International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 11; Issue 07 (C); July 2022; Page No.1287-1291
DOI: http://dx.doi.org/10.24327/ijcar.2022.1291.0287

Research Article

A COMPARATIVE ANALYSIS OF OXIDATIVE STRESS IN BENIGN BONE TUMORS BEFORE AND AFTER SURGERY

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A R T I C L E I N F O

Article History:

Received 10th April, 2022
Received in revised form 2nd May, 2022
Accepted 26th June, 2022
Published online 28th July, 2022

Keywords:

Oxidative Stress; Bone tumors; MDA; SOD; Antioxidants;

A B S T R A C T

Background: Oxidative stress has been implicated in cancer development increased level of reactive oxygen species (ROS) that disrupts the intracellular reduction-oxidation (redox) balance. Bone tumors constitute 1% of all tumors and benign are more common than malignant. This study was conducted to measure the levels of oxidant stress and antioxidant mechanism in benign bone tumors before and after surgical intervention.

Methods: 16 patients with biopsy proven benign bone tumors treated at a single centre by a single surgeon were included in this study. 16 healthy controls were also included in the study. Serum Malondialdehyde (MDA) levels were determined to assess oxidative stress while antioxidant status was evaluated using superoxide dismutase (SOD). Two samples were taken – One at the time of diagnosis and another 6 month after surgical intervention.

Results: Patients with benign bone tumors showed significant increase in plasma MDA Levels ($p < 0.05$) while significant decreases was noted in SOD levels ($p < 0.05$). Similarly significant association was found between healthy individuals and patients with benign bone tumors ($p < 0.05$) in terms of decreased SOD levels and plasma MDA levels.

Conclusions: In conclusion, an increase in oxidative stress and decrease in antioxidant status is observed in bone tumors which is significantly affected after surgical intervention. Further studies on manipulation of redox balance in patients with bone tumors, benign as well as malignant, can act as useful approach in early diagnosis or designing management strategies for bone tumors.

INTRODUCTION

Bone tumors are classified on the basis of cell origin. Benign bone tumors are more common than malignant tumors. Benign lytic lesions of bone occur most commonly during the first three decades of life and few of them like non-ossifying fibroma, simple bone cyst, enchondroma, bone infarcts etc may be asymptomatic and may be found incidentally on imaging for separate complaints, but they also can present with mild pain and localized swelling of short duration [1]. Further there are certain locally aggressive lesions like giant cell tumor, chondroblastoma, chondromyxoid fibroma, aneurysmal bone cysts and osteoblastomas [2].

Once an accurate diagnosis has been made on basis of radiographs and biopsy one can plan an appropriate line of treatment. Many benign bone tumors are treated by extended curettage including the use of adjuvants, such as liquid nitrogen, phenol, high speed burr, polymethyl methacrylate, or thermal cautery to extend destruction of tumor cells. The defect after curettage can be filled with autologous bone graft, allografts and bone substitutes [3,4].

Various evidences exist supporting the role of oxidants in the development of cancers. In bone tissues, recent studies have demonstrated Reactive oxygen species (ROS) generation as a key modulator of bone cell function and that the pathophysiology of mineralised tissues is influenced by oxidative stress. However, apart from the known risk factors, the function of oxidative stress and its outcome with treatment measures remain to be explored further.

The imbalance between the pro-oxidants and antioxidants in favour towards the former gives rise to oxidative stress that leads to carcinogenesis. Increased ROS formation and decreased efficiency of the antioxidant defence causes the permanent alteration of bimolecular structures (DNA, proteins and lipids) and functions [5]. The reactive intermediates, produced by oxidative stress, alter the structure of the membrane and cause lipid peroxidation of polyunsaturated fatty acids (PUFAs). Free radicals incurred during lipid peroxidation have some local effects due to its short life but their degradation products may serve as 'oxidative stress second messengers' due to the longer half-life and their ability to diffuse from the place of their origin. These decomposition products, mainly aldehydes such as malonaldehyde, hexanal, 4-hydroxynonenal or acrolein, gain a lot of attention because they are the most reactive compounds. Malondialdehyde (MDA) is one of the most common and most harmful products

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of lipid peroxidation, which may lead to cell damaging, reacting with the free amino groups of proteins and nucleic acids, with the target mutagenic activity at the site of guanine in the DNA sequence [6]. Therefore, determination of MDA can be used to estimate the intensity of oxidative stress or damage caused by lipid per-oxidation.

Superoxide ($O_2^{\bullet-}$) is a potent oxidizing agent. Excessive amounts lead to a cascade of reactions causing damage to important biological macromolecules such as DNA, lipids, and proteins. Excess superoxide plays a role in the pathogenesis of many disease states including cancers, cardiovascular disorders, and neurodegenerative diseases [7].

Superoxide dismutases (SODs) are necessary antioxidant enzymes that protect cells from reactive oxygen species (ROS). SODs perform their bio-protective role by converting superoxide into oxygen and hydrogen peroxide. Decreased levels of SODs can cause cancer of the lung, colon, and lymphatic system, as well as neurodegenerative diseases Our study aims to determine the role of oxidative stress in benign bone tumor patients by comparing the level of MDA and SOD before and after surgical intervention. This would provide a better understanding of the role of ROS in bone tumor that could lead to the development of new therapeutic strategies.

MATERIALS AND METHODS

This study was carried out in a tertiary care centre after obtaining approval from Institutional Ethical Committee. The subjects were divided into 3 groups:

Group I: Healthy controls (n=16)
Group II: Patients with benign bone tumors (n=16) before and after surgery.
Informed written consent was obtained from all patients. Control group was age and sex matched irrespective of age and gender.

Besides plain X-ray, computerized tomography (CT) scan and magnetic resonance imaging (MRI) all the patients underwent biopsy for establishing the diagnosis and staging of the tumours. Patients with any other chronic disease or on dietary supplements were excluded from the study.

Venous blood sample was collected under all aseptic precautions from all the patients at the time of diagnosis. Similar samples were collected from healthy controls also. Another sample was taken 6 month post surgical intervention. Serum sample was analyzed for routine biochemical investigations the same day and was stored at -20ºC in separate aliquots.

Measurement of Oxidative Damage-

Determination of Lipid Peroxidation

Serum MDA was analyzed by colorimetric method using the principle that 2- thiobarbituric acid (TBA) reacts with MDA when heated at acid pH. The optical density of the complex TBA-MDAwas then recorded at 535 nm. The concentration of MDA was determined using an MDA standard curve. Results were expressed as micromoles MDA per litre plasma [8,9].

Measurement of Antioxidant Status

Determination of Superoxide Dismutase Activity

Serum SOD activity was measured by enzymatic method on Randox autoanalyzer. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with INT (2-(4-iodophenyl)-3-(-4-nitrophenol)-5-phenyltetrazolium chloride) to form red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction if INT under the condition of the assay. The SOD activity was measured using the linear regression equation from the standard curve. Results were expressed as units per millilitre plasma [10,11].

Routine Biochemistry parameters were estimated on autoanalyzer (Rx Suzuka, United Kingdom) using kits by Randox. The data was compiled and analysed using Chi square test, Fisher's exact test, student t-test and ANNOVA F-test. Relationship between variables was analyzed using Pearson's correlation coefficient. A p value of <0.05 was considered statistically significant.

RESULTS

Demography

Out of 14 patients with benign tumors, 11 (58.8%) were males and 5 (31.2%) were females while in control group, there were 10 males and 6 females. The gender ratio was comparable in both the groups ($p>0.05$). The mean age of the patients was found to be 27.4±7.6 years ranging from16-48 years while that of controls was 21.3±5.8 years ranging from 19-62 years.

Table 1 Demographic profile of study population

Parameters	Benign Bone Tumors	Control Group
Total number (n)	16	16
Sex	Males – 11 Females- 5	Males-10 Females-6
Mean Age	27.4±7.6 years	21.3±5.8

Histopathological diagnosis and treatment

Table 2 Histopathological diagnosis and surgical intervention done

Diagnosis	Location	Treatment
Giant Cell Tumor (n=8)	Knee(n=5)	EC + Bone Cement (n=4) EC + Autograft (n=1)
	Wrist (n=3)	EC + Autograft (n=3)
Aneurysmal Bone Cyst (n=4)	Humerus (n=3)	EC + Autograft (n=2) EC + Bone substitutes (n=1)
	Proximal Tibia (n=1)	EC + Autograft
Enchondroma (n=3)	Metacarpals (n=2) Phalanx (n=1)	EC + Autograft
Chondroblastoma (n=1)	Proximal Tibia (n=1)	Extended Curettage only

EC: Extended Curettage

Extended curettage remained the mainstay of the treatment for all the lesions. Giant Cell tumor of the knee was the most encountered lesion which was treated by extended curettage and defect was filled with bone cement in 4 patients and allograft from ipsilateral iliac crest in one patient. Wrist lesions were managed by extended curettage and bone graft. Patients with aneurysmal bone cyst were managed with autograft (n=3)

and artificial bone substitute (n=1). Lesions of metacarpals and phalanges i.e. Enchondroma were also managed by Extended Curettage and autograft while one patient with Chondroblastoma was managed by Extended curettage only. All the patients were on regular follow up till 1 year.

Oxidative stress & Antioxidant status

The levels of MDA and SOD in different groups are presented in table 1. The difference in the serum levels of both the parameters was found to be highly significant in group III as compared to group I ($p<0.001$) and significant as compared to group II ($p<0.05$) while the difference between group I and group II was not statistically significant ($p>0.05$). A negative non-significant correlation was found between MDA and SOD levels ($r=-0.068$, $p=0.661$) in group III while the correlation in group I ($r=0.076$, $p=0.902$) and group II ($r=0.210$, $p=0.430$) was also not statistically significant.

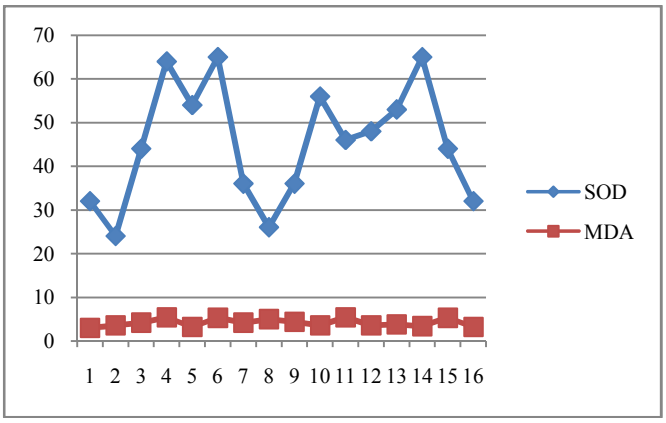

Figure 3 showing correlation between MDA levels and SOD levels in Group II (Benign bone tumors- Post Surgery)

Table 3 Comparison of MDA levels and SOD levels in different groups.

		Group I Healthy controls (n=16)	Group II Benign bone tumors- Pre Surgery (n=16)	Group III Benign bone tumors- Post Surgery (n=16)	P value between Group I & II	p value between Group I & III	p value between Group II & III
MDA (µmol/L)	Mean±S.D	2.46±1.10	5.72±3.2	3.4±2.1	0.0005	0.1359	0.0397
	Range	1.02-3.4	3.24-8.52	2.85-5.33			
SOD (IU/mL)	Mean±S.D	168±32	88±34	42±22	0.0075	0.0523	0.0439
	Range	118-230	47-98	21-65			

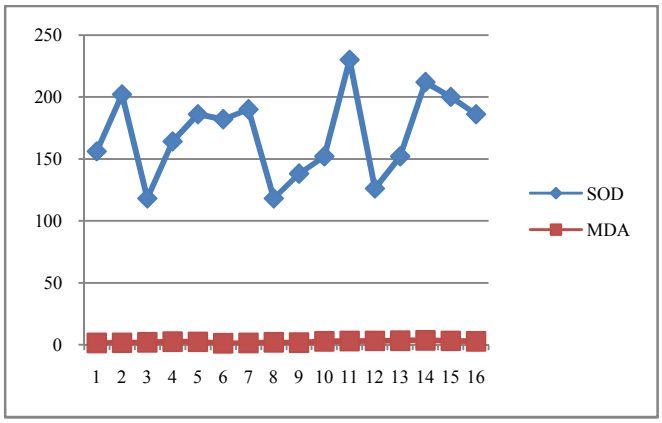

Figure 1 showing correlation between MDA levels and SOD levels in Group I (Healthy Controls).

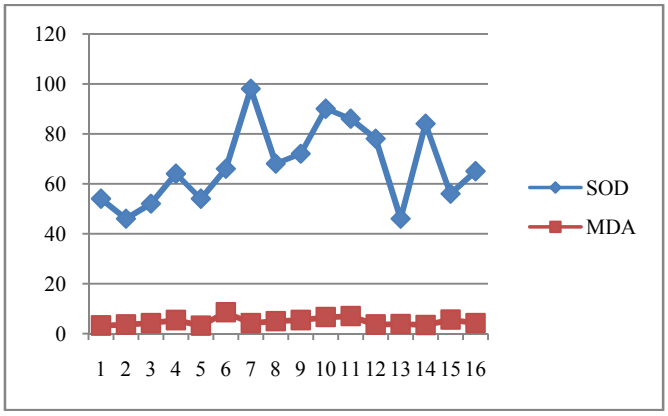

Figure 2 showing correlation between MDA levels and SOD levels in Group II (Benign bone tumors- Pre Surgery)

DISCUSSION

This is a rare study indicating the presence of oxidative stress and diminished antioxidant status in benign bone tumors before and after surgical intervention.

The role of oxidative stress in cancer development is complex and not well-defined. Free radicals are involved in most of the cell process regulation especially active form of these free radicals tends to indicate oxidative stress in a cell. The antioxidative system prevents the organism from the damage caused by oxidative stress. Imbalance between oxidants-antioxidant systems have been investigated in many malignancies including bony malignancies [5]. Several studies have shown increased oxidative stress and decreased antioxidant status in various malignancies like breast, gastric, cervical carcinoma [12,13,14]. Oxidative stress and its correlation with bone tumors have been studied in two studies only till now

Table 4 showing levels of MDA and SOD in bony tumors.

Study (Year)	Type of Cancer	Serum MDA levels	Serum SOD levels
Nathan *et al* (2011)[5]	Bone & Soft tissue Sarcomas (n=47)	7.30 ± 4.10	34 ± 34
Dhupper *et al* (2022)[15]	Bone tumors		
	Benign (n=14)	5.72±3.2	78±34
	Malignant (n=14)	7.40±4.10	42±22

Till now no study have been conducted to measure the levels of oxidative stress and its effect after surgical intervention. Few studies been mentioned in literature to study the association of surgery and oxidative stress. In a study conducted by Leimkühler *et al* in 2022 concluded that patients with lower preoperative serum free thiol levels, indicating a decrease in extracellular antioxidant capacity and therefore an increase in systemic oxidative stress, are more likely to develop postoperative complications and show a longer in

hospital stay than patients with higher serum free thiol levels[16].

Decreased antioxidant status as measured SOD levels was observed in bone tumors, benign as compared to healthy controls results of which are consistent with the literature. Possible explanation for the same could be due to exhaustation of antioxidant reserves in the attempt to counteract the DNA, lipid and protein damage. Another explanation could be the elevated DNA, lipid and protein oxidation may have occurred as a result of a weak defense system. Similar reductions have also been observed in breast cancer, cervical cancer, gastric cancer and many more.

Further the decrease in SOD levels was found to be statistically significant between group I and III ($p=0.000413$) and between groups II and III ($p= 0.0407$) while the difference was not significant between groups I and II ($p>0.05$). Nathan *et al* in his study also noticed significant reduction in antioxidant enzymes, SOD and CAT in patients with bone and soft tissue sarcoma ($p = 0.000$) which are consistent with findings of our study.

Limitations of this study include relatively small sample size in each subgroup analyzed, and susceptibility of MDA to artifacts as it can react with aldehydes other than MDA [17], and DNA and protein oxidation were not evaluated. Finally, the oxidative stress levels found in serum in our study population may not reflect cellular concentrations, and results should be interpreted with caution.

Strengths of this study include the prospective design, homogenous population from a socioeconomic and geographic standpoint, the exclusion of patients with metastatic disease, and robust statistical analysis controlling for confounding variables such as sex, age. The novelty of our findings is strength of this study since it can potentially identify future biomarkers for screening and early detection of bone tumors and potential targets for individualized treatment.

CONCLUSION

To summarize, present study measured oxidative stress (MDA levels) and antioxidant status (SOD levels) in benign bone tumors and studied the same after surgical intervention. Effect of surgical intervention on oxidative stress and antioxidant levels is less researched area till now. A small sample size may be the limitations of present study but novelty of this study is strength of the same.

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How to cite this article:

Vasudha Dhupper *et al* (2022) 'A Comparative Analysis of Oxidative Stress In Benign Bone Tumors Before And After Surgery', *International Journal of Current Advanced Research,* 11(07), pp. 1287-1291.
DOI: http://dx.doi.org/10.24327/ijcar.2022.1291.0287

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