Up-Regulation of Heat Shock Proteins (HSPs) in Osteoporosis in Young Females of Reproductive Age

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Abstract:

Background: Osteoporosis is the most common bone pathology in the world. It has a multifactorial pathophysiology which is only partially understood. Recently there has been some interest in the role of HSPs in the pathophysiology of osteoporosis but the available data are limited.

Objectives: Aim of this study is to correlate relationship between heat shock proteins (HSPs) and osteoporosis by comparing serum levels of HSP-27, 70 and 90 in osteoporotic patients with controls (healthy individuals).

Methodology: A cross sectional comparative study was thus designed. Fifty (50) self-reported healthy age matched controls were recruited from local population. Fifty (50) patients were diagnosed according to the protocol of The International Society for Clinical Densitometry. Premenopausal women presenting with fragility fractures and BMD Z-score less than or equal to –2 were diagnosed as osteoporosis patients. Five ml of blood was taken and serum was separated for biochemical analysis of HSP-27, HSP-90, HSP-70 using human ELISA kits (Bio-Vendor). Statistical analysis was performed by SPSS statistics 17.0.

Results: Mean values of HSP-27, HSP-70 and HSP-90 were raised in osteoporotic females in comparison with controls at p=0.001, p=0.04 and p=0.02 respectively.

Conclusion: Our study expands on role of HSPs in bone homeostasis. Future studies may further explore HSPs as therapeutic target in osteoporosis

Keywords: HSP-70, HSP-27, HSP-90, heat shock protein, osteoporosis, bone homeostasis

Introduction

Osteoporosis literally means "porous bone". It is reported to be the most common bone pathology in the world. It is marked by reduced bone mineral density (BMD) which leads to bone fragility increased risk of bone fractures [1]. According to the World Health Organization (WHO), osteoporosis should be diagnosed when BMD is 2.5 standard deviations (SDs) or lower than the average value for young healthy women (T-score≤ 2.5) [2].

Osteoporosis has a multifactorial pathophysiology which is only partially understood. It underlying mechanisms include genetic, hormonal, inflammatory and mechanical factors. Bone homeostasis is a dynamic of remodeling a balance between the process of resorption and deposition. In youth, deposition outweighs resorption leading to skeletal growth. Peak mineral bone density is achieved at 18 to 25 years, at which point, bone resorption equals deposition. The degree of peak mineral density (PMD) is determined by multiple factors including genetics, nutritional status and physical activity. As age progresses resorption outweighs deposition, bone mineral density reduces and a person may develop osteoporosis if BMD falls below a critical point [3].

The risk of osteoporosis is minimal in women of reproductive age group and increases dramatically after menopause when estrogen levels fall [4]. Estrogen controls bone mineral density via receptors present on osteocytes, osteoblasts, osteoclasts as well as immune cells. Over all it inhibits bone resorption and stimulates bone deposition leading to increased bone mineral density [5] However in some women bone mineral density may decrease even before menopause. Risk factors include vitamin D deficiency, smoking, sedentary life style and genetic factors causing low baseline bone mineral density. Endocrinal disorders like primary renal or liver failure and liver, connective tissue disorders like osteogenesis imperfecta, and certain drugs may also contribute to premenopausal menopause [6, 7].

One of the most common causes of osteoporosis in younger women is vitamin D deficiency. Chronic vitamin D deficiency can lead to secondary hyperparathyroidism, which in turn causes increased resorption, osteoporosis and increased risk of fractures. It has been shown that vitamin D supplements with or without Calcium can improve bone mineral density and reduce the incidence of fractures [8]. Calcium is a vital raw material for bone formation. Vitamin D contributes to bone mineral density by maintaining calcium levels of the blood. However long term studies have also shown that there is no direct correlation between calcium intake and bone mineral density, therefore there might be other mechanisms by which vitamin D is contributing to bone homeostasis [9].

Osteoporosis and the role of heat shock proteins (HSPs) in bone metabolism have been of great interest in the recent years. Heat shock proteins (HSPs) are molecular chaperons that protect the cells from high temperature and other form of stress by regulating various physiological activities [10]. HSPs also function in maintenance of immune system against various infections, cancer and auto-immune disorders [11]. According to some recent researches HSPs play an important role in bone homeostasis. HSP70 promotes RANKL/RANK pathway mediated osteoclastic bone resorption and also stimulates osteogenesis via ERK and Wnt/β-catenin pathways. Similarly HSP90 stimulates osteoclasts formation. Small HSPs (sHSPs) especially HSP27 regulate osteoblasts differentiation. HSPs also play important roles against osteoblasts autophagy and functions in protection of osteoporosis of bone tissues. Considering their extensive roles in bone homeostasis, HSP-based drugs are being proposed by the internal researchers as possible therapeutic agents for osteoporosis. [10, 12].

These studies are available on HSPs and their relation with osteoporosis are limited therefore; aim of study is to enhance concept of relation between HSPs and osteoporosis by comparing serum levels of HSP27, 70 and 90 in osteoporotic patient with controls.

Methodology

A cross sectional comparative study was designed. A sample size of 47 per group was calculated using the study of Daswani et al.[13] the medians and IQR were converted to means and standard deviation [14] and then sample size was calculated using formula for comparison of means. Fifty (50) patients were selected and screened at Aga Khan Hospital Lahore. This

research work was approved by the "Research and Ethics Committee" at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. %). Diagnosed premenopausal females of osteoporosis were recruited. Fifty (50) self-reported healthy controls with matched ages were also recruited from local population. The patients were diagnosed according to the criteria of The International Society for Clinical Densitometry (ISCD). Premenopausal women presenting with fragility fractures and BMD Z-score less than or equal to –2 were diagnosed as osteoporosis patients.

5 ml blood sample was taken from antecubital vein using aseptic technique. Blood was centrifuged at 4000 RPM for 10 minutes and serum was separated for biochemcial analysis of HSP-27, HSP-90, HSP-70 using human ELISA kits (Bio-Vendor). Statistical analysis was performed by SPSS statistics 17.0. Normality was tested by Shapiro Wilk tests. Mean levels of were compared by independent samples Students 't' test.

Results

The demographic data of the osteoporotic females and the matched controls is shown in figures 1-3. Osteoporosis had less significant difference on weight and age, whereas, BMI was much observed in case of subjects as compared to control.

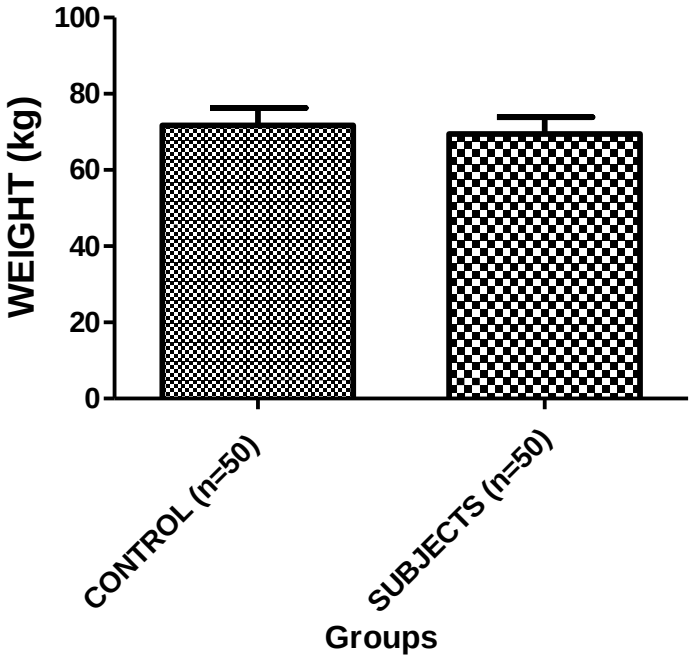

Figure 1: Demographic weight data of osteoporosis young females of reproductive age.

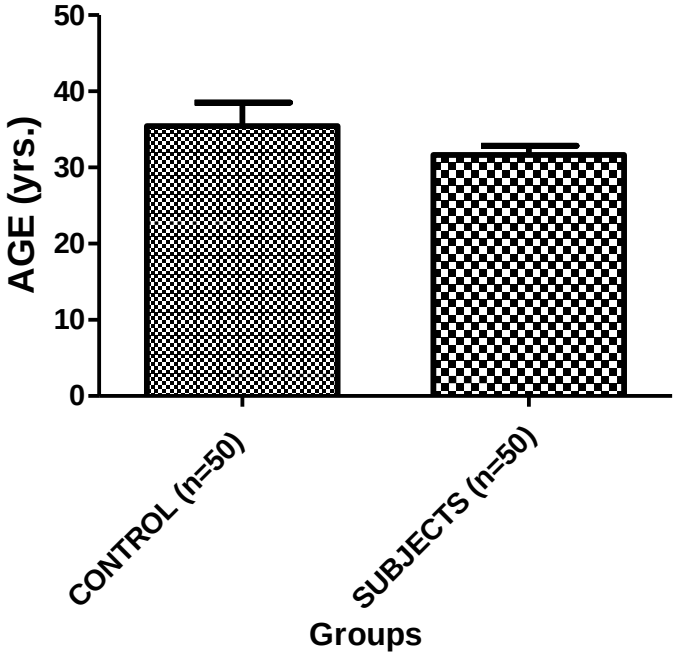

Figure 2: Demographic age data of osteoporosis young females of reproductive age.

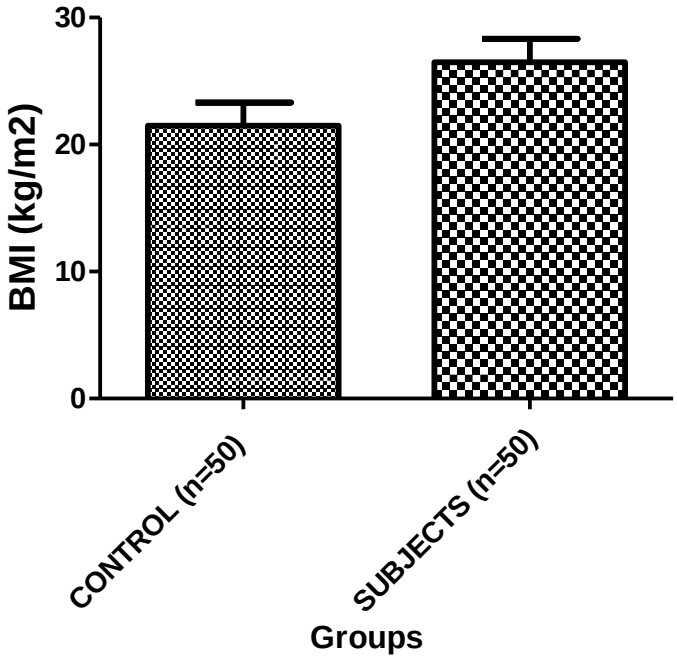

Figure 3: Demographic BMI data of osteoporosis young females of reproductive age.

The mean values of HSP-27, HSP-70 and HSP-90 are shown in figure 4. Values of heat shock proteins HSP-27, HSP-70 and HSP-90 were statistically significantly raised in osteoporotic females as compared to controls at p=0.001, p=0.04 and p=0.02 respectively.

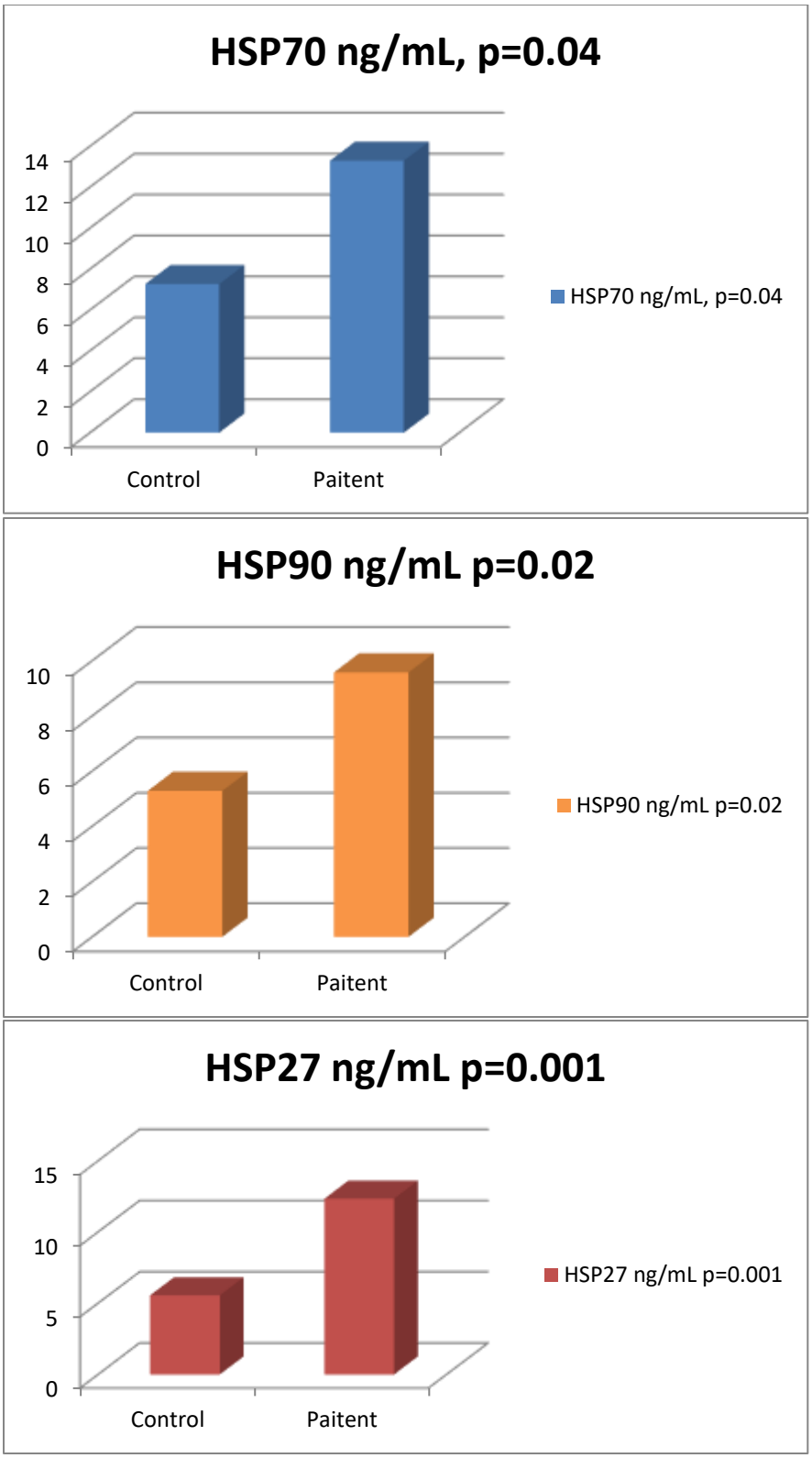

Figure 4: Serum levels of HSP-70, HSP-90 and HSP-27 in osteoporosis and matched controls

Discussion

Our research shows that serum levels of HSP27, HSP70 and HSP90 are significantly raised in premenopausal osteoporotic females as compared to healthy women of the same average age. Of the three, HSP27 showed the most significant association with osteoporosis.

HSP27 has been the main concern of study regarding bone metabolism [11]. The level of HSP27 is found to be elevated in young women with osteoporosis (12.35± 2.99) as compared to control (5.56±0.325). Our results are consistent with observations of Daswani et al. In 2016 they compared serum HSP-27 levels in premenopausal and post-menopausal women with low versus high BMD. They also found that HSP-27 levels were raised in women with decreased level of BMD in both pre-menopausal and post-menopausal groups [12]. Another study reported that HSP27 protein expression in monocytes was higher in women with low BMD in monocytes in both premenopausal and postmenopausal patients. Furthermore, it was shown that pHSP27 increased migration of monocytes towards bone milieu. As osteocastic activity is increased in osteoporosis the authors proposed that that HSP27 could be pivotal in the pathophysiology of osteoporosis [15, 16].

The current study also revealed a higher levels of HSP70 in osteoporotic females as compared to healthy controls. This is line with previous studies which suggest a role for HSP27 in improving bone physiology. It has been shown that HSP70 activates alkaline phosphatase which promotes the mineralization of mesenchyme stem cells in human [17]. It has also been demonstrated HSP70 prevents the apoptosis of osteoblasts and osteocytes [10]. To further support this hypothesis there is evidence that that HSP70 inhibitors slow down the EGF triggered migration of osteoblasts via inhibition of p44/p42 MAP kinase and Akt [18].

We also found raised HSP90 levels in sera of patients as compared to healthy subjects. Although no studies have yet commented on the serum levels of HSP90 is osteoporosis patients; there have been basic experimental studies on role of HSP90 in bone homeostasis. It has been reported that the inhibition of HSP90 by 17 AAG (17-allylamino-17 demethoxygelanamycin) helps reduce glucocorticoid-induced bone loss by enhancing osteogenesis. Furthermore HSP90 may be involved in osteoporosis by regulating the development of senescence associated secretory phenotype (SASP) cells in the bone. This phenotype of senescent cells secretes pro-inflammatory

factors which might promotes bone resorption. It is already shown that HSP90 along with other cytoplasmic and nuclear factors regulates the development of SASP [19-20]. It is known that aging, promotes the development of SASP phenotype senescence cells in the bone. It is further shown that age-related bone loss may be controlled by reducing the number of senescent cells in the bone. The raised levels of HSP90 may be related to increased load to senescence associated secretory phenotype (SASP) cells in the bone. However further studies should look into this correlation.

Conclusion:

Our study expands on the role of Heat Shock Proteins in bone homeostasis. Future studies may further explore HSPs as therapeutic target in osteoporosis

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