ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Useful markers to predict the risk of Diabetic and Non-Diabetic osteomyelitis

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ABSTRACT

Osteomyelitis is the local or generalized infection of the bone and bone marrow, usually caused by bacteria and fungi. It can be induced by trauma, surgery, by direct extension from a nearby infection or via the blood stream. The present work involves the estimation of total antioxidant status, Phosphodiesterase level and the activity of Super oxide dismutase in diabetic and nondiabetic osteomyelitic patients. This study involved 100 osteomyelitic patients with and without diabetes diagnosed at Thejaswini hospital, Mangalore and 100 healthy individuals. About 5 ml of venous blood was collected and used for the estimation of Biochemical parameters. There is a significant increase **(**p=0.0001) in serum Phosphodiesterase level, whereas the Total antioxidant and Superoxide dismutase declines significantly **(**p=0.0001 and **(**p=0.0002) in both non diabetic and diabetic osteomyelitis compared to normal subjects. Diabetes mellitus causes not only hyperglycaemia but oxidative stress, resulting mainly enhanced production of reactive oxygen species. Total antioxidant, SOD and phosphodiesterase are the useful markers to assess the diabetic Osteomyelitis.

Key words: Diabetic Osteomyelitis, Antioxidant, Phosphodiesterase, Superoxide dismutase

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INTRODUCTION

Osteomyelitis is the local or generalized infection of the bone and bone marrow, usually caused by bacteria and fungi. The common causative agent responsible for Osteomyelitis includes Staphylococcus species, Salmonella, Mycobacterium species, Pseudomonas etc. Staphylococcus infection predominates today and before the era of antibiotics. It occurs when an infection develops in a bone or spreads to a bone from another area of the body. The infected bone may deteriorate and form a pocket of pus in response to the infection. For the occurrence of Osteomyelitis, a situation that makes bones vulnerable must be present. For instance, trauma to the bone or to the soft tissue around the bone, such as puncture wound provides useful environment for Osteomyelitis. Diabetic foot ulcers give infections a route to enter bone or nearby tissue. The microenvironment determines the infections. The developing Osteomyelitis increases with impaired immune function, extensive tissue damage or reduced blood supply to the affected area. The infection may be acute or chronic and may persist intermittently for years. Patients with diabetes, haemodialysis, spleenectomy adds to greater risk.

Diabetes mellitus (DM) causes not only hyperglycaemia but oxidative stress, resulting mainly enhanced production of mitochondrial reactive oxygen species (ROS). Oxidative stress occurs in some illnesses like persistent hyperglycemia caused through diabetes induces ROS production by glucose autoxidation [1, 2], activation of protein kinase C, and increase flux through the hexosamine pathway [3]. Oxidative stress has been also associated with diabetic status in animals and humans [4-8]. Oxidative stress induced by hyperglycemia leads to the activation of stress-sensitive signalling pathways, which worsen both insulin secretion and action, and promote the development of type 2 diabetes mellitus (T2DM) [9]. Free radicals are reactive chemical species that have an odd number of electrons. Since they are highly reactive, their lifetimes generally are very short, their very existence has often been clouded in acrimonious debate [10]. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant- based drugs/formulations for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades [11].

The human body does contain an array of antioxidant defence mechanisms (non-enzymatic and enzymatic antioxidants) to remove harmful ROS as soon as they are formed and to prevent their deleterious effects [12].

Super oxide dismutase (SOD) catalyzes the destruction of the O2- free radical. It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals [13, 14]. A major class of antioxidant enzymes involved in maintaining homeostatic levels of ROS, such as superoxide, are represented by the superoxide dismutase (SOD). SODs are a family of metalloenzyme that catalyze the dismutation of O_2 to H_2O_2. Mammalian cells possess three distinct forms of SODs; extracellular SOD (EC-SOD) present in the extracellular spaces, manganese SOD, found exclusively in the mitochondria, and copper-zinc SOD located in the cytoplasm [15,16]. EC-SOD is the principal enzymatic antioxidant in extracellular spaces [17] and plays an important role in the protection of mammals against super oxide.
Phosphodiesterase are another group of enzymes which catalyze the hydrolysis of phosphodiesters to phosphomonoesters. They exist both intracellularly and extracellularly

in a wide variety of tissues and organisms [18-21]. Intracellular Phosphodiesterases play a role in signal transduction by regulating the cellular concentrations of cyclic nucleotides [22]. But, to our knowledge, regarding the prognostic role of Phosphodiesterase, total antioxidant and super oxide dismutase in osteomyelitis is less documented. In an attempt to find an indicator that predicts the severity of infection in osteomyelitis, we evaluated the prognostic impact of Serum total antioxidant, Super oxide dismutase (SOD), Phosphodiesterase concentrations.

MATERIALS AND METHODS

This work was carried out in the central research laboratory of A.B. Shetty Memorial Institute of Dental Sciences, after getting approval from the ethical committee of the institution. Indian subjects aged 30-70 years with non-diabetic Osteomyelitis and Diabetic Osteomyelitis were recruited for the study. The study included 100 Osteomyelitis cases including male and females, were divided into 3 groups who are diagnosed at Thejaswini hospital, Mangalore. There were 100 healthy controls in each group. The osteomyelitis cases were divided into 2 groups. Group B involves patients suffering from osteomyelitis without Diabetes; Group C involves Diabetic patients suffering from Osteomyelitis. Group A involves normal healthy participants. About 5 ml of venous blood was collected and used for the study.

Serum Total antioxidant level was measured by phosphomolybdic acid method [21]. Super oxide dismutase (SOD) activity was assayed using nitro blue tetrazolium (NBT) method [23] and Phosphodiesterase was assayed using PNPP method [24].

Statistical analysis

All the data obtained were expressed as Mean ± SD. The data were statistically analyzed by one-way ANOVA. P value <0.05 was considered as the level of significance.

RESULTS

Diabetes mellitus (DM) causes not only hyperglycaemia but oxidative stress, resulting mainly enhanced production of mitochondrial reactive oxygen species (ROS). The oxidation state was assessed by measuring Total antioxidants and activity of antioxidant enzymes (SOD) in the blood serum Diabetic and nondiabetic osteomyelitic patients including age and sex matched normal controls. The concentration of Phosphodiesterases, which play a role in signal transduction by regulating the cellular concentrations of cyclic nucleotides, was also estimated. The recruited subjects were divided into Group A containing normal subjects, Group B containing patients with Non-diabetic osteomyelitis and Group C containing patients with diabetic osteomyelitis. Phosphodiesterase level was significantly increased ($p=0.0001$) in Group B and Group C as compared to Group A whereas the level of Total antioxidant and Superoxide dismutase was significantly decreased ($p=0.0001$ and $p=0.0002$) in Group B and Group C as compared to Group A respectively. In our study we found that there is a significant increase in serum Phosphodiesterase level ($p<0.0001$) in Group B and Group C as compared to Group A (Table-1, Fig-1) whereas the level of Total

ISSN: 0975-8585

antioxidant and Superoxide dismutase was significantly decreased ($p<0.0001$ and $p<0.0002$) respectively in Group B and Group C as compared to Group A (Table-1, Fig-2 and Fig-3).

Table-1: Concentration of Phosphodiesterase, Total Antioxidant and Superoxide dismutase in normal individuals and patients with and without diabetic osteomyelitis. Data are expressed as Mean± SD, n=100 in each groups.

Parameters	Group A	Group B	Group C
Total antioxidant (µg of α-tocopherol /ml)	261.10 ± 18.60	147.40 ± 22.08	100.50 ± 10.78
Phosphodiesterase (IU)	166.50 ± 12.50	260.50 ± 12.72	339.60 ± 18.59
Superoxide dismutase (units/mg protein)	1.61 ± 0.31	0.949 ± 0.14	0.293 ± 0.08

Note: Group A contains normal subjects, Group B contains patients with Non-diabetic osteomyelitis and Group C contains patients with diabetic osteomyelitis. Phosphodiesterase level was significantly increased ($p=0.0001$) in Group B and Group C as compared to Group A whereas the level of Total antioxidant and Superoxide dismutase was significantly decreased ($p=0.0001$ and $p=0.0002$) in Group B and Group C as compared to Group A respectively.

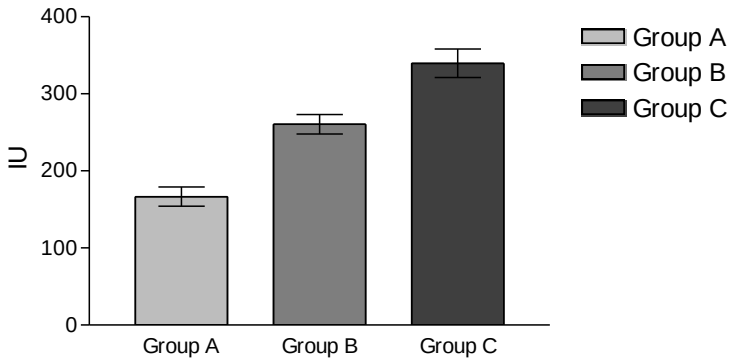

Fig-1: Comparison of Phosphodiesterase level in Group A with normal subjects, Group B having patients with Non-diabetic osteomyelitis and Group C includes patients with diabetic osteomyelitis. n=100 in each groups.

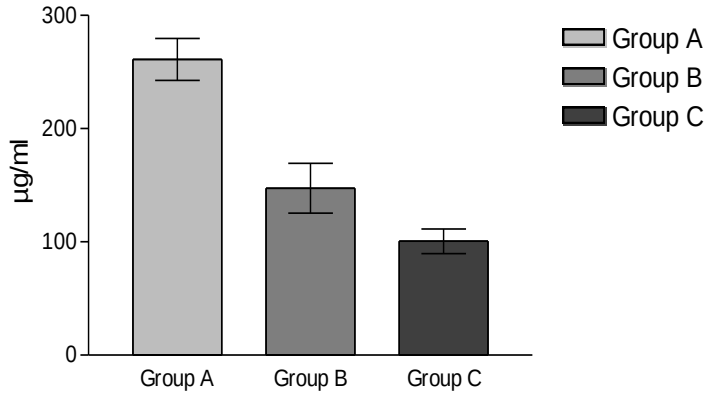

Fig-2: Comparison of Total Antioxidant level in Group A with normal subjects, Group B having patients with Non-diabetic osteomyelitis and Group C includes patients with diabetic osteomyelitis. n=100 in each groups.

ISSN: 0975-8585

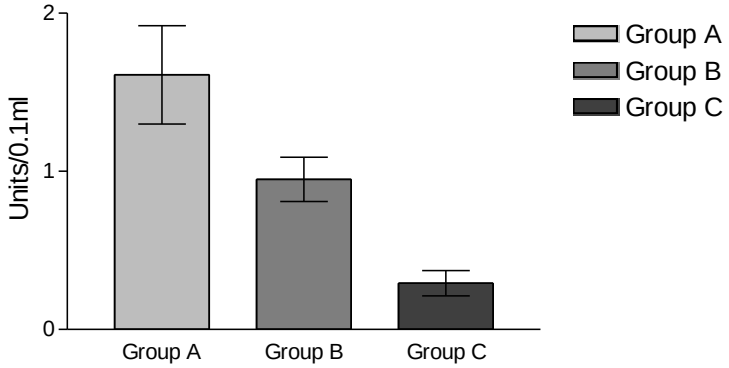

Fig-3: Comparison of SOD level in Group A with normal subjects, Group B having patients with Non-diabetic osteomyelitis and Group C includes patients with diabetic osteomyelitis. n=100 in each groups.

DISCUSSION

Diabetes mellitus is a disorder that primarily affects the micro vascular circulation. In the extremities, micro vascular disease due to "sugar-coated capillaries" limits the blood supply to the superficial and deep structures. Pressure due to ill-fitting shoes or trauma further compromises the local blood supply at the micro vascular level, predisposing the patient to infection. The infection may involve the skin, soft tissues, bone, or all of these tissues. Diabetes also accelerates macro vascular disease, which is evident clinically as accelerating atherosclerosis and/or peripheral vascular disease. Most diabetic foot infections occur in the setting of good dorsalis pedis pulses; this finding indicates that the primary problem in a diabetic foot infection is micro vascular compromise. Impaired micro vascular circulation hinders white cell migration into the area of infection and limits the ability of antibiotics to reach the site of infection in an effective concentration. Diabetic neuropathy may be encountered in conjunction with vasculopathy. This may allow for incidental trauma that goes unrecognized (e.g., blistering, penetrating foreign body). In chronic osteomyelitis, a sequestrum and involucrum form; these represent islands of infected bone. Bone fragments that are isolated have no blood supply. Administered antibiotics do not penetrate the devascularized infected bone fragments; they can enter the area of osteomyelitis only via the remaining blood supply. Therefore, antibiotic therapy alone cannot cure patients with chronic osteomyelitis without surgical debridement to remove these isolated infected elements. Surgical debridement is essential to remove the infected bony fragments that the antibiotics cannot reach so that affected areas can be treated with antimicrobial therapy.The role of antioxidants in diabetic patients is controversial. In our study the decrease in the levels of total antioxidant and SOD occur with the progression of lipid peroxidation. The lowered values of total antioxidant might be due to its functions as an important component of cellular defence against oxygen toxicity. Oxidative stress occurs in some illnesses like persistent hyperglycemia caused through diabetes induces ROS production by glucose autoxidation [1, 2], activation of protein kinase C, and increase flux through the hexosamine pathway [3]. Oxidative stress induced by hyperglycemia leads to the pathways, which worsen both insulin secretion and action, and promote the development of type 2 diabetes mellitus [9].Total antioxidant and SOD shows that acute phase response and oxidative stress may be playing a major role in the patho physiology of both micro vascular and macro vascular complications and possibly cause various forms of tissue damage in non-diabetic Osteomyelitis and diabetic Osteomyelitis.

Decline in the total antioxidant, SOD and increase in Phosphodiesterase this might be due to the severity of infection. Hence our study demonstrates that the concentration of Total antioxidant, SOD and Phosphodiesterase were the useful markers in predicting the risk of diabetic and non diabetic osteomyelitis.

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