

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Comparative Study of oxidative stress in Diabetic and Non-diabetic osteomyelitis.

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ABSTRACT

Osteomyelitis occurs when an infection develops in a bone or spreads to a bone from another area of the body. It is caused by bacteria or fungi. The infected bone may deteriorate and form a pocket of pus in response to the infection. An enzyme of the innate immune system, myeloperoxidase [MPO] exhibits a wide array of proatherogenic effects. Lipid peroxidation [LPO] is a free radical-related process which in biological systems may occur under enzymatic control. The latter form is associated mostly with cellular damage as a result of oxidative stress, which also involves cellular antioxidants like Vitamin-C [Vit C]. The present study involves the estimation of Myeloperoxidase activity, lipid peroxidation and Vit C in diabetic and non diabetic osteomyelitis. We observed a significantly high Myeloperoxidase activity and lipid peroxidation and decreased levels of Vitamin-C in diabetic osteomyelitis when compared to non diabetic osteomyelitis.

Key words: Osteomyelitis, Myeloperoxidase, Lipid peroxidation and Vitamin-C

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INTRODUCTION

Osteomyelitis is an infection of the bone or bone marrow. In general, microorganisms may infect bone through the bloodstream, contiguously from local areas of infection or penetrating trauma, including iatrogenic causes such as joint replacements or internal fixation of fractures. Once the bone is infected, leukocytes enter the infected area, and, in their attempt to engulf the infectious organisms, release enzymes that lyse the bone. Pus spreads into the bone's blood vessels, impairing their flow, and areas of devitalized infected bone, known as sequestra, form the basis of a chronic infection [1]. When it is chronic it can lead to bone sclerosis and deformity. Staphylococcus aureus is the organism most commonly isolated from all forms of osteomyelitis. Bloodstream-sourced osteomyelitis is seen most frequently in children, and nearly 90% of cases are caused by Staphylococcus aureus.

Myeloperoxidase, a member of the heme peroxidase super family, is contained in azurophilic granules of neutrophils and monocytes. It is released upon leukocyte activation, contributing to innate immunity. MPO alters the biological function of surrounding proteins and lipids by halogenation, nitration and oxidative cross-linking [2]. In the cardiovascular system MPO has been linked to the development of atherosclerotic disease and phenotypic transition of stable to vulnerable plaque [3, 4]. Recently, it was demonstrated in an animal model that MPO contributes to adverse remodelling and left ventricular dilatation after acute myocardial infarction, and may thus contribute to the development of heart failure [5, 6].

Lipid peroxidation has been shown to cause a profound alteration in the structural integrity and functions of cell membranes. Free radical-induced lipid peroxidation has been implicated in the pathogenesis of several pathological disorders [7]. Oxidation of low-density lipoprotein particles and cytotoxic effects of lipid peroxides enhance the formation of foam cells and atherosclerotic lesion. Several reactive oxygen species and lipid peroxidation products are produced in physiological quantities in the human body, but it has been well established that over-production of ROS occurs at sites of chronic inflammation [8].

Vitamin C is a water soluble antioxidant and essential nutrient for humans and certain other animal species, in which it functions as a vitamin. It protects the body against oxidative stress [9]. Vitamin C may also be able to regenerate other antioxidants such as vitamin E [10]. It is a common enzymatic cofactor in mammals used in the synthesis of collagen. Vitamin C acts as an electron donor for important enzymes [11].

But, to our knowledge, there is yet very little documented record in the literature of the prognostic role of MPO, lipid peroxidation and vitamin-C in osteomyelitis. In an attempt to find an indicator that severity of infection prediction in osteomyelitis, we evaluated the prognostic impact of Serum MPOx, lipid peroxidation and vitamin-C.

MATERIALS AND METHODS

This work was carried out in the central research laboratory of Nitte University, after getting approval from the concerned institutional ethical committee. Indian subjects aged 35-60 years with osteomyelitis and diabetic osteomyelitis were recruited for the study. The study included 150 participants [including male and females] divided into 3 groups of 50 each diagnosed for osteomyelitis with and without diabetes. Group A involves patients suffering from osteomyelitis; Group B involves patients suffering from diabetic osteomyelitis and group C involves normal subjects. Venous blood samples were collected and used for the study. Serum Myeloperoxidase and lipid peroxidation level was measured by the method of Matheson et al [12] and TBA method respectively [13]. Vit C was estimated by DNPH method [14].

Statistical analysis

All the results were expressed as Mean \pm SD. The data were statistically analyzed by one-way ANOVA test. The p value <0.05 was considered as the level of significance.

RESULTS

There is a significant increase in serum Myeloperoxidase and lipid peroxidation whereas vitamin-C level declines significantly in both non diabetic and diabetic osteomyelitis compared to normal subjects and also there is a significant increase of serum Myeloperoxidase and lipid peroxidation levels and decrease of vitamin-C levels in diabetic osteomyelitis when compare to non diabetic osteomyelitis [Table-1, Graph-1,2,3].

Table-1: Level of Myeloperoxidase activity, lipid peroxidation and vitamin-C levels in different groups. Data are expressed as Mean \pm SD, n=50 in each groups.

Parameters	Group A	Group B	Group C
Myeloperoxidase[pmol/l]	180.4 \pm 71.89	310.7 \pm 71.03	116.5 \pm 67.96
Lipid peroxidation[μ M/L]	2.302 \pm 0.061	2.592 \pm 0.133	1.701 \pm 0.170
Vitamin C[mg/dl]	0.868 \pm 0.294	0.421 \pm 0.129	1.605 \pm 0.411

Note: Group A contains patients with osteomyelitis, Group B contains patients with diabetic osteomyelitis and Group C contains normal subjects. There were significant differences at $p < 0.05$.

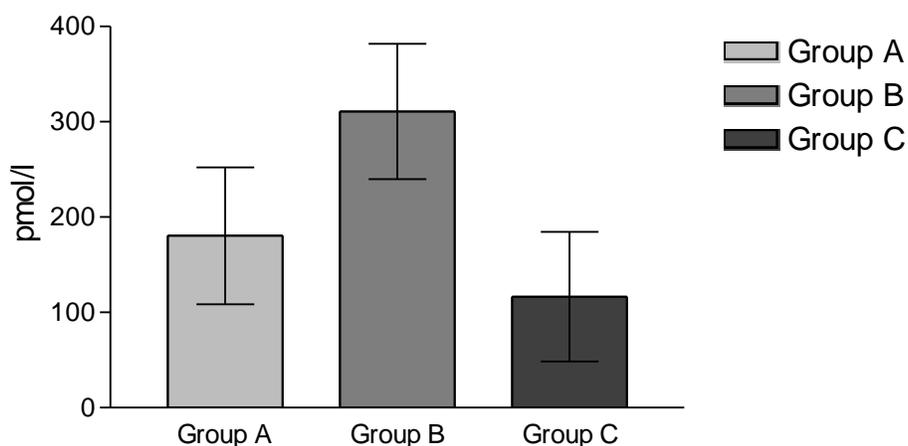


Fig:1- Myeloperoxidase level in Group A [patients with osteomyelitis], Group B [patients with diabetic osteomyelitis] and Group C [normal subjects].

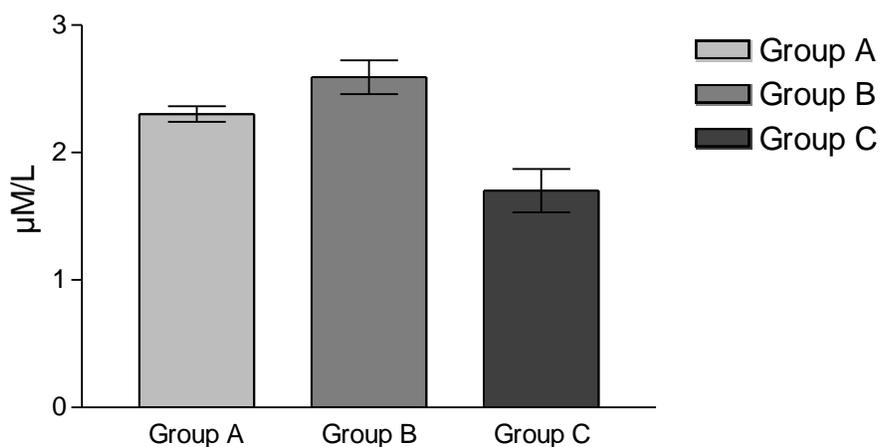


Fig:2- MDA level in Group A [patients with osteomyelitis], Group B [patients with diabetic osteomyelitis] and Group C [normal subjects].

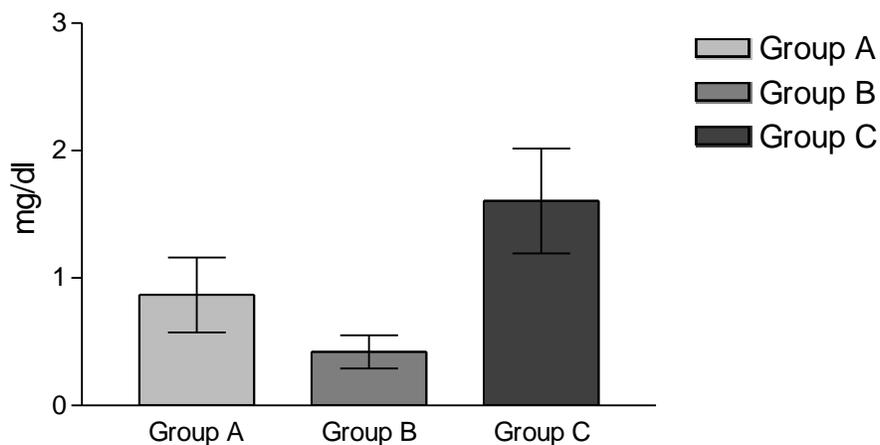


Fig:3- Vitamin-C level in Group A [patients with osteomyelitis], Group B [patients with diabetic osteomyelitis] and Group C [normal subjects].



DISCUSSION

Oxidative stress is due to a disturbance in the balance between the production of ROS and the efficiency of the antioxidant defense. In other words, oxidative stress results if excessive production of ROS overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defense [15].

The results of this study indicate that MPO has a strong prognostic impact on the risk of major cardiovascular events in osteomyelitis patients. Indeed, patients with MPO serum concentrations 310.7 ± 71.03 pM/l had a much greater risk of developing myocardial infarction. Myeloperoxidase can exert a plethora of proatherogenic effects, including oxidation of lipoproteins and induction of vascular dysfunction [16-21]. MPO plays a role in a range of events involving the initiation, propagation, and clinical complications of atherosclerosis in addition to the generation of atherogenic lipoproteins [22]. Elevated MPO levels predict severity of infection. Therefore, increased serum levels of MPO seem to precede myocardial injury and thus may be considered a predictor of vulnerable plaques.

Lipids, especially polyunsaturated fatty acids, are very susceptible to free radical attack, which can initiate lipid peroxidation [23]. The increase in the levels of MDA in our study indicates that lipid peroxidation is taking place in patients of diabetic and non diabetic osteomyelitis. Lipid peroxidation appears to be a major source of endogenous DNA damage in humans and may serve as a futuristic diagnostic and prognostic tool in diabetic and non diabetic osteomyelitis. The end product of lipid peroxidation, malondialdehyde increases due to its high severity of infection in osteomyelitis and diabetic osteomyelitis.

Vit C radical scavenging antioxidant [24], present in all cells, can also act as a reducing agent. This free radical scavenger protects the cell against the toxic oxygen free radicals. In our study the significant decrease in the levels of Vit C occurs with the progression of lipid peroxidation. The lowered values of Vit C indicates that the severity of infection. Vit C level decreases by scavenging the free radicals and prevent the lipid peroxidation.

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