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ORIGINAL ARTICLE

Protective Effects of Omega-3 Fatty Acid on Salt Induced Microscopic Changes in Femur of Female Sprague Dawley Rats

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ABSTRACT

Osteoporosis is a major risk factor for fracture affects an enormous number of people of both genders worldwide.
Objectives: To evaluate the shielding effect of omega-3 fatty acids on high salt induced histological findings in femur of rats.
Study Design: Randomized Control Trial.
Methodology: Female rats (n=30) were divided into three groups. Group A recieved high salt diet (8%NaCl) while group B recieved omega-3-treated salt loaded diet receiving 260 mg/kg body weight with 8% NaCl solution (8 weeks), control group received standard diet. Tissue from mid shaft and proximal end of femur was obtained to study the osteoblast number, mid cortical bone thickness and trabecular bone architecture.
Statistical analysis: SPSS software, v 21 analyzed data.
Results: Protective effects were seen in Omega-3 fatty acid supplemented experimental group B with increase in osteoblast number, mid cortical bone thickness and increase in microstructure of trabeculae.
Conclusion: We concluded that dietary nutrient like omega-3 fatty acid is a helpful tool in eliminating adverse effects of salt on bones by enhancing osteoblastic activity thus reducing its remodeling.
Keywords: Bone, Osteoblast, Omega-3 fatty acid, Salt and Trabeculae.

INTRODUCTION

Osteoporosis is a major risk factor for fractures that affect an enormous number of people of both genders globally[1]. Its a silent bone disease that has features like reduced bone quality, increased bone fragility leading to increase susceptibility towards fractures and disruption of micro-architectural integrity[2]. According to an estimate, this health issue causes more than 2 million fractures/year, putting additional burden on economy and society[3]. Bone loss due to aging is a current health issue that increases risk of fractures thus forcing to imply new, instant prevention strategies.

According to previous studies, humans have been consuming salt less than 0.25 g/day for several years. However, the average salt intake is approximately 9-12 g/d whereas WHO recommends that sodium intake should be <2 g/day. Health issues like hypertension, cardiovascular disease (CVD), weak bones and osteoporosis do arise in patients taking high salt diet[4].

Previous research showed that salt intake controls urinary calcium excretion by upregulating intestinal absorption along-with mobilizing calcium from bone[5]. Individuals frequently exceed dietary recommendations for salt[6]. Salt addiction results in many deaths/year globally by aggravating hypertension and osteoporosis[7] as human dietary salt intake has increased up to tenfold, food processing being the most substantive source[8].

Osteoporosis is a disease of bones and joints that have many reasons. Numerous micro and macro dietary nutrients like salt usually affects bone health by various mechanisms like controlling rate of bone metabolism, preventing bone loss and fractures through less osteoclastic activity and increasing bone formation by osteoblasts[9].

Nevertheless, omega-3 fatty acid long chain, regulates bone metabolism thus prevent osteoporosis. They produce protective effects through various mechanisms like decreasing prostaglandins expression, modulation of pro-inflammatory cytokines and improve calcium accretion in bone[10]. They also stimulate bone growth by altering structure of growth plate, thus improves trabecular and cortical morphology and bone quality[11]. Among post-menopausal women, bone mineral density improved as a result of fatty acids supplementation as evident by previous studies[12].

Objectives: To evaluate the shielding effect of omega-3 fatty acids on high salt induced histological findings in femur of rats.

METHODOLOGY

It was randomized control trial study. Data was collected from took place in Islamic International Medical College, Anatomy department, Rawalpindi after ethical approval. Animals enrolled were female Sprague Dawley rats (n=30)**.** They were 10-12 weeks old. Group A: They were fed high salt diet (8%NaCl). Group B: They recieved omega-3-treated salt loaded diet receiving 260 mg/kg body weight omega-3 by oral gavage with consumption of 8% NaCl solution for 8 weeks, Group C (control group): Diet was not altered. At the end of experiment, they were dissected while removing left femora. Decalcification was performed. Mid shaft and proximal end of femur provided the tissue to study the osteoblast number, mid cortical bone thickness and trabecular bone architecture. Histological slices of specimen were prepared by H&E staining for microscopy.

Statistical Analysis: Data analyzed by SPSS v-21.0. Quantitative data analysis was expressed as mean ± SD. ANOVA and Pearson Chi Square tests were used for analysis with P-value ≤0.05 was considered significant.

RESULTS

Number of osteoblasts in femur among different study groups were presented as Mean ± SD . Significant difference in osteoblast number between different groups was noticed (p<0.05) as shown in Table-1. Histologically, osteoblasts in femur were shown by figure-1

Table-1: Osteoblast Number in Femur among Groups as Mean

Parameters	Groups	Mean ± SD	P value
Osteoblast Number	A	7.2±1.0801	0.000*
	B	8.11±0.6315	
	C	9.3800±0.77	

*Statistically significant

Results (Table-2) clearly indicated that there was a decrease in number of osteoblasts in group-A while increase in number of osteoblast due to omega-3 fatty acids in group-B.

Table-2: Multiple Comparison of Mean Osteoblasts Number in Femur

Groups	C	A	B
Mean difference	2.18	1.27	-0.91
p value	0.0001*	0.0027*	0.029*

*Statistically significant

The thickness of cortical bone of femur among different study groups were presented as Mean ± SD. The results were significant (p<0.05) among different groups as shown by Table-3.
The mean cortical thickness between groups (Fig-2) showed significant difference having p<0.05 as shown in Table-4.

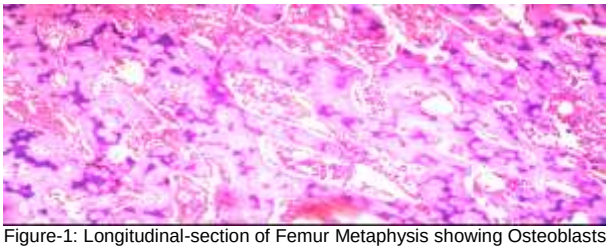

Figure-1: Longitudinal-section of Femur Metaphysis showing Osteoblasts

Table-3: Cortical Bone Thickness in Femur among Groups as Mean

Parameters	Groups	Mean ± SD	P value
Cortical Bone Thickness	A	36.20±9.7616µm	0.003*
	B	43.15±8.2531µm	
	C	48.75±6.0976µm	

*Statistically significant

Table-4: Multiple Comparison of Mean Cortical Thickness (µm) Of Femur

Groups	C	A	B
Mean difference	12.55	5.6	-6.95
p value	0.0008*	0.0295*	0.0298*

*Statistically significant

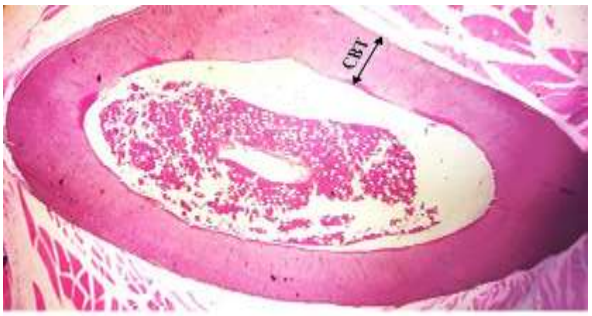

Figure-2: Cross-section of mid shaft Femur showing cortical bone thickness

Score of trabecular change of femur among different study groups was presented as Mean ± SD as shown by Table-5.

Table-5: Cortical Bone Thickness in Femur among Groups as Mean

Parameters	Groups	Mean ± SD	P value
Trabecular Change of Femur	A	1.6±0.6992	0.000*
	B	0.90±0.5676	
	C	0	

*Statistically significant

DISCUSSION

Bone is a tissue which changes under the influence of certain circumstances and has a variable organization just to fulfill diverse functional requirements[13]. Our results showed that decreased number of osteoblasts in group-A were due to deleterious effects of high salt content in diet thus were in line with previous work[14]. Certain parameters like decreased ALP activity, lesser production of growth factors and low bone formation showed decrease osteoblasts count that ultimately results in osteoporosis.

Alkaline phosphatase (ALP) is a marker of osteoblastic activity in bones as documented previously[15,16]. However, one researcher reported that low calcium and ALP levels effect bone integrity after salt loaded diet in rats[17]. ALP levels are useful in determining osteoblastic activity, hence used in animal models of experimental induced osteoporosis[17].

In the present study, increase in the osteoblasts number of group B, was also validated by one researcher who reported that Omega-3 has many beneficial effects like increased cortical and trabecular bone thickness as well as decreased osteoclasts number. He also demonstrated that it improves the disturbed bone status imposed by salt loading[15]. The cortical bone thickness is an important parameter to evaluate bone quality and strength[18]. Decrease trabecular architecture has was shown previously by osteoporosis[19,20]. Our results showed that group-A had widening of inter-trabecular spaces with reduction in trabecular thickness. Similar findings with adverse effects of high salt on trabecular structure have been reported previously[15].

Limitations: The study has few limitations as well. The size of the sample was not enough to generalize the results. Limited resources were available.

CONCLUSION

We concluded that salt loaded diet exerted detrimental effects on bones due to decrease in osteoblast number and mid cortical bone thickness thus causing bone loss with weakness. Omega-3 fatty acid is a helpful tool in eliminating adverse effects of salt on bones by enhancing osteoblastic activity thus reducing its remodeling.

Authors' Contribution:

KA&NM: Conceptualized the study, analyzed the data, and formulated the initial draft.

SA&AY: Contributed to the histomorphological evaluation.

IZ&MZ: Contributed to the analysis of data and proofread the draft.

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REFERENCES

1. Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. European journal of rheumatology. 2017;4(1):46.
2. Elbossaty W. Mineralization of Bones in Osteoporosis and Osteomalacia. Ann Clin Lab Res. 2017;5(4):201.
3. Hiligsmann M, Evers SM, Sedrine WB, Kanis JA, Ramaekers B, Reginster J-Y, et al. A systematic review of cost-effectiveness analyses of drugs for postmenopausal osteoporosis. Pharmacoeconomics. 2015;33(3):205-24.
4. Bhardwaj P, Rai D, Garg M. Zinc improves the bone mechanical strength in ovariectomized rat model by restoring bone composition and hydroxyapatite crystallite dimension. Vitam Miner. 2016;5:137.
5. Abou-Saleh H, Ouhtit A, Halade GV, Rahman MM. Bone Benefits of Fish Oil Supplementation Depend on its EPA and DHA Content. Nutrients. 2019;11(11):2701.
6. Carbone L, Johnson KC, Huang Y, Pettinger M, Thomas F, Cauley J, et al. Sodium intake and osteoporosis. Findings from the Women's Health Initiative. The Journal of Clinical Endocrinology & Metabolism. 2016;101(4):1414-21.
7. Kim Y, Kim H-Y, Kim JH. Associations between reported dietary sodium intake and osteoporosis in Korean postmenopausal women: The 2008-2011 Korea national health and nutrition examination survey. Asia Pacific Journal of Public Health. 2017;29(5):430-9.
8. He FJ, MacGregor GA. Reducing population salt intake worldwide: from evidence to implementation. Progress in cardiovascular diseases. 2010;52(5):363-82.
9. Aparicio A, Rodríguez-Rodríguez E, Cuadrado-Soto E, Navia B, López-Sobaler A, Ortega R. Estimation of salt intake assessed by urinary excretion of sodium over 24 h in Spanish subjects aged 7–11 years. European journal of nutrition. 2017;56(1):171-8.
10. Tekol Y. Salt addiction: A different kind of drug addiction. Medical hypotheses. 2006;67(5):1233-4.
11. Legetic B, Campbell N. Reducing salt intake in the Americas: pan American health Organization actions. Journal of health communication. 2011;16(sup2):37-48.
12. Maggio M, Artoni A, Lauretani F, Borghi L, Nouvenne A, Valenti G, et al. The impact of omega-3 fatty acids on osteoporosis. Current pharmaceutical design. 2009;15(36):4157-64.
13. Rousseau JH, Kleppinger A, Kenny AM. Self-reported dietary intake of omega-3 fatty acids and association with bone and lower extremity function. Journal of the American Geriatrics Society. 2009;57(10):1781-8.
14. Bonnet N, Ferrari SL. Effects of long-term supplementation with omega-3 fatty acids on longitudinal changes in bone mass and microstructure in mice. The Journal of nutritional biochemistry. 2011;22(7):665-72.
15. Koren N, Simsa-Maziel S, Shahar R, Schwartz B, Monsonego-Ornan E. Exposure to omega-3 fatty acids at early age accelerate bone growth and improve bone quality. The Journal of nutritional biochemistry. 2014;25(6):623-33.
16. Watkins BA, Li Y, Lippman HE, Feng S. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. Prostaglandins, leukotrienes and essential fatty acids. 2003;68(6):387-98.
17. Yatabe MS, Yatabe J, Takano K, Murakami Y, Sakuta R, Abe S, et al. Effects of a high-sodium diet on renal tubule Ca 2+ transporter and claudin expression in Wistar-Kyoto rats. BMC nephrology. 2012;13(1):160.
18. Varagic J, Ahmad S, Brosnihan KB, Habibi J, Tilmon RD, Sowers JR, et al. Salt-induced renal injury in spontaneously hypertensive rats: effects of nebivolol. American journal of nephrology. 2010;32(6):557-66.
19. Shady AM, Nooh HZ. Effect of black seed (Nigella sativa) on compact bone of streptozotocin induced diabetic rats. Egyptian Journal of Histology. 2010;33(1):168-77.
20. Zidan RA, Elnegris HM. Effect of homocysteine on the histological structure of femur in young male albino rats and the possible protective role of folic acid. Journal of Histology & Histopathology. 2015;2(1):16.