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Role Of Oxiative Stress In Osteomyelitis Patients.

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ABSTRACT

Osteomyelitis (OM) is the local or generalized infection of bone and bone marrow, usually caused by bacteria. It can be introduced by trauma, surgery, by direct extension from a nearby infection or via blood stream**.** The present work involves the estimation of total antioxidant level, Phosphodiesterase (PDE) level , Nitric oxide (NO) level, Vitamin C level, and Malondialdehyde (MDA) level, in diabetic , non-diabetic, and CRP +VE osteomyelitic patients. This study involved 140 osteomyelitic patients with and without diabetes diagnosed and CRP +VE at Ava's College of Medical Laboratory Technology, Moodabidri, compared with healthy individuals. There is a significant increase ($p< 0.0001$) in PDE level and MDA level ,in each diabetic , non-diabetic , and CRP +VE osteomyelitic patients compared to healthy control . Whereas the levels of Vitamin C, Nitric oxide, and total antioxidant had decline significantly ($p<0.0001$). These results recommend that there occurs an imbalance between oxidants and antioxidants, especially an increase in oxidative stress in Osteomyelitis patients.

Keywords: Osteomyelitis, oxidative stress, anti oxidant.

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INTRODUCTION

Osteomyelitis is a bone infection caused by bacteria. The common causative factor responsible for osteomyelitis includes *Staphylococcus spp.*, *Salmonella*, *Mycobacterium spp*, *Pseudomonas* etc. Osteomyelitis may be acute or chronic and may persist sporadically for years. Diabetes and other diseases such as Haemodialysis and spleenection adds to risk of gaining osteomyelitis. Acute bacterial osteomyelitis (OM) in bone and bone marrow infection, that is still associated with significant disability and poor clinical outcome [1]. In children, it is often caused by *Staphylococcus aureus* spread by hematogenous dissemination from some other site of inflammation and, occasionally, from inflamed neighboring tissues or by direct penetration through the open fracture wound. The disease is accompanied by synthesis of proinflammatory cytokines, activation and mobilization of phagocytic cells, thrombosis, necrosis, bone sequestration, and formation of a new bone at the site of infection. OM is seen more in males than females. Despite treatment, up to 30% of bone infections become chronic [2]. Pus spreads into the bone's blood vessels, delaying the blood flow, and areas of devitalised infected bone, known as sequestra, form the basis of a chronic infection. A new bone is formed around the areas of necrosis called an involucrum [3]. Chronic contagion outcomes in various sequelae similar chronic sinuses with reveled bone, decrease of structural wholeness and growth disorder [4]. OM may be owing to intracellular bacteria (within bone cells). Bacteria may abscond and attack other bone cells. Intracellular bacteria go with resistant to some antibiotics guiding to adversity in disposal of this disease and the infection becomes chronic. The tibia, femur, humerus, vertebra, the maxilla and mandibular bodies are particularly sensitive to OM in as much as of the singularly of their blood quantity [5]. Others have determined chronicity, as a wound with revealed bone, positive bone cultures, and drainage for six months [6]. Vitamin C is the primary non-enzymatic water-soluble antioxidant [7] and a controller of bone cells metabolism and viability. In bond tissues, vitamin C caters as a cofactor of lysine and proline hydroxylase, concerned in collagen creation. In vitro studies propose that vitamin C can contain activity and decrease the endurance rate of osteoclasts [8] while improving the survival [9] and distinction of osteoblasts [10]. In addition, supervision of antioxidant vitamins A, E, and C was shown to hasten bone curing after long-bone fixative surgery by declining oxidative injury and encouraging osteogenic activity [11]. Neutrophils are the first line of host immune defense against many bacterial infections and they play a important role in the pathogenesis of OM. Circulating neutrophils are increased in OM and they accumulate at the focus of bone infection, along with bacteria. Serum and bone fragments from these patients have increased levels of inflammatory cytokines [12]. Pro-inflammatory cytokines such as interleukin (IL-1) and tumour necrosis factor (TNF-α) cause activation of the inducible isoform of nitric oxide synthase (iNOS or iNOS-2), and nitric oxide (NO) derived from this pathway stimulates the bone loss [13,14]. Also in the presence of bacteria, increased production of NO occurs due to more endothelial nitric oxide synthase (eNOS or eNOS-3) activity [15]. NO leads to increased reactive oxygen species (ROS) production which damages lipids, proteins, etc leading to oxidative stress. Inducible and endothelial NO causes bone loss by increasing bone resorption, and may account for the osteolysis that is characteristic of OM. Intracellular Phosphodiesterases (PDE) play a role in signal transduction by regulating the cellular concentrations of cyclic nucleotides [16]. Myeloperoxidase (MPO) is an oxidative enzyme with antibacterial activity, which employs H2O2 to create hypochloric acid and other toxic materials in neutrophil phagolysosomes. It has been reported that MPO plays substantial functions in chronic processes such as neurodegenerative diseases and atherosclerosis [17,18]. Malondialdehyde (MDA) the end product of lipid peroxidation, is a excellent symbol of free radical–mediated damage and oxidative stress [19].

MATERIAL AND METHODS

Patients:

The study involves 150 diagnosed osteomyelitis infected patients (19 – 88 years) including male and females, were divided into 3 groups (G2,G3,and G4) , confirmed clinically, microbiologically, and biochemically from the department of Orthopedics Ava's Health Center, Modabidri. K.S.Hegde Medical Collage, Deralaktte and Thejaswini Hospital, Manglore , Dakshina Kannada District, Karnataka. The control (G1) consist of 50 normal healthy individuals. Fasting venous blood is obtained from both groups, put into plain tubes and estimation were done for the following parameters: Myeloperoxidase, Nitric oxide, Vitamin C, Manlondialdehyde, Calcium, Phosphodiesteras, Total antioxidant.

Nitric oxide (NO):

The activity of Nitric oxide was estimated by method of Griese [20]. The measurement was done using sulphanilamide and N-(1-naphthyl) – Ethylene diamine dihydrochloride solution at the absorbance of 550 nm. [20].

Vitamin C:

This method measures the total of L- ascorbic acid and 2,3 – diketogluconic acid and is based upon the measurement of the color product formed by coupling of 2,4- dinitrophenyl hydrazine with ketonic group of oxidized ascorbic acid at 520 nm. The activity of phosphodiesterase can be measured using Bis-p-nitrophenyl phosphate sodium salt, Tris HCL buffer at the absorbance of 405 nm [24]. The activity of vitamin C test was estimated by the method of Dinitro Phenyl Hydrazine (DNPH) [21,22,23].

Total Antioxidant:

The activity of total antioxidant was estimate by method of phosphomolybdenum. The measurement was done using appropriate volume of reagent solution and Ethanol at absorbance of 695nm[25].

Malondialdehyde (MDA):

Malondialdehyde formed by the breakdown of polyunsaturated fatty acids serves as convenient index to determine the lipid peroxidation. So this Malondialdehyde reacts with Thiobabuteric acid to give red colour which is read 1t 535nm. The measurement was done using TBA-TCA-HCL reagent[26].

Phosphodiesterase (PDO) :

Phosphodiesterase are the enzymes which catalyse the hydrolysis of phosphodiester to phosphomonoesters. Phosphodiesterase was assayed using PNPP method [27].

STATISTICAL ANALYSIS

All the results were expressed as mean± SD. The statistical analysis was done by using the independent t-test. The p-values (<0.0001) was considered as highly significant.

RESULTS

The recruited subjects were divided into four groups Group 1 (G1) containing normal subjects, Group 2 (G2) containing patients with Non-diabetic osteomyelitis , Group 3 (G3) containing patients with diabetic osteomyelitis, and Group 4(G4) with CRP +VE patients osteomyelitis.

Table [1] shows that mean serum MDA in patients 2.072 ± 1.076, 2.2 ± 1.31, 2.6 ± 1.461 (µ mol/l) in G 2,G3 and G4 respectively and controls(G1) were 1.6 ± 1.9 (µ mol/l); PDE in patients 245.8 ± 127.09 , 350.27 ± 135.54, 242.68 ± 61.088 (IU) in G 2,G3 and G4 respectively and controls (G1) were 166.8 ± 139.06 (IU). MDA , and PDE levels were significantly higher in the OM patients (p< 0.0001) as compared to that in the controls.

While , level of NO levels in patients were 36.15 ± 15.05, 34.44 ± 12.24, 37.5 ± 17.512 (µ mol/l) in G 2,G3 and G4 respectively and controls (G1) were 52.0 ±13 (µ mol/l) ; Vitamin C in Patients 0.163 ± 0.020, 0.242 ± 0.029, 0.120 ± 0.0024 (mg\dl) in G 2,G3 and G4 respectively and controls(G1) were 0.4 ± 1.5 (mg\dl) ; total AO in patients 87± 21.96, 66.4 ± 13.303, 82.0 ± 7.441(µg\dl) in G 2,G3 and G4 respectively and controls (G1) were 270 ± 380 (µg\dl) . The level of NO, Ascorbic acid, Calcium and total AO was significantly decreased (p< 0.0001) in OM patients as compared to that in the controls, table [1].

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Table 1: Concentration of MDA, PDE, NO, Vitamin C, and Total Antioxidant in normal individuals and OM patients

Parameters	Groups			
	G1	G2	G3	G4
	Control	Non-diabetic Osteomyelitis	diabetic Osteomyelitis	CRP +VE Osteomyelitis
Malondialdehyde (MDA)	1.6 ± 1.9 µM\l	2.072 ± 1.076	2.2 ± 1.31	2.6 ± 1.461
Phosphodiesterase (PDE)	166.8 ± 139.06 IU	245.8 ± 127.09	350.27 ± 135.54	242.68 ± 61.088
Nitric oxidas (NO)	52.0 ±13 µM\l	36.15 ± 15.05	34.44 ± 12.24	37.5 ± 17.512
Vitamin C	0.4 ± 1.5 mg\dl	0.163 ± 0.020	0.242 ± 0.029	0.120 ± 0.0024
Total antioxidant (AO)	270 ± 380 µg\dl	87± 21.96	66.4 ± 13.303	82.0 ± 7.441

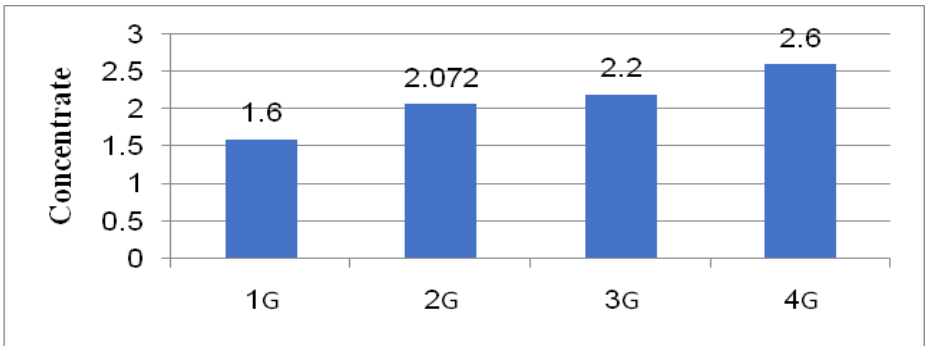

Fig 1: Comparison of Malondialdehyde (MDA)in Group G1 with Groups having patients G2,G3,G4
G1= control , G1 = Non-diabetic osteomyelitis, G3= diabetic osteomyelitism G4= CRP +VE osreomyleitis.

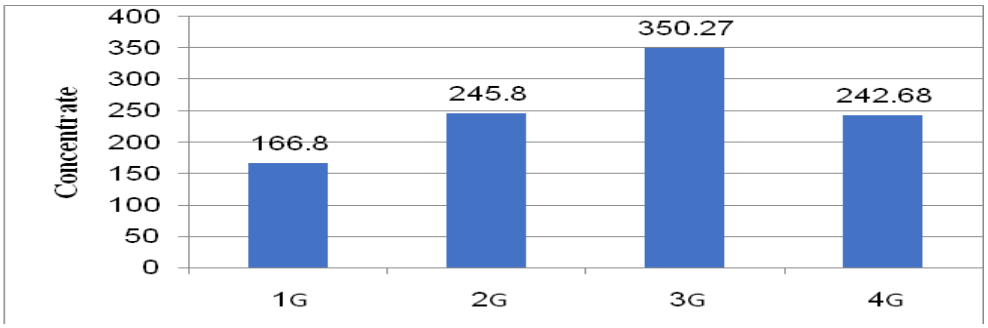

Fig 2: Comparison of Phosphodiesterase level in Group G1 with Groups having patients G2,G3,G4
G1= control , G1 = Non-diabetic osteomyelitis, G3= diabetic osteomyelitism G4= CRP +VE osreomyleitis.

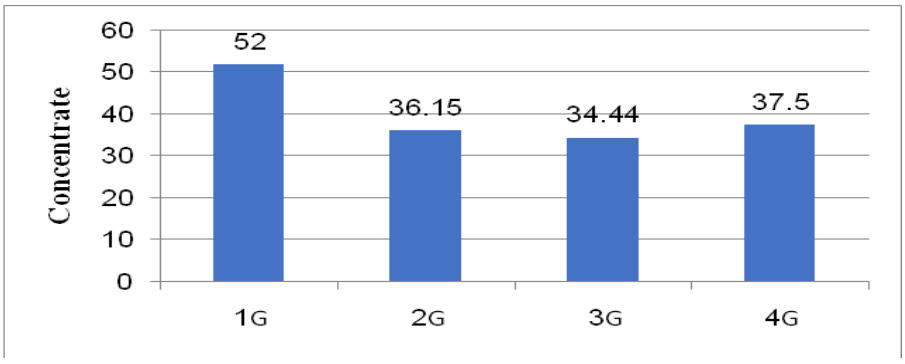

Fig 3: Comparison of Nitric oxid (NO) level in Group G1 with Groups having patients G2,G3,G4
G1= control , G1 = Non-diabetic osteomyelitis, G3= diabetic osteomyelitism G4= CRP +VE osreomyleitis.

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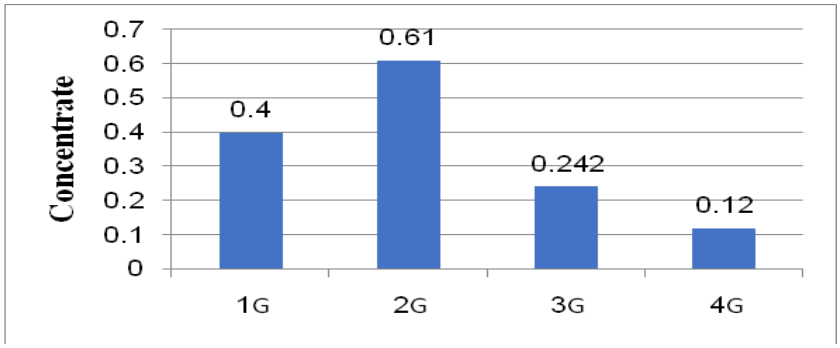

Fig 4: Comparison of Vitamin C level in Group G1 with Groups having patients G2,G3,G4
G1= control , G1 = Non-diabetic osteomyelitis, G3= diabetic osteomyelitism G4= CRP +VE osreomyleitis.

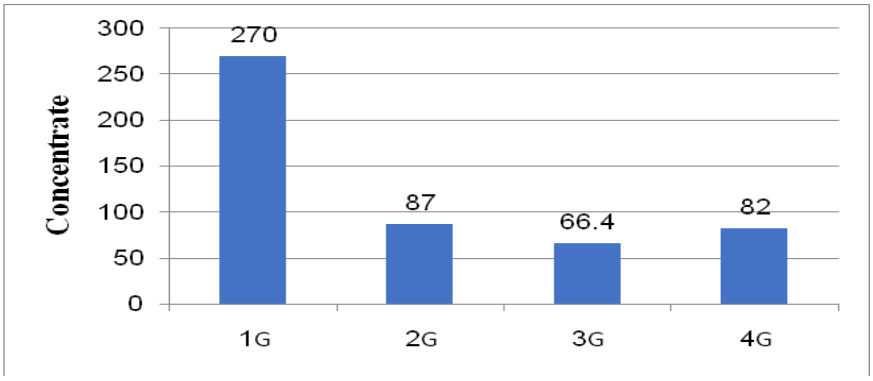

Fig 5: Comparison of Total oxidants level in Group G1 with Groups having patients G2,G3,G4
G1= control , G1 = Non-diabetic osteomyelitis, G3= diabetic osteomyelitism G4= CRP +VE osreomyleitis

DISCUSSION

MDA levels were significantly raised ($p<0.0001$) in patients of OM as compared to controls. The increase in the levels of MDA in this study indicates that lipid peroxidation occurs in patients of OM. The last product of lipid peroxidation, MDA increases due to its high seriousness of infection in OM. The present study was in harmony with the watching of some searchers [28] who have established that there is a significant increase in lipid peroxidation. Studies such as [29] on the activity of paraoxonase and arylesterase in OM patients and showed rise in concentrations of lipid peroxide observed in OM patients revealed to be associated to the rise in oxidative stress and inflammatory conditions present in these patients, and may reason a much more severe status of the disease. Other studies [30] found that lipid peroxidation is an important factor to access the cellular damage. Lipid peroxidation in terms with malondialdehyde (MDA) level was significantly ($p < 0.05$) increased in lymphocytes due to VSSA and VRSA infection as compared to control mice group,which was significantly ($p < 0.05$) decreased due to treatment of nanoconjugated Vancomycin. Ascorbic acid is a radical scavenging antioxidant present in all cells and can also act as a reducing factor [31]. Thus protects the cell against the toxic oxygen free radicals. The lowered values of Vit. C indicate the severity of infection. Ascorbic acid level decreases by scavenging the free radicals and preventing lipid peroxidation. The findings in the present study were in accordance with the observation of various authors like [28] who had detected serum ascorbic acid levels in osteomyelitis patients. Serum nitrite levels were also significantly elevated ($p<0.0001$) in patients of osteomyelitis as compared to controls. Nitric oxide (NO) was produced by the induced isoform of NO synthase (NOS) is an weighty arbitator of inflammation. Peroxynitrite, a cytotoxic oxidant formed from the reaction of nitric oxide (NO) and superoxide is a arbitator of cellular damage in is chaemia/reperfusion damage, shock and inflammation. Peroxynitrite initiates lipid peroxidation and this mechanism also provides to superoxide radical and NO mediated cytotoxicity [32]. In Swiss male mice [30] found that NO level was significantly ($p < 0.05$) increased in lymphocytes due to VSSA and VRSA infections as compared to control group mice, which was significantly ($p < 0.05$) reducted due to treatment of nanoconjugated vancomycin. Other studies[10] carried out on OM patients and establish that OM patients

homozygous for the NOS3 (27-bp repeat, intron 4 polymorphism) 4 allelomorph are more sensitive to OM and showed significantly higher serum NO levels as compared to controls.

CONCLUSION

The work showed that there are significant changes in all parameters when compared to the control. Diabetic complication aggravates the severity of Osteomyelitis.

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