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Zinc Supplementation Mitigates High Salt Diet-Induced Bone Damage: A Histological Evaluation of Osteocyte Apoptosis

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Abstract

Objective: This study aims to evaluate the protective role of zinc supplementation against bone damage induced by a high-salt diet, with a specific focus on osteocyte apoptosis in rats.

Methods: This was an investigational study conducted at the Army Medical College, Rawalpindi, Pakistan, over eight weeks. Thirty female Sprague-Dawley rats were randomly divided into three groups: control (Group C), high-salt diet (Group A), and high-salt diet with zinc supplementation (Group B). Group C received a standard diet, Group A received a diet with 8% sodium chloride, and Group B received the high-salt diet plus zinc supplementation at 50 mg/kg body weight daily via oral gavage. After eight weeks, femurs were harvested, processed, and stained with hematoxylin and eosin. Osteocyte apoptosis was assessed by counting empty lacunae and apoptotic bodies under light microscopy.

Results: The high-salt diet group (Group A) exhibited a significantly higher density of apoptotic osteocytes compared to the control group (mean ± SD: 2.3166 ± 0.820 vs. 1.3666 ± 0.431 per unit area; p=0.0005). Zinc supplementation in Group B resulted in a significant reduction in osteocyte apoptosis compared to both the high-salt diet group (mean ± SD: 1.7000 ± 0.492 per unit area; p=0.0087) and the control group (p=0.0009). These findings indicate that zinc supplementation effectively reduces osteocyte apoptosis caused by high salt intake.

Conclusion: Zinc supplementation significantly mitigates the harmful effects of a high-salt diet on bone health by reducing osteocyte apoptosis. This suggests its potential as a therapeutic intervention to counteract salt-induced bone damage and prevent related diseases such as osteoporosis.

Keywords: Zinc, salt, osteocytes, apoptosis, osteoporosis.

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1. Introduction

Osteoporosis represents a major global health issue that affects millions of people worldwide and is expected to increase due to the ageing population and social conditions. Osteoporosis is responsible for the largest number of fractures worldwide each year and results in high morbidity, mortality and medical costs.[1] Osteoporosis, a progressive bone disease, is characterized by a decrease in bone mass, bone strength and deterioration of strong bone microstructure. This predisposes people to fractures, which can have a significant impact on overall health and well-being.[2]

Bone homeostasis, the subtle balance between bone formation and bone resorption, is carefully maintained through a coordinated process known as bone remodelling. This remodelling process involves interactions between bone-resorbing osteoclasts and bone-forming osteoblasts; this results in bone integrity and helps coordinate strength and stiffness. Disruption of this essential homeostasis, characterized by abnormal bone fragility, can lead to enhanced osteogenesis and a heightened risk of fracture.[3] Osteocytes have been identified as critical regulators of bone remodelling. These unique cells have a powerful network of dendritic processes that convert mechanical signals into biochemical signals; these signals modulate the activity of osteoblasts and osteoclasts through the expression of different factors such as sclerostin and RANKL.[4] A diet high in sodium chloride (salt) is a triggering factor for osteoporosis and fractures. High salt consumption is associated with increased calcium in the urine, resulting in decreased calcium absorption and accelerated bone loss. This underlines the significance of limiting dietary sodium intake and ensuring adequate calcium intake for bone health, especially in individuals with high salt consumption.[5]

Zinc, an important mineral, has various effects on bone turnover. It acts as a cofactor for many enzymes involved in the differentiation, proliferation and

elimination of osteoblasts, resulting in bone formation.[6] Additionally, zinc exhibits anti-inflammatory properties by suppressing osteoclast activity and regulating the expression of osteoprotegerin (OPG), an important inhibitor of osteoclastogenesis. A recent study showed the potential of zinc supplementation to reduce age-related bone loss and osteoporosis.[7]

Given the negative effects of high salt intake on bone health and the literature supporting the benefits of zinc for bone health, this study aimed to investigate the potential protective role of zinc against salt-induced bone loss. The theory is that zinc supplementation may reduce the negative effects of high salt intake on bone metabolism, providing strong bone protection by modulating the activity of osteoblasts and osteoclasts.

2. Materials & Methods

This investigational study obtained ethical approval from the Institutional Review Board of Army Medical College, Rawalpindi, Pakistan. Thirty female Sprague-Dawley rats, aged three months, were procured from the National Institute of Health (NIH), Islamabad. Upon arrival, the animals were housed in standard polypropylene cages with wire mesh lids and allowed a one-week acclimatization period in a controlled environment. The environmental conditions maintained a 12-hour light/dark cycle, temperature range of 21-25°C, and humidity of 40-60%. During the acclimatization phase, the animals had ad libitum access to standard laboratory rodent chow and tap water.

The rats were randomly assigned to three experimental groups of ten animals each. Group C was the control group and received the standard laboratory diet. Group A was given a high-salt diet prepared by adding 8% sodium chloride (NaCl) to the normal diet. Group B (also had high salt) received zinc supplementation at a dose of 50 mg/kg body weight, based on previous studies on the effects of zinc in a rat model.8 The zinc solution was administered by oral gavage once daily throughout the eight-week study.

At the end of the eight-week study period, animals were euthanized according to an approved protocol. The left femur was carefully dissected, cleaned, and placed in a neutral 10% solution of Formalin for 48 hours. Decalcification was performed using a 5-10% aqueous solution of nitric acid for 12-24 hours, followed by routine tissue processing and embedding in paraffin wax. Sections with a thickness of 5 µm were obtained using a rotary microtome for subsequent histological analyses. Histological examination was performed by hematoxylin and eosin (H&E) staining. For the detection and quantification of osteocyte apoptosis, empty lacunae were identified under light microscopy at a magnification of 400x (10x eyepiece and 40x objective). In addition to empty lacunae, morphological features suggestive of apoptosis, such as condensed and fragmented nuclei with marginated chromatin, were also considered for identification.9 The number of apoptotic osteocytes within the femoral cortex was quantified along the four anatomical axes (anterior, posterior, medial, and lateral), as depicted in the provided schematic diagram. Briefly, measurements were taken across the entire cortical width, from the periosteal to the endocortical surface, for each anatomical axis.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 27. Quantitative data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was employed for inter-group comparisons. Intra-group comparisons were conducted using a paired-sample t-test. The Pearson Chi-Square test was utilized for analyzing qualitative data. A p-value less than 0.05 was considered statistically significant.

3. Results

Microscopic examination of the femoral diaphysis revealed significant differences in the density of apoptotic osteocytes across the three experimental groups (Fig. 1). As illustrated in Figure 1, the control group (Group C) exhibited the lowest density of apoptotic osteocytes (mean ± SD: 1.3666 ± 0.431 per unit area). In contrast, the high-salt diet group (Group A) displayed a marked increase in the number of apoptotic osteocytes compared to the control group (mean ± SD: 2.3166 ± 0.820 per unit area; p=0.0005). Notably, zinc supplementation in Group B resulted in a significant reduction in the density of apoptotic osteocytes compared to the high-salt diet group (mean ± SD: 1.7000 ± 0.492 per unit area; p=0.0009 compared to Group C and p=0.0087 compared to Group A). These findings are further corroborated by the statistical analyses presented in Tables 1 and 2.

Table 1: FEMUR Mean density of apoptotic osteocytes per unit area

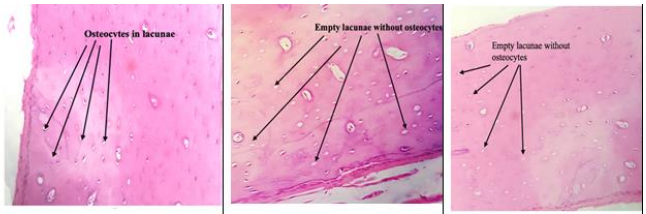
Groups	C	A	B
Mean score	1.3666	2.3166	1.7000
Standard Deviation	0.4316	0.8208	0.4928
SEM	0.1111	0.2119	0.1272
p-value		0.004	

Table 1 summarizes the mean density of apoptotic osteocytes per unit area in the femoral diaphysis for all experimental groups.

Table 2: FEMUR Differences in apoptotic osteocyte density between the groups

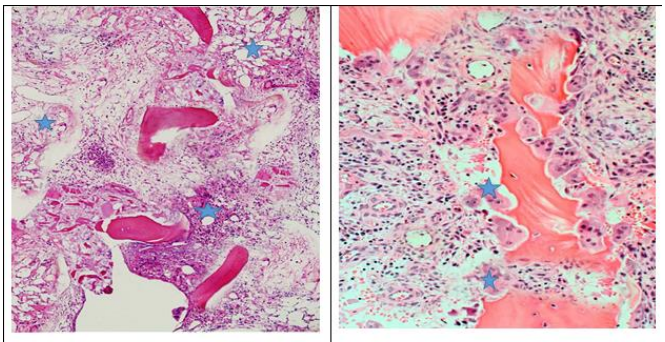
Groups	C	A	B
Mean difference	-0.950	-0.333	0.616
p-value	0.0005*	0.0009*	0.0087*

Table 2 presents the results of multiple comparisons, highlighting the statistically significant differences in apoptotic osteocyte density between the groups ($p<0.05$ for all comparisons).



Control group (C) Longitudinal section of Femur showing Osteocytes in their lacunae H&E, X40	High-salt diet group (A). Longitudinal section of Femur showing apoptotic osteocytes in the form of empty lacunae H&E, X40	Zinc-supplemented group (B) Longitudinal section of Femur showing apoptotic osteocytes in the form of empty lacunae H&E, X40

Figure 1: Photomicrographs of apoptotic osteocytes in the femoral diaphysis stained with hematoxylin and eosin (H&E). A) Control group (Group C). B) High-salt diet group (Group A). C) Zinc-supplemented group (Group B).



High-salt diet group (A). The longitudinal section of the Femur shows more apoptotic osteocytes (star) in the form of empty lacunae H&E, X40	Zinc-supplemented group (B) Longitudinal section of the Femur showing fewer apoptotic osteocytes (star) in the form of empty lacunae H&E, X40

Figure 2: Photomicrographs of apoptotic osteocytes in the femoral diaphysis stained with hematoxylin and eosin (H&E). High-salt diet group (Group A). C) Zinc-supplemented group (Group B)

4. Discussion

The present study investigated the protective effects of zinc supplementation against high dietary salt-induced bone impairment in the long bones of rats, as assessed by the quantitative histological parameter of osteocyte apoptosis. The findings suggest that zinc supplementation can mitigate the deleterious effects of excessive salt intake on bone health.

Bone is a highly dynamic tissue that constantly changes in response to various stimuli. The maintenance of healthy bone is determined by the delicate balance between bone formation by osteoblasts and bone resorption by osteoclasts. This process is known as bone remodelling.[10] An imbalance in this integrated system that supports high bone density can lead to osteoporosis, a progressive metabolic bone disease that has been identified as a major health problem and affects more than 200 million people worldwide.[11]

This study used osteocyte apoptosis as a histological measure to assess the extent of bone damage caused by high salt intake. Osteocyte apoptosis has been shown to play a role in various experimental models of osteoporosis, with increased apoptotic osteocytes in the vertebrae.[12] Microscopic examination of cortical bone revealed a significantly higher number of free lacunae, indicative of apoptotic osteocytes, in the high-salt diet group (Group A) compared to the control group. The current study supports previous research showing the negative effects of high salt intake on bone health.[13] High sodium chloride (NaCl) intake is linked to increased levels of calcium and parathyroid hormone (PTH) in the urine, leading to increased bone wasting and bone loss.[14] Zinc-treated group (Group B) showed a significant reduction in the number of apoptotic osteocytes compared with the control group and the high salt group. It was comparable to a previous study.[15] This finding is also supported by the previous study which demonstrated the antiapoptotic effect of zinc in a mouse model.[16] The protective role of zinc in maintaining bone structure is consistent with previous reports on the anabolic and bone-forming effects of zinc supplementation, as evidenced by a reduction in osteocyte apoptosis. Zinc plays an important role in bone metabolism, and its deficiency plays a role in promoting bone resorption.[17] Zinc supplementation has been shown to improve bone mechanical capacity in ovariectomized rats by restoring bone marrow and hydroxyapatite crystallite measurements. In addition, zinc has a

synergistic effect on both bone stimulation and regulation of bone resorption in ovariectomized rats, ultimately increasing bone mass.[15] Few studies have shown that oral zinc treatment has a protective effect on osteogenesis. Zinc prevents the effects of various environmental factors that can negatively affect bones at a younger age. Zinc supplementation has been linked to partially or completely preventing the negative effects of cadmium toxicity on bone growth.[17] The results of our study suggest that zinc supplementation may prevent the negative effects of high salt intake on bone histology. The increase in osteocyte apoptosis in high-salt rats and the decrease in apoptosis after zinc supplementation are consistent with previous research showing that zinc supplementation can significantly ameliorate bone loss, as established by the morphological features of the structure decrease in the number of osteoclasts and continuous proliferation of osteocytes. The important role of zinc in bone metabolism has also been proven by its role in bone formation, collagen matrix synthesis, mineralization and bone regulation. Zinc promotes bone formation by inducing the expression of transcription factor 2 (Runx2), a key regulator of osteoblast differentiation.[17] Zinc also exhibits anti-inflammatory properties by inhibiting osteoclast-like cells and reducing bone loss. Zinc modulates the RANKL/RANK/OPG pathway, a critical cascade signalling pathway involved in bone remodelling.[17] As bone substitute materials rich in zinc ions gain popularity in the field of bone grafting technology and new treatments, research is still needed to find a method that can be used to stimulate bone formation and improve osseointegration.[15]

5. Conclusion

Osteocyte apoptosis plays an important role in bone remodelling and mineralization. These studies have shown that high salt intake triggers apoptosis of osteocytes, which compromises bone integrity. Zinc supplementation appeared to attenuate these effects, reducing osteocyte apoptosis and preserving osteocyte survival, although not completely. These findings demonstrate the therapeutic potential of zinc in counteracting the adverse effects of high salt exposure on bone health by reducing osteocyte apoptosis. Further research may develop zinc-based strategies to maintain bone integrity and prevent bone diseases such as osteoporosis.

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K.A, A.Q, N.M, M.M.K, S.I, A.A - Analysis/Interpretation/Discussion
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