of free radical oxidation in the blood plasma of patients with burns and osteomyelitis. This trend is expressed equally (+ 27.8% and + 27.1% relative to practically healthy people, respectively; $p < 0.05$ for both cases). On the contrary, in the group of patients with osteomyelitis in red blood cells, the indicator values does not differ from that of the control level. However, in severely burned patients, its moderate increase was recorded (+ 8%; $p < 0.05$ relative to practically healthy people). This indicates the presence of certain features of a shift in oxidative metabolism in the diseases under consideration.

Moreover, the variability of changes is manifested in the total antioxidant activity of blood plasma

(Figure 2). It should be noted that both burns and chronic osteomyelitis show a decrease in antioxidant potential of the plasma. Together with an increase in the intensity of free radical processes, this will allow us to record the formation of oxidative stress in both diseases. Simultaneously, the severity of the decrease in the total antioxidant activity is not the same. In particular, in osteomyelitis, the reduction in the indicator level is 20.4%. In contrast, in patients with burns, a decrease in the parameter value was recorded by 7.8% ($p < 0.05$ for both cases compared to practically healthy people). Simultaneously, the antioxidant activity of blood plasma in severely burned patients is 1.16 times higher than in osteomyelitis ($p < 0.05$). Analysis of the level of malonic dialdehyde in blood plasma and red blood cells also allowed us to verify the specificity of the implementation of oxidative stress in burns and chronic osteomyelitis (Figure 3 and Figure 4). Indeed, in the blood plasma, the concentration of the secondary product of lipid peroxidation increases only in patients with chronic osteomyelitis (by 25.6% relative to the control group; $p < 0.05$), while in patients with burns, it remains at the level of practically healthy people (Figure 3).

On the contrary, the concentration of malonic dialdehyde in red blood cells increases in representatives of both groups (osteomyelitis and burns), but this trend is expressed differently (Figure 4). A more distinct increase in the red blood cell level of the compound was observed in the burn group (1.54-fold increase compared to healthy group; $p < 0.05$) than in the chronic osteomyelitis group (+ 39.1%; $p < 0.05$). It should be noted that the concentration of malonic dialdehyde in burns is significantly higher than in osteomyelitis (by 10.5%; $p < 0.05$).

According to the assessment results of the level of other lipid peroxidation products, less significant changes were revealed (Figure 5). A moderate increase was found only for diene conjugates, and these changes were expressed almost equally in patients with burns and osteomyelitis (+ 9.7 and + 12.8% relative to the control group, respectively; $p < 0.05$).

We also evaluated the state of the enzyme antioxidant systems in the patient groups (Figure 6 and Figure 7). It was found that the pathologies had a multidirectional effect on the activity of erythrocyte superoxide dismutase (Figure 6). Thus, in chronic osteomyelitis, moderate activation of the enzyme was noted (by 10.6% relative to practically healthy people; $p < 0.05$). In patients with burns, there was a significant inhibition of the enzyme (by 20.3%; $p < 0.05$).

Analysis of red blood cell catalase activity revealed activation only in chronic osteomyelitis (1.25-fold

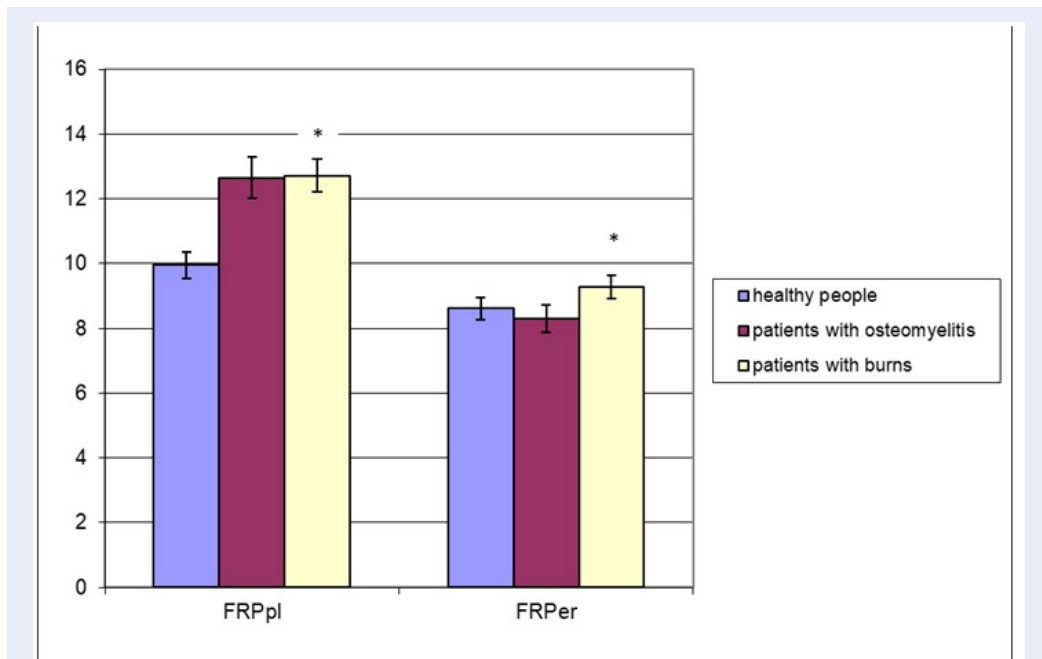


Figure 1: The intensity of free radical processes in blood plasma and erythrocytes in healthy people and patients with chronic osteomyelitis and burns (** – level of statistical differences to healthy people; $p < 0.05$).

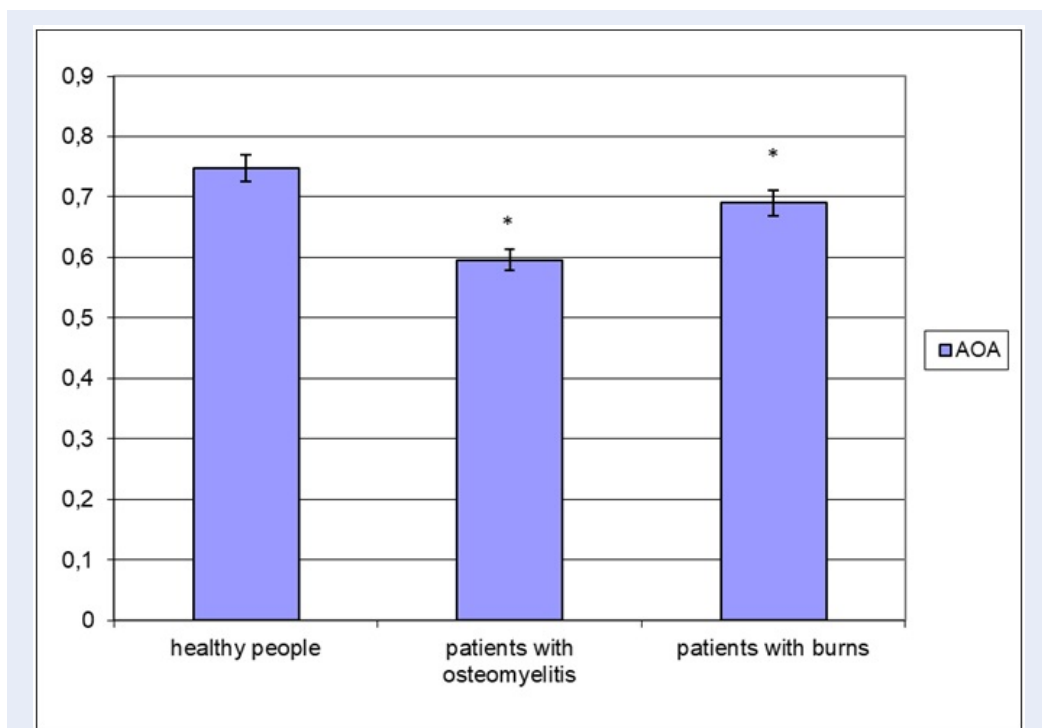


Figure 2: Total antioxidant activity in blood plasma and erythrocytes in healthy people and patients with chronic osteomyelitis and burns (** – level of statistical difference when compared to healthy people; $p < 0.05$).

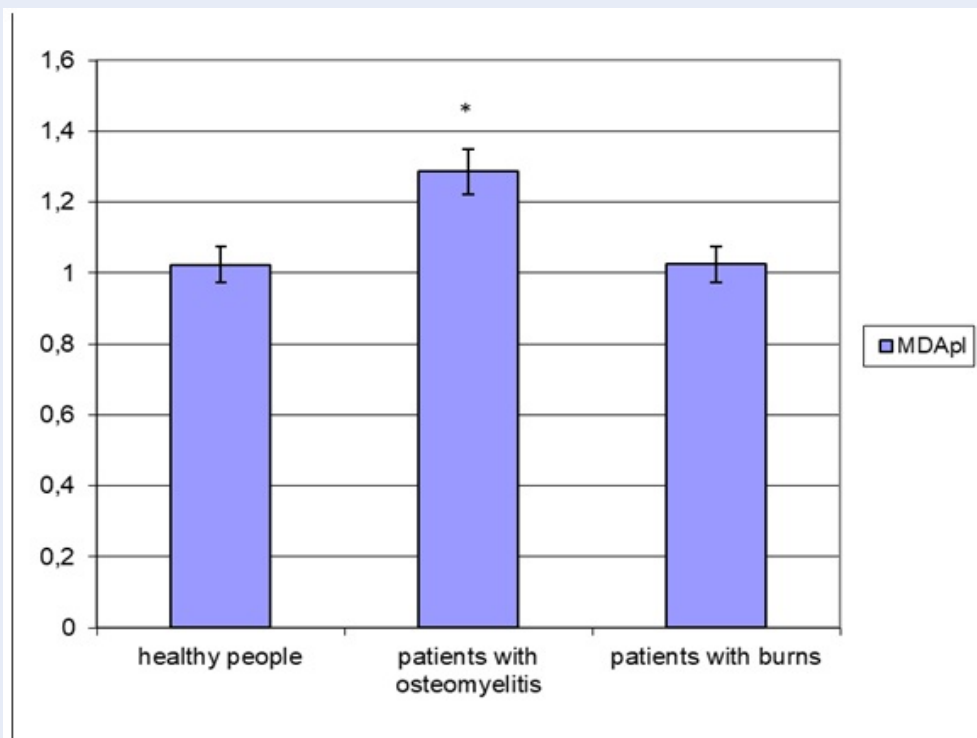


Figure 3: Malonic dialdehyde level in blood plasma in healthy people and patients with chronic osteomyelitis and burns (***) – level of statistical differences to healthy people; $p < 0.05$).

greater than that of the control group; $p < 0.05$), while in severely burned patients, the enzyme's properties and function remained at the physiological level.

DISCUSSION

It is known that oxidative stress is a universal pathological process (syndrome) that develops in various diseases and conditions⁸⁻¹⁷. Simultaneously, there is relatively little information about the specificity of changes in the balance of free radical processes and antioxidant systems in blood and tissues formed within the framework of oxidative stress in specific pathologies^{1,4,5}. Therefore, this study attempted to establish the features of specificity for oxidative stress in traumatic pathologies (e.g. burns and chronic osteomyelitis).

The study demonstrated the general signs and features of the implementation of oxidative stress in osteomyelitis and burns. Indeed, the presence of this syndrome in both pathological conditions was confirmed by the detection of characteristic laboratory shifts, including the intensification of free radical oxidation in the blood plasma, as well as suppression of its antioxidant reserves. These results confirm our previously published results¹⁸⁻²⁵ and those of other

authors^{19,20,23,26,27}. At the same time, the persistent nature of these disorders is emphasized by the detection of increased levels of diene conjugates which increase about equally in patients with burns and osteomyelitis.

On the other hand, there are some features of oxidative stress in the evaluated pathological conditions. Particularly, we suggest that the changes are more related to free radical reactions occurring in the blood plasma in chronic osteomyelitis. This is evidenced by the absence of shifts in Fe-induced biochemiluminescence in the membranes of red blood cells in this pathology and a more pronounced decrease in the total antioxidant activity in blood plasma than in patients with burns. In addition, in osteomyelitis, an increase in the plasma concentration of malonic dialdehyde was observed, which was absent in severely burned patients. Finally, the synchronization of the pathological process in osteomyelitis contributes to the formation of compensatory rearrangements of the enzyme antioxidant system^{23,24}, as evidenced by a moderate increase in the activity of erythrocyte superoxide dismutase and catalase. We assume that this circumstance is responsible for the lower severity of

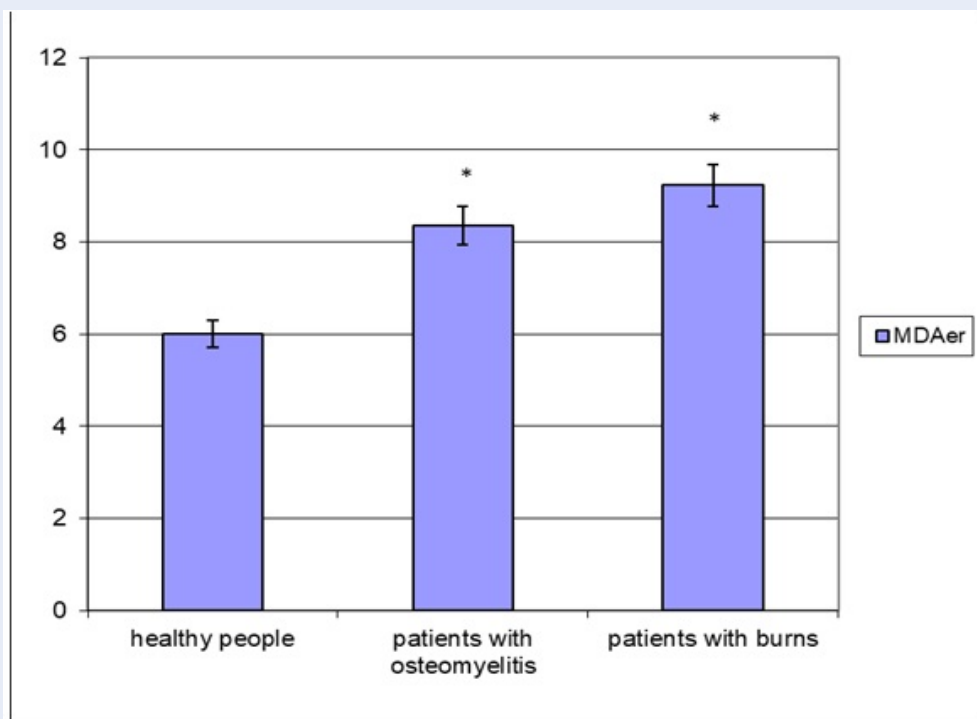


Figure 4: Malonic dialdehyde level in erythrocytes in healthy people and patients with chronic osteomyelitis and burns ("*" — level of statistical differences to healthy people; $p < 0.05$).

oxidative metabolism changes in the red blood cells of patients with chronic osteomyelitis.

On the contrary, in burn victims, changes in free radical processes fully affect both blood plasma and red blood cells. This is indicated by a significant increase in the light sum of Fe-induced biochemiluminescence as an integral indicator of the intensity of lipid peroxidation and other free radical reactions in blood plasma and red blood cells. Also, this pathological condition (burn) is characterized by a more pronounced increase in the red blood cell concentration of malonic dialdehyde, in combination with an increase in the level of diene conjugates, than in osteomyelitis. This may indicate that the process is active. In addition, it should be emphasized that an acute thermal burn injury does not involve the inclusion of compensatory mechanisms, in particular, enzyme antioxidant systems^{18,20,21,26,28}. This leads to a rapid depletion of their reserves during the conditions of intensification of free radical formation. This is indicated by the inhibition of erythrocyte superoxide dismutase activity detected in burns, which ensures the formation of distinct signs of oxidative stress in the red blood cells of patients in this group.

CONCLUSION

The study herein enabled the verification of oxidative stress in patients with burns and chronic osteomyelitis. The specificity of the formation of the pathological syndrome (oxidative stress) was found. We have shown that in osteomyelitis, since it is a long-lasting process, changes in the balance of free radical generation and the activity of the antioxidant system are compensatory and mostly relate to changes in blood plasma. On the contrary, in burn victims, oxidative stress signs have a maladaptive character. Oxidative stress is seen in blood plasma and red blood cells, accompanied by a pronounced depletion of enzyme antioxidant system reserve. The findings of the oxidative stress features may prove useful in determining a different approach to the correction of pathological conditions, such as burns and osteomyelitis^{5,25,28-30}.

ABBREVIATIONS

AOA: antioxidant activity

DC: diene conjugates

TC: triene conjugates

ShB: Schiff bases

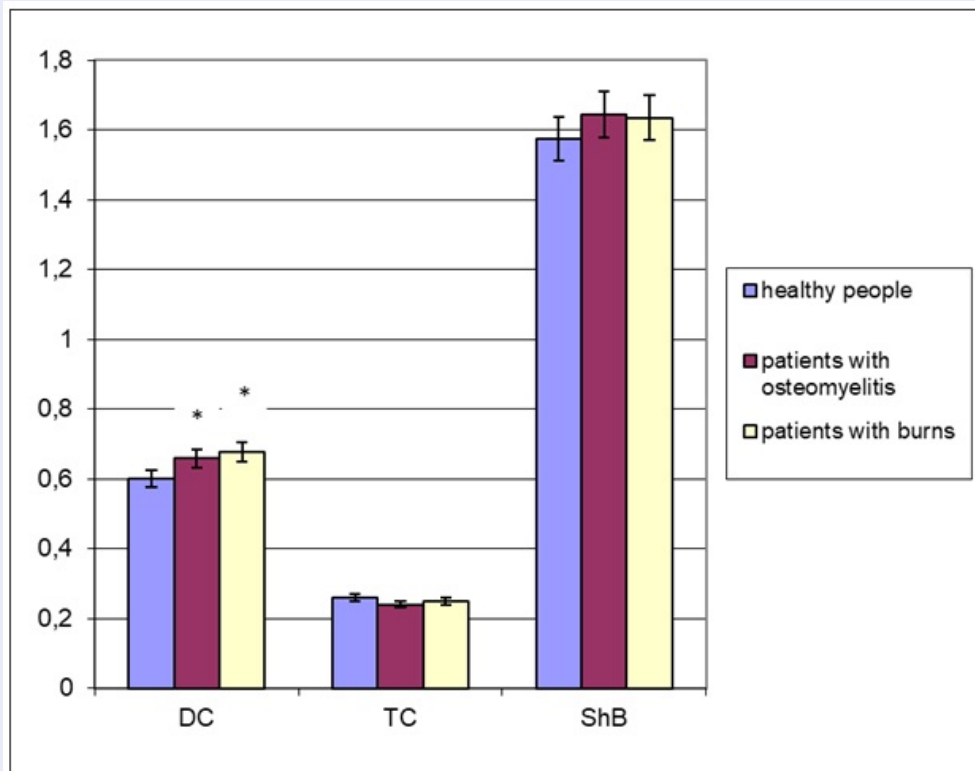


Figure 5: Level of diene (DC) and triene conjugates (TC) and Schiff bases (ShB) in blood plasma in healthy people and patients with chronic osteomyelitis and burns (*# – level of statistical differences compared to healthy people; $p < 0.05$).

AUTHOR'S CONTRIBUTIONS

A.K.M., V.I.Z. and A.G.S. contributed to the conceptualization and design of the study, the analysis and interpretation of data. A.S.P. carried out the selection and supervision of patients. K.L.B. and A.G.S. performed laboratory tests. They were drafting the article and revising the article critically for important intellectual content. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL

The study was approved by Local Ethic Committee of Privolzhsky Research Medical University (no. 2 from

18/02/2018).

CONSENT FOR PUBLICATION

The authors hereby consents that the Publisher publishes the Work.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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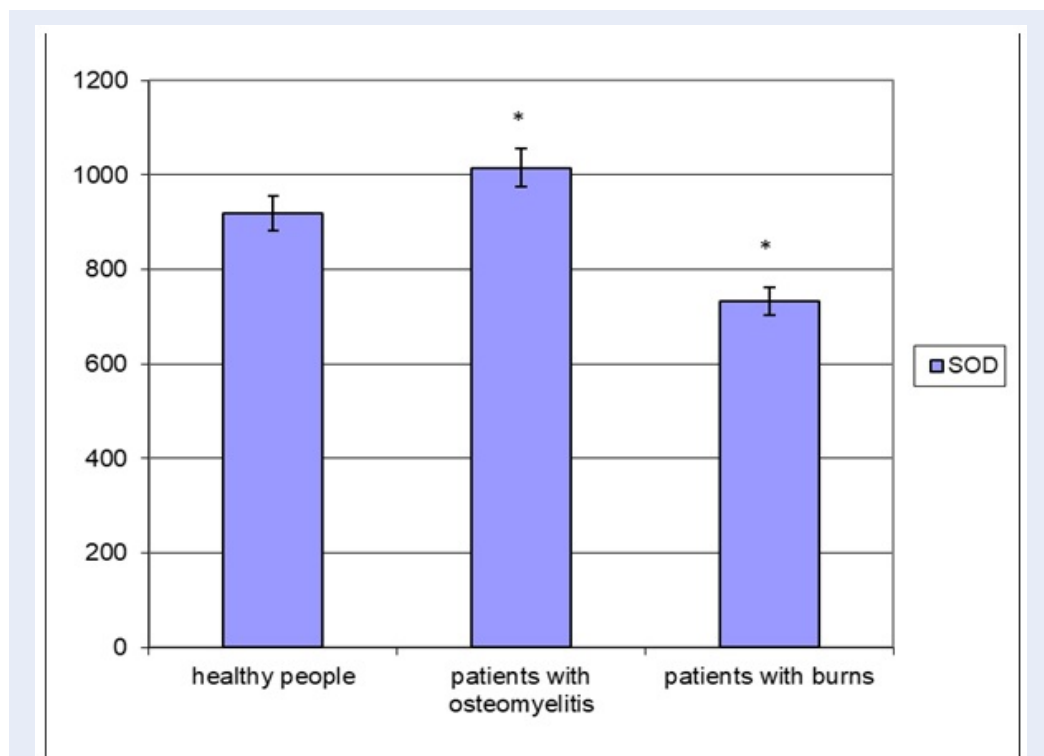


Figure 6: Superoxide dismutase activity in erythrocytes in healthy people and patients with chronic osteomyelitis and burns (***) – level of statistical differences compared to healthy people; $p < 0.05$).

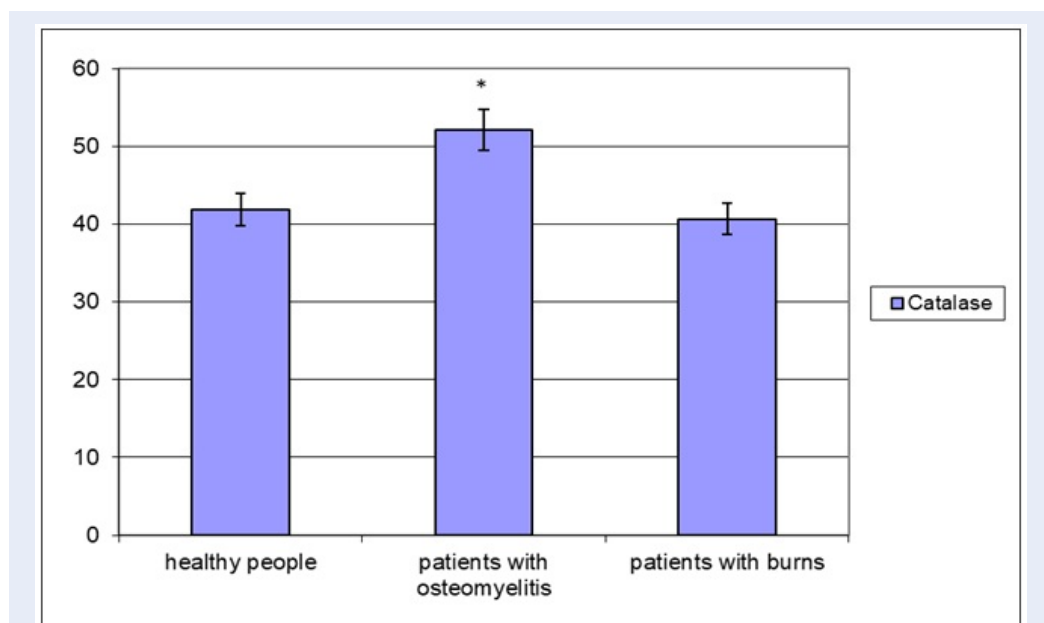


Figure 7: Catalase activity in erythrocytes in healthy people and patients with chronic osteomyelitis and burns (***) – level of statistical differences compared to healthy people; $p < 0.05$).

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