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Research Article

Association of Heat Shock Protein 20 with Biochemical and Inflammatory Markers in Osteoporosis Patients: Implications for Cardiovascular Risk

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Abstract

Osteoporosis is a disease caused by decrease in bone density and deterioration of bone structure, that have different causes. the current study showed the interaction between osteoporosis and cardiovascular diseases by testing patient samples and using Heat Shock Protein 20 as a biochemical marker. This study was conducted at Al-Hussein Teaching Hospital in Karbala and some external laboratories in the Holy Karbala Governorate that have 53 cases, of which 33 were osteoporosis diagnosed with and 20 were healthy controls and the participants age ranged from 20 to 60 years. however, the result of the current study showed there is a significant increase in age and BMI, with more females and smokers in contrast to the control group. Most patients lived in urban areas, and their educational and social status varied. the result of laboratory showed decrease in levels of vitamin D3, zinc, magnesium, iron, and vitamin B12 in patients, while CRP and ALP levels were higher significant suggested possible inflammation. also, the TSH levels were increased, indicated thyroid function alterations. while the Calcium levels remained stable between groups. the heat shock protein levels were elevated in patients with osteoporosis, so that this biochemical marker associated with the disease. Conclusions: Osteoporosis patients were older, had higher BMI, and were indicated lifestyle and demographic risk factors. They had decrease in levels of vitamin D3, zinc, magnesium, iron, and B12 levels, with increased in CRP and ALP, that indicated inflammation and bone issues. the increase in TSH level may suggested dysfunction in thyroid, but the level of calcium remained stable. the high level in Heat Shock Protein 20 (HSP20) marker could reflect a protective response to inflammation and stress, potentially linking osteoporosis and cardiovascular health, warranting further research.

1. Introduction

Osteoporosis, recognized as the most common metabolic bone disease, impact about 200 million individuals worldwide and is defined by a reduction in bone mineral density (BMD). These changes are related to elevated the risk of fragility fractures [1]. The osteoporosis disease, is commonly asymptomatic Before a fracture, while the fracture can be caused post life-threatening [2]. Fractures in the spine and hip particularly caused severe pain and can lead to mortality, 20% of patient with osteoporotic hip fractures dying within six months [3]. Recent research showed a strong correlation between osteoporosis and cardiovascular diseases (CVDs), as each disease was common risk factors for similar pathophysiological mechanisms, such as chronic inflammation, oxidative stress, and endothelial dysfunction [4]. In Addition,

vascular calcification as a risk factor for CVD disease has been correlated to bone demineralization, suggesting a potential "bone-vascular axis" that connects both diseases [5].

Heat-shock proteins (Hsp) are the one type of proteins commonly classified according to their molecular weight and have three different families. The first group comprises major Hsps Proteins with molecular weight of 60, 70, 90, 110 kDa. The minor Hsps is the second group that induced by glucose deprivation such as the glucose-related proteins. The last group included low molecular weight Hsps (20 kDa) [6]. However, Hsp20 is the only small heat shock protein that undergoes phosphorylated by PKA/PKG at Ser16 [7, 8]. Hsp20 is expressed in various tissues while is predominantly present in smooth, skeletal and cardiac muscle., This protein caused relaxation In smooth muscle, while its role in skeletal muscle is understood [9].

2. Methods

2.1. Study subject

The study was conducted at Al-Hussein Teaching Hospital in Karbala and several external laboratories in the Holy Karbala Governorate and included 53 cases, of which 33 were diagnosed with osteoporosis and 20 were healthy controls.

2.2. Participants

Fifty-three Iraqi individuals undergoing health assessments between January and June 2024 were included in the research. Additionally, twenty healthy adults without osteoporosis symptoms served as controls. The samples examined were patients suffering from osteoporosis receiving drug treatment.

2.3. Inclusion and Exclusion Criteria

Participants comprised in research underwent health assessments and met the following criteria: they were between 20 and 60 years of age and undergoing osteoporosis examinations for medical reasons. Those Patients with osteopenia or metabolic bone disorders and other disease are excluded. Participants were excluded if they were pregnant or lactating, and some patients are smokers.

2.4. Assessment criteria

Participants underwent comprehensive health assessments, including blood chemistry (vitamin D3, zinc, CRP, magnesium, Iron, ALP, vitamin B12, calcium), fasting blood sugar, and TSH.

2.5. BMI Calculation

Body mass index (BMI) was calculated by dividing the individual's self-reported body weight (kg) by their height squared (m). Body mass index (BMI) was classified based on the WHO physical status classification17: underweight (BMI $<$18.5 kg/m^2), normal weight (BMI 18.5-25 kg/m^2), overweight (BMI 25-30 kg/m^2), or obese (BMI $>$30 kg/m [10].

2.6. Laboratory Analyses

Blood samples were collected via venipuncture using sterile syringes of 5mL. Each sample was immediately transferred into designated tubes. The blood was allowed to clot at room temperature for 10 minutes, then centrifugation at 6000 rpm for 15 minutes. The resulting serum was then stored frozen at -80 ℃ for subsequent laboratory analysis.

Determination of Vitamin D: Vitamin D is measured in the laboratory using immunoassay techniques such as VIDAS. A specific reagent binds to 25-hydroxyvitamin D in the sample, generating a detectable signal proportional to its concentration. The assay is automated using analyzers bioMérieux VIDAS, providing results within approximately 30-40 minutes.

Determination of zinc: A colorimetric reaction was used to determine the concentration of zinc in the serum. The level of electrolyte in serum was supplied by Biolabo SA, France.

Determination of CRP: C-reactive protein (CRP) is determined in the laboratory by utilized Immunoturbidimetric Assay. In the immunoturbidimetric method, CRP in the sample binds to specific antibodies, forming immune complexes that increase turbidity, which is measured spectrophotometrically.

Determination of magnesium: Magnesium is measured using the Colorimetric Spectrophotometry Method. In the colorimetric method, magnesium reacts with a metallochromic dye (such as Xylidyl Blue) to form a colored complex, with absorbance measured spectrophotometrically. Automated analyzers Mindray BS-200 provide rapid and accurate results.

Determination of Iron: Iron is measured in the laboratory using Colorimetric Spectrophotometry. The test involves releasing iron from transferrin, reducing it to the ferrous form, and reacting it with a chromogen to produce a colored complex, which is measured at a specific wavelength.

Determination Fasting Blood Sugar: A colorimetric reaction was used to assess the content of fasting blood sugar in serum. Biolabo SA of France offered the fasting blood sugar level in serum.

Determination of ALP: A colorimetric reaction was used to determine the concentration of ALP in the serum. Biolabo SA, France supplied the level of electrolyte in serum.

Determination of vitamin B12: Vitamin B12 is measured in the laboratory using immunoassay techniques such as VIDAS. The test involves binding vitamin B12 in the sample to specific intrinsic factor-coated reagents, generating a luminescent signal proportional to its concentration.

Determination of TSH: The TSH (Thyroid Stimulating Hormone) test using the VIDAS system is performed through the ELFA (Enzyme-Linked Fluorescent Assay) technique, which relies on enzyme fluorescence detection for accurate measurement.

Determination of calcium: the calcium level in blood analyzed by Colorimetric reaction in serum. The level of electrolyte in serum was measured by Biolabo SA, France.

2.7. Statistical Analysis

The data were supplied by utilizing Statistical Package for Social Sciences (SPSS) software version 26.0. Significance testing involved calculating descriptive statistics such as means and standard deviations for comparisons between patient and control subgroups. Data visualizations were created using Microsoft Office 2016 Excel. All statistical tested were conducted with a significance level set at $P < 0.05$.

3. Results

3.1. Patient Characteristics

The study included 53 individuals were classified into a control group and osteoporosis patient group. As shown in Table 1, the patients had an average age of 38 ± 2.579 years, in contrast to the other group with an average age of 21.6 ± 1.208 years. However, the BMI of osteoporosis patients was 23.51 ± 0.30, compared to 21.57 ± 1.30 in healthy group with a significant variation. In this study notable the different gender distribution, which the females constituting a majority percentage of the patients (55.6%) compared to males (44.4%), while, the control group has balanced gender distribution comprised 50% male and 50% female individual. Also, the study demonstrated the smoking habits that comprised 33.3% of the patients but none of the participate in control group identified as smoking. Regarding residential locations, the majority of the patients (85.2%) resided in urban areas, but few patients 14.8% resided in rural areas. An educational background tested showed that 40.7% of patients were students of undergraduate, 40.7% held a bachelor's degree, and 18.5% had postgraduate studies. Furthermore, the study analyzed social status, relived that 66.7% of patients were married, while 33.3% were single. These findings is the key of demographic differences between the patient and control groups.

Table 1: Comparison of the clinical characteristics between Patients with Osteo disease and control groups

Clinical characteristics	**Mean ± SE**	
	Patient N=33	**Control N=20**
Age (year)	38 ± 2.579*	21.6 ± 1.208
BMI (kg/m^2)	23.51 ± 0.30*	21.57 ± 1.309
Gender	Male (44.4%) Female (55.6%)	Male (50%) Female (50%)
Level of Education	Undergraduate studies (40.7%) Bachelors Degree (40.7%) Postgraduate (18.5%)	Bachelors (100%)
Residence	City (85.2%) Rural Area (14.8%)	City (100%)
Smoking	Yes (66.7%) No (33.3%)	No (100%)
Social status	Single (33.3%) Married (66.7%)	Single (100%)

Laboratory parameters values between patients and the control group revealed significant differences in several parameters, as shown in Table 2. Specifically, Vitamin D3 levels were 16.57 ± 2.066 in osteoporosis patients versus 55.62 ± 1.84 in controls. Similarly, zinc levels were lower in patients 64.89 ± 5.431 compared to controls 95 ± 7.239 while the concentration of CRP significantly higher in patients (39.97 ± 14.70) compared to controls (8.8 ± 0.37), suggesting chronic inflammation. Magnesium concentration were also decrease in patients (1.45 ± 0.25) compared to controls (2.48 ± 0.16). Iron levels were decreased in patients (36.83 ± 6.13) in contrast to the control group (75 ± 12.45). Glucose levels were slightly lower in patients (131.75 ± 19.93) compared to controls (77.4 ± 2.50). Additionally, ALP levels were significantly higher in patients (137.25 ± 32.73) compared to controls (61.2 ± 8.83).

However, significant differences were observed in Vitamin B12 levels were reduced in patients 142 ± 28.49 compared to controls 674.8 ± 106.61. While, the TSH levels were higher in patients 3.74 ± 1.51 compared to controls 2.88 ± 0.80, suggesting potential thyroid function alterations. Calcium levels remained relatively similar between the two groups, with patients showing 9.44 ± 0.25 and controls 9.48 ± 0.31. These results indicate significant changes in several biochemical markers in patients, which may be related to the disease pathophysiology.

Table 2: Comparison of clinical tests between patients and control group

Test	Mean ± Std	
	Patient	**Control**
Vitamin D3 (ng/mL)	16.57 ± 2.066*	55.62 ± 1.845
Zinc (µg/dL)	64.89 ± 5.431*	95 ± 7.239
CRP (mg/L)	39.9667 ± 14.69608*	8.8 ± 0.37417
Magnesium (mg/dL)	1.4488 ± 0.24735	2.48 ± 0.15937
Iron (µg/dL)	36.83 ± 6.129*	75 ± 12.45
Glucose (mg/dL)	131.75 ± 19.926 *	77.4 ± 2.502
ALP (U/L)	137.25 ± 32.727*	61.2 ± 8.834
Vitamin B12 (pg/mL)	142 ± 28.49*	674.8 ± 106.614
TSH (µIU/mL)	3.7412 ± 1.508848*	2.88 ± 0.796492
Calcium (mg/dL)	9.438 ± 0.2456	9.48 ± 0.3121

3.2. Evaluation of biomarkers associated with osteoporosis

Figure 1, shows the Heat shock protein 20 test levels between the studied groups. As illustrated in these figures, there are significant increases (p Heat shock protein 20 levels were assessed as a specific biomarker between the studied groups. Our data demonstrated a significant elevation ($p < 0.05$) in HSP 20 levels 11.582 ± 2.77 pg/m) in patients diagnosed with Osteoporosis in contrasted to the healthy control group (0.7550 ± 0.365 pg/mL) (Figure 1).

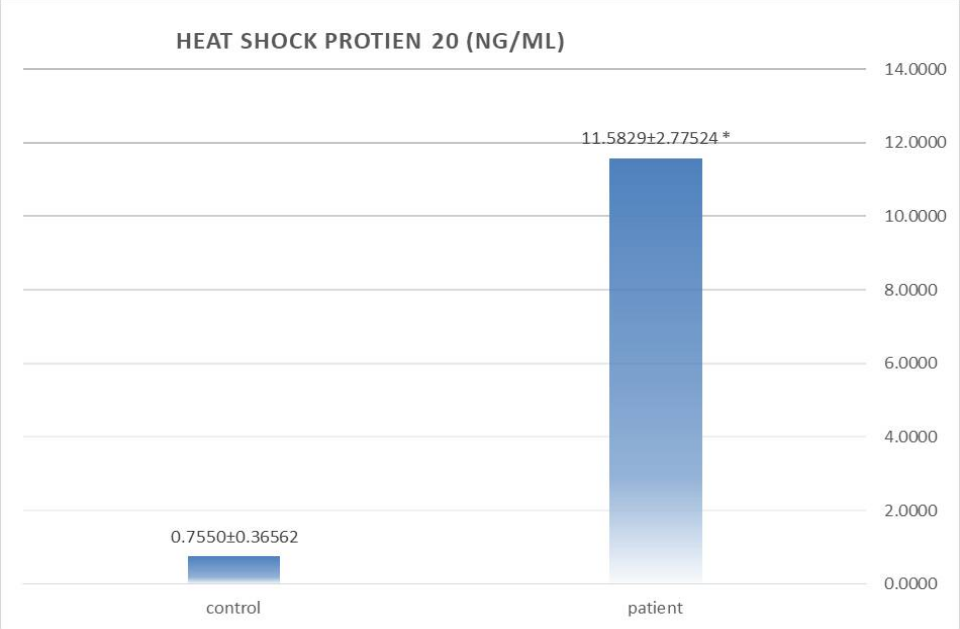

Figure 1: Comparison of the HSP-20 between Groups of osteoporosis disease and healthy group * P <0.05 statistically significant with control group

4. Discussion

Ageing is a significant risk factor for osteoporosis. An earlier study by Pignolo et al., [11] found that aging leads to a shift in mesenchymal stem cell differentiation towards adipogenesis, reducing osteoblast function [11]. Studies indicate that bone mineral density (BMD) significantly decreases with age, particularly in the lumbar spine and femoral neck, increasing osteoporosis incidence [12].

Previous studies showed that diet, physical activity, and smoking habits can also contribute to bone health, independent of BMI [13]. Studies show that a significant percentage of individuals with normal BMI still present with osteoporotic BMD. Furthermore, in one research, 48.7% of women have normal BMI with osteoporotic BMD [14, 15]. Other researches demonstrated that osteoporosis developed in normal-weight individuals can needed for monitoring regularly [16]. nutritional deficiencies, hormonal changes and lifestyle factors in postmenopausal women were the important risk factors for Osteoporosis disease because the estrogen level decline after menopause and caused resorption of bone increased and bone formation decreased, which leading to higher fracture risk [17, 18]. In addition, low calcium intake, deficiency of vitamin D, and genetic predisposition were the other factors effected this condition increased [19, 20]. So that, the decrease in dietary calcium and vitamin D concentration among women, contributing to osteoporosis [21]. A previous study indicated that osteoporosis is inerasably developed in urban settings due to several factors such as genetic, environmental, and lifestyle factors. However, insufficient consumption of fruits and vegetables caused decrease calcium intake that common in urban diets [22]. Other study relived that Malnutrition and obesity also play important roles in bone healthy [23]. High rates of smoking and alcohol consumption further exacerbate the risk of osteoporosis [23].

The current study found that 66.7% of patients were married, and 33.3% were single. Infante et al., [24] found that sedentary lifestyles, often seen in married individuals due to family responsibilities, can contribute to bone density loss [24]. While these factors highlight the

risks associated with osteoporosis in married individuals, it is also important to consider that not all married individuals will experience these issues. Osteoporosis is a multifactorial condition that significantly increases with age due to various intrinsic and extrinsic factors.

Current study consistent with Sabirov et al., [25], who found that vitamin D is essential for calcium and phosphorus metabolism, and crucial for bone mineralization. Deficiency in vitamin D is linked to an increased risk of low bone mineral density and fractures [25]. Elderly individuals are particularly vulnerable due to reduced synthesis and activation of vitamin D [25]. A study by Zofková *et al.* [26] demonstrated that zinc is vital for bone formation and mineralization; its deficiency can slow bone mass increase and accelerate loss post-menopause [26].

However, the low level of Magnesium associated with osteoporosis disease directly by acting on formation of crystal and indirectly by effecting on the secretion and activity of parathyroid hormone and by induced low-grade inflammation. Multiple studies link low magnesium status with osteoporosis risk [27]. One study found that women with osteoporosis had significantly lower magnesium levels than those without [28]. Another study found a link between low serum magnesium and osteoporosis of the spine [29]. Magnesium supports bone structure and influences calcium metabolism; inadequate levels can lead to osteoporosis [30].

Iron is play important role in collagen formation that is essential for bone strength [26]. Also, vitamin B12 is contributed to bone cell function and the levels decrease caused osteoporosis disease [30]. Other studies were consistent with the current study demonstrated that diabetes mellitus is an important factor caused of osteoporosis because the Insulin hormones that regulates osteoblast and osteoclast activity and effected on bone building and resorption [31]. The mineral deposition impaired and decline in osteoblast numbers was the risk factor of insulin deficiency and bone integrity. Abnormal glucose metabolism (AGM), characterized by blood glucose elevation that linked to osteoporosis [31]. Li *et al.* [12] demonstrated a decreased prevalence of osteoporosis in people with T2DM, while Park *et al.* [32] relived a similar association while without considering diabetes status. Wang *et al.* [33] resulted that increase in insulin resistance related with higher osteoporosis risk which suggesting a link between hyperglycemia and osteoporosis.

Previous studies demonstrated that high glucose levels inhibit the expression of key osteogenic markers, including ALP, osteoglycin (OGN), and Runx2, leading to reduced osteoblast proliferation and mineralization [29]. In vitro studies indicate that high glucose conditions can decrease ALP activity by approximately 50%, suggesting a significant impairment in mineralization capacity [34]. High glucose can enhance ALP expression during early osteoblast differentiation but may lead to decreased expression in later stages, indicating a time dependent effect on mineralization [35]. Another study found that the important biomarker for bone metabolism and BMD was alkaline phosphatase (ALP), the different in its concentration can affected on repair, remodeling and growth of bones so that, the decrease the mass of bone or decline in bone formation caused Osteoporosis. ALP important hydrolyzes phosphate esters induced formation of bone that essential phosphate for hydroxyapatite deposition and the inhibition in concentration caused inhibitory effect on bone salt formation, and induced bone formation [36, 37]. Thus, it can serve as a bone turnover marker.

The current study was consistent with Francisqueti-Ferron et al. [21], who found that increased serum ALP concentration may demonstrated an elevated risk of osteoporosis or redused bone density and could potentially serve as a biomarker for the diagnosis and treatment of osteoporosis [21]. Bone changes involve two processes: bone modeling and bone remodeling. Reducing bone remodeling or an imbalance between bone synthesis and absorption can lead to bone diseases such as osteoporosis [21, 38].

The current study was consistent with Wang et al. [33] who found HSP20 enhances osteoblast differentiation while inhibiting osteoclast activity, promoting bone formation [39]. Studies have shown that small heat shock proteins, including HSP20/HSPB6, participate in bone metabolism by regulating osteoblast and osteoclast activity, suggesting their involvement in osteoporosis development and bone remodeling [40]. Reduced expression of protective cellular proteins, including heat shock proteins, has been associated with increased bone resorption, lower bone mineral density, and higher fracture risk in osteoporosis patients [41].

The HSP20/HSPB6 has necessary role in health and disease, as highlighted by Reddy et al. [42]. Several studies have demonstrated that HSP20 has cardioprotective properties [43]. Overexpressing HSP20 caused improved cardiac outcomes after a drug induced heart injury [43] or even in the diabetic heart [44]. HSP20 is also a vasorelaxant [45], also declined vasospasms and thrombosis associated with vein grafting [46, 47].

Another study found that HSP20 reduces oxygen/glucose deprivation/reperfusion-induced organelle damage and cellular apoptosis, indicating neuroprotective effects [48]. It is abundant in stressed cells [49] and expressed in many tissues, including the brain and heart [43]. The study by Fan et al., [50] found that HSP20 exerts anti-inflammatory and anti-apoptotic effects in vascular endothelial cells, reducing atherosclerosis progression [50]. It inhibits vascular smooth muscle cell (VSMC) calcification, a key factor in arterial stiffness observed in osteoporosis patients [51]. Also, HSP20 enhances nitric oxide (NO) production, improving endothelial function and preventing hypertension [52].

In cardiac myocytes, HSP20 acts as a chaperone protein, binding to PDK1 for nuclear transport [53], and its phosphorylation affects the cytoskeleton structure [54]. HSP20's function can be affected when it forms dimers under heat [55]. As a heat shock protein, HSP20 enhances cell survival under stress, protecting the heart against apoptosis, remodeling, and ischemia/reperfusion injury [56]. HSP20 levels increase under conditions like ischemia, exercise, doxorubicin treatment, and β adrenergic stimulation [57–60]. It plays a crucial role in heart protection, with increased expression in failing hearts, suggesting a compensatory mechanism [43, 60, 61]. HSP20 is emerging as a potential biomarker for assessing both osteoporosis and CVD risk. Circulating HSP20 Levels: Reduced HSP20 levels correlate with low BMD and arterial calcification. Exosome-derived HSP20 plays a role in intercellular communication between vascular and bone cells [62]. Gene therapy and pharmacological modulation of HSP20 are being explored for osteoporosis and CVD prevention [63].

5. Conclusion

In conclusion, our findings support the notion that osteoporosis is a multifaceted condition influenced by various factors, including age, gender, smokers, those with higher BMI. lower levels of vitamin D3, zinc, magnesium, iron, and vitamin B12, alongside higher CRP, ALP, and TSH levels, suggesting possible effects on bone metabolism and inflammation. The elevated HSP20 levels in osteoporosis patients showed a correlation between cellular stress and disease progression. Furthermore, increased HSP20 levels can be used as suitable biomarkers and indicate a possible association between osteoporosis and cardiovascular disease. However, further research is needed to understand the mechanisms linking these circulating biomarkers to osteoporosis.

Article Information

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Ethical Approval: The study was approved by the Ethical Committee of the University of Kufa, Iraq. All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and the Helsinki Declaration.

Informed Consent: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author.

Clinical Trial Registration: Not applicable.

Reporting Guidelines Statement: This observational study was conducted in accordance with the STROBE reporting guidelines.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

References

[1] T. L. Yang, H. Shen, A. Liu, S. S. Dong, L. Zhang, F. Y. Deng, Q. Zhao, H. W. Deng, and R. R. Recker. A road map for understanding molecular and genetic determinants of osteoporosis. *Nature Reviews Endocrinology*, 16(2):91–103, 2020. URL https://doi.org/10.1038/s41574-019-0282-7.

[2] B. Liang, G. Burley, S. Lin, and Y. C. Shi. Osteoporosis pathogenesis and treatment: Existing and emerging avenues. *Cellular and Molecular Biology Letters*, 27(1):72, 2022. URL https://doi.org/10.1186/s11658-022-00371-3.

[3] C. L. Leibson, A. N. Tosteson, S. E. Gabriel, J. E. Ransom, and L. J. Melton. Mortality, disability, and nursing home use for persons with and without hip fracture: A population-based study. *Journal of the American Geriatrics Society*, 50(10):1644–1650, 2002. URL https://doi.org/10.1046/j.1532-5415.2002.50455.x.

[4] M. N. Weitzmann and R. Pacifici. Inflammation and osteoporosis. *Nature Reviews Rheumatology*, 19(1):25–40, 2023. URL https://doi.org/10.1038/s41584-022-00868-2.

[5] J. L. Pérez-Castrillón, M. Rodríguez-García, C. García-Morales, J. M. Gómez, and C. R. López. The paradox of vascular calcification and osteoporosis. *Current Osteoporosis Reports*, 21(1):12–26, 2023. URL https://doi.org/10.1007/s11914-023-00758-3.

[6] C. Garrido, C. Paul, R. Seigneuric, and H. H. Kampinga. The small heat shock proteins family: The long forgotten chaperones. *International Journal of Biochemistry Cell Biology*, 44(10):1588–1592, 2012. URL https://doi.org/10.1016/j.biocel.2012.02.022.

[7] A. Beall, D. Bagwell, D. Woodrum, T. A. Stoming, K. Kato, A. Suzuki, H. Rasmussen, and C. M. Brophy. The small heat shock-related protein, HSP20, is phosphorylated on serine 16 during cyclic nucleotide-dependent relaxation. *Journal of Biological Chemistry*, 274 (16):11344–11351, 1999. URL https://doi.org/10.1074/jbc.274.16.11344.

[8] H. V. Edwards, R. T. Cameron, and G. S. Baillie. The emerging role of hsp20 as a multifunctional protective agent. *Cell Signalling*, 23 (9):1447–1454, 2011. URL https://doi.org/10.1016/j.cellsig.2011.04.009.

[9] M. Ba, C. A. Singer, M. Tyagi, and C. M. Brophy. HSP20 phosphorylation and airway smooth muscle relaxation. *Cell Health and Cytoskeleton*, 1:27–42, 2009. URL https://doi.org/10.2147/CHC.S4222.

[10] A. Prista, J. A. R. Maia, A. Damasceno, and G. Beunen. Anthropometric indicators of nutritional status: Implications for fitness, activity, and health in school-age children and adolescents from Maputo, Mozambique. *The American Journal of Clinical Nutrition*, 77 (4):952–959, 2003. URL https://doi.org/10.1093/ajcn/77.4.952.

[11] R. J. Pignolo. Aging and bone metabolism. *Comprehensive Physiology*, 13(1):4355–4386, 2023. URL https://doi.org/10.1002/cphy.c220012.

[12] C. Li, J. Liu, R. L. Xia, Q. Song, X. L. Cui, and A. J. Chao. The prevalence of osteoporosis in postmenopausal women in urban Tianjin, China and its related factors. *Menopause*, 30(7):774–780, 2023. URL https://doi.org/10.1097/GME.0000000000002204.

[13] S. Agarwal and R. B. Uppin. Effect of obesity on osteoporosis: A DEXA scan based report in urban population of Belagavi. *Journal of the Scientific Society*, 43(2):67–69, 2016. URL https://doi.org/10.4103/0974-5009.182596.

[14] B. K. Wijaya. Relationship between body mass index and bone mass density as a risk factor for osteoporosis in women. *International Journal of Scientific and Research Publications*, 12(4):533–537, 2022. URL GoogleScholar:https://scholar.google.com/scholar?q=Relationship+between+Body+Mass+Index+and+Bone+Mass+Density+as+a+Risk+Factor+for+Osteoporosis+in+Women.

[15] H. Humaryanto and A. Syauqy. The profile of body mass index and bone mass density scan as osteoporosis risk factor among female. *Jurnal Kedokteran Brawijaya*, 30(3):218–222, 2019. URL https://doi.org/10.21776/ub.jkb.2019.030.03.13.

[16] C. Geng and C. Chen. Association between serum insulin-like growth factor 1 and osteoporosis risk in Parkinson's disease: A cross-sectional study. *Neurological Sciences*, 45(11):5291–5296, 2024. URL https://doi.org/10.1007/s10072-024-07605-6.

[17] S. Charde, M. Charde, and A. Kumar. A comprehensive review on postmenopausal osteoporosis in women. *Cureus*, 15(11):e48582, 2023. URL https://doi.org/10.7759/cureus.48582.

[18] C. H. Cheng, L. R. Chen, and K. H. Chen. Osteoporosis due to hormone imbalance: An overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. *International Journal of Molecular Sciences*, 23(3):1376, 2022. URL https://doi.org/10.3390/ijms23031376.

[19] A. V. Khadilkar and R. M. Mandlik. Epidemiology and treatment of osteoporosis in women: An Indian perspective. *International Journal of Women's Health*, 7:841–850, 2015. URL https://doi.org/10.2147/IJWH.S54623.

[20] I. Akkawi and H. Zmerly. Osteoporosis: Current concepts. *Joints*, 6(2):122–127, 2018. URL https://doi.org/10.1055/s-0038-1660790.

[21] F. V. Francisqueti-Ferron, A. J. T. Ferron, J. L. Garcia, C. C. V. A. Silva, M. R. Costa, C. S. Gregolin, F. Moreto, A. L. A. Ferreira, I. O. Minatel, and C. R. Corrêa. Basic concepts on the role of nuclear factor erythroid-derived 2-like 2 (Nrf2) in age-related diseases. *International Journal of Molecular Sciences*, 20(13):3208, 2019. URL https://doi.org/10.3390/ijms20133208.

[22] T. Baruah and P. Bora. Osteoporosis and osteopenia among a group of population of Guwahati City. *Assam. International Journal of Science and Research*, 3(11):2319–7064, 2012. URL GoogleScholar:https://scholar.google.com/scholar?q=Osteoporosis+and+osteopenia+among+a+group+of+population+of+Guwahati+City+Assam.

[23] M. Mousavi, B. Pakzad, and M. Ebrahimian. Investigating the decreased bone density prevalence in people with common risk factors referred to Al-Zahra Educational-Medical Center in Isfahan City during 2018 and 2019. *Journal of Shahid Sadoughi University of Medical Sciences*, 30(9):5225–5235, 2022. URL GoogleScholar:https://scholar.google.com/scholar?q=Investigating+the+decreased+bone+density+prevalence+in+people+with+common+risk+factors.

[24] M. Infante, M. Caprio, and A. Fabbri. Introduction: Causes and risk factors for male osteoporosis. In A. Ferlin and S. Migliaccio, editors, *Male osteoporosis*, pages 1–12. Springer, Cham, 2020. URL https://doi.org/10.1007/978-3-030-25820-0_1.

[25] I. S. Sabirov, S. S. Khasanova, A. B. Asykpaeva, E. Madzhidova, A. A. Matkerimov, and F. A. A. Rizk Al-Kasabi. Vitamin D deficiency and osteoporosis: Focus on elderly and senile age. *Bulletin of Science and Practice*, 10(12):342–351, 2024. URL https://doi.org/10.33619/2414-2948/109/43.

[26] I. Zofková, P. Nemcikova, and P. Matucha. Trace elements and bone health. *Clinical Chemistry and Laboratory Medicine*, 51(8):1555–1561, 2013. URL https://doi.org/10.1515/cclm-2012-0868.

[27] C. Palacios. The role of nutrients in bone health, from A to Z. *Critical Reviews in Food Science and Nutrition*, 46(8):621–628, 2006. URL https://doi.org/10.1080/10408390500466174.

[28] Y. Zhang, M. Li, P. Lou, M. Zhang, D. Shou, and P. Tong. miRNA-seq analysis of high glucose induced osteoblasts provides insight into the mechanism underlying diabetic osteoporosis. *Scientific Reports*, 14:13441, 2024. URL https://doi.org/10.1038/s41598-024-64391-z.

[29] Y. Zhang, H. Wang, J. Wang, et al. The prevalence and treatment rate trends of osteoporosis in postmenopausal women: Results from the National Health and Nutrition Examination Survey 2005–2018. *PLOS ONE*, 18(9):e0290289, 2023. URL https://doi.org/10.1371/journal.pone.0290289.

[30] A. R. Gaby and J. V. Wright. Nutrients and osteoporosis. *Journal of Nutritional Medicine*, 1(1):63–72, 1990. URL https://doi.org/10.3109/13590849009003136.

[31] C. Conte, S. Epstein, and N. Napoli. Insulin resistance and bone: A biological partnership. *Acta Diabetologica*, 55(4):305–314, 2018. URL https://doi.org/10.1007/s00592-018-1111-0.

[32] S. K. Park, J. Y. Jung, C. M. Oh, J. M. Choi, M. H. Kim, E. Ha, et al. Fasting glucose level and the risk of incident osteoporosis in Koreans. *Bone*, 142:115690, 2021. URL https://doi.org/10.1016/j.bone.2020.115690.

[33] H. Wang, Y. Liu, and J. Zhang. Low bone mineral density is associated with increased risk of arterial stiffness and atherosclerosis. *Cardiovascular Research*, 117(11):2394–2405, 2021. URL https://doi.org/10.1093/cvr/cvab095.

[34] J. S. Cunha, V. M. Ferreira, E. Maquigussa, et al. Effects of high glucose and high insulin concentrations on osteoblast function in vitro. *Cell and Tissue Research*, 358(1):249–256, 2014. URL https://doi.org/10.1007/s00441-014-1913-x.

[35] A. Takeno, I. Kanazawa, K. Tanaka, et al. High glucose promotes mineralization via bone morphogenetic protein 4-Smad signals in early stage of osteoblast differentiation. *Diabetology International*, 12(2):171–180, 2021. URL https://doi.org/10.1007/s13340-020-00463-5.

[36] Z. Chen, T. J. Klein, R. Z. Murray, R. Crawford, J. Chang, C. Wu, and Y. Xiao. Osteoimmunomodulation for the development of advanced bone biomaterials. *Materials Today*, 19(6):304–321, 2016. URL https://doi.org/10.1016/j.mattod.2015.11.004.

[37] L. L. Ihde, D. M. Forrester, C. J. Gottsegen, S. Masih, D. B. Patel, L. A. Vachon, and E. A. White. Sclerosing bone dysplasias: Review and differentiation from other causes of osteosclerosis. *RadioGraphics*, 31(7):1865–1882, 2011. URL https://doi.org/10.1148/rg.317115093.

[38] L. G. Raisz. Pathogenesis of osteoporosis: Concepts, conflicts, and prospects. *The Journal of Clinical Investigation*, 115(12):3318–3325, 2005. URL https://doi.org/10.1172/JCI27071.

[39] X. Wang, H. Tokuda, D. Hatakeyama, K. Hirade, M. Niwa, H. Ito, and O. Kozawa. Mechanism of simvastatin on induction of heat shock protein in osteoblasts. *Archives of Biochemistry and Biophysics*, 415(1):6–13, 2003. URL https://doi.org/10.1016/S0003-9861(03)00208-4.

[40] K. Hang, C. Ye, E. Chen, W. Zhang, D. Xue, Z. Pan, and J. Shen. Role of the heat shock protein family in bone metabolism. *Cell Stress and Chaperones*, 23(6):1153–1164, 2018. URL https://doi.org/10.1007/s12192-018-0932-z.

[41] S. Bolamperti, I. Villa, and A. Rubinacci. Bone remodeling: An operational process ensuring survival and bone mechanical competence. *Bone Research*, 10:48, 2022. URL https://doi.org/10.1038/s41413-022-00219-8.

[42] V. S. Reddy, S. K. Madala, J. Trinath, and G. B. Reddy. Molecular chaperone functions of small heat shock proteins. In *Heat Shock Proteins in Human Diseases*, pages 31–50. Springer, 2018. URL https://doi.org/10.1007/978-3-319-73344-7_2.

[43] G. C. Fan, X. Ren, J. Qian, Q. Yuan, P. Nicolaou, Y. Wang, W. K. Jones, G. Chu, and E. G. Kranias. Novel cardioprotective role of a small heat-shock protein, hsp20, against ischemia/reperfusion injury. *Circulation*, 111(14):1792–1799, 2005. URL https://doi.org/10.1161/01.CIR.0000160851.41872.C6.

[44] X. Wang, B. Zingarelli, M. O'Connor, P. Zhang, A. Adeyemo, E. G. Kranias, and G. C. Fan. Overexpression of Hsp20 prevents endotoxin-induced myocardial dysfunction and apoptosis. *Basic Research in Cardiology*, 104(4):385–396, 2009. URL https://doi.org/10.1007/s00395-009-0006-z.

[45] C. R. Flynn, P. Komalavilas, D. Tessier, J. Thresher, E. E. Niederkofler, C. M. Dreiza, R. W. Nelson, A. Panitch, L. Joshi, and C. M. Brophy. Transduction of biologically active motifs of the small heat shock-related protein HSP20 leads to relaxation of vascular smooth muscle. *The FASEB Journal*, 17(11):1358–1360, 2003. URL https://doi.org/10.1096/fj.02-1069fje.

[46] E. C. McLemore, D. J. Tessier, C. R. Flynn, E. J. Furnish, P. Komalavilas, J. Thresher, L. Joshi, W. Stone, and C. M. Brophy. Transducible recombinant HSP20 inhibits vasospasm and platelet aggregation. *Journal of Vascular Surgery*, 39(3):700–709, 2004. URL https://doi.org/10.1016/j.jvs.2003.10.050.

[47] D. J. Tessier, P. Komalavilas, A. Panitch, L. Joshi, and C. M. Brophy. Transduction of a phosphorylated small heat shock-related protein produces vascular relaxation. *Proceedings of the National Academy of Sciences*, 101(17):6621–6626, 2004. URL https://doi.org/10.1073/pnas.0306797101.

[48] J. Zhong, X. Li, C. McNamee, A. P. Chen, A. A. Baccarelli, and G. C. Fan. Protective effects of HSP20 against oxygen-glucose deprivation/reperfusion-induced Golgi fragmentation and apoptosis in primary neurons. *Oxidative Medicine and Cellular Longevity*, 2015, . Google Scholar:1–10, 2015. URL https://doi.org/10.1155/2015/627145.

[49] J. Li, C. Zhang, Y. Xing, J. S. Janicki, M. Yamamoto, X. L. Wang, and T. Cui. Up-regulation of HSP20 protects against oxidative stress and apoptosis in cardiomyocytes. *Cell Stress and Chaperones*, 17(4):485–493, 2012. URL https://doi.org/10.1007/s12192-011-0327-6.

[50] L. Fan, Y. Zhang, X. Liu, and H. Chen. Heat shock protein 20 attenuates endothelial inflammation and apoptosis in atherosclerosis. *Journal of Cellular and Molecular Medicine*, 27(8):1120–1132, 2023. URL https://scholar.google.com/scholar?q=Heat+shock+protein+20+attenuates+endothelial+inflammation+and+apoptosis+in+atherosclerosis.GoogleScholar.

[51] Y. Chen, S. Wang, X. Zhao, and J. Liu. HSP20 suppresses vascular smooth muscle cell calcification through modulation of osteogenic signaling pathways. *Vascular Pharmacology*, 149:107134, 2023. URL https://doi.org/10.1016/j.vph.2023.107134.

[52] Q. Sun, H. Li, W. Zhang, and M. Xu. HSP20 improves endothelial function by enhancing nitric oxide production and reducing vascular dysfunction. *Life Sciences*, 330:121982, 2023. URL https://doi.org/10.1016/j.lfs.2023.121982.

[53] Y. Y. Sin, T. P. Martin, G. S. Wills, S. Currie, and G. S. Baillie. Small heat shock protein 20 (HSP20) interacts with phosphorylated Akt and protein kinase D1. *Cell Signalling*, 27(4):673–681, 2015. URL https://doi.org/10.1016/j.cellsig.2014.12.019.

[54] C. M. Dreiza, C. M. Brophy, P. Komalavilas, E. J. Furnish, L. Joshi, J. E. Murphy-Ullrich, M. von Rechenberg, Y. S. Ho, B. Richardson, N. Xu, Y. Zhen, J. M. Peltier, A. Panitch, and J. D. Walters. Transducible heat shock protein 20 (HSP20) phosphopeptide inhibits intracellular contractile signaling. *Journal of Biological Chemistry*, 280(26):24698–24705, 2005. URL https://doi.org/10.1074/jbc.M501307200.

[55] R. van Montfort, C. Slingsby, and E. Vierling. Structure and function of the small heat shock protein/-crystallin family of molecular chaperones. *Advances in Protein Chemistry*, 59:105–156, 2001. URL https://doi.org/10.1016/S0065-3233(01)59004-X.

[56] C. M. Dreiza, P. Komalavilas, E. J. Furnish, C. R. Flynn, M. R. Sheller, C. C. Smoke, L. B. Lopes, C. M. Brophy, and L. Joshi. The small heat shock protein, HSPB6, in muscle function and disease. *Cellular Signalling*, 22(1):1–7, 2010. URL https://doi.org/10.1016/j.cellsig.2009.08.013.

[57] J. Qian, X. Ren, X. Wang, P. Zhang, W. K. Jones, J. D. Molkentin, G. C. Fan, and E. G. Kranias. Blockade of Hsp20 phosphorylation exacerbates cardiac ischemia/reperfusion injury in mice. *Circulation Research*, 105(12):1223–1231, 2009. URL https://doi.org/10.1161/CIRCRESAHA.109.206854.

[58] M. O. Boluyt, J. L. Brevick, D. S. Rogers, M. J. Randall, A. F. Scalia, and Z. B. Li. Changes in the rat heart proteome induced by exercise training: Increased abundance of HSP20. *Proteomics*, 6(10):3154–3169, 2006. URL https://doi.org/10.1002/pmic.200500697.

[59] T. Dohke, A. Wada, T. Isono, M. Fujii, T. Yamamoto, T. Tsutamoto, M. Horie, and T. Ono. Proteomic analysis reveals significant alternations of cardiac small heat shock protein expression in congestive heart failure. *Journal of Cardiac Failure*, 12(1):77–84, 2006. URL https://doi.org/10.1016/j.cardfail.2005.08.004.

[60] G. C. Fan, G. Chu, B. Mitton, Q. Song, Q. Yuan, and E. G. Kranias. Small heat-shock protein Hsp20 phosphorylation inhibits -agonist-induced cardiac apoptosis. *Circulation Research*, 94(11):1474–1482, 2004. URL https://doi.org/10.1161/01.RES.0000129181.96957.DE.

[61] G. C. Fan, Q. Yuan, G. Song, Y. Wang, G. Chen, J. Qian, X. Zhou, Y. J. Lee, M. Ashraf, and E. G. Kranias. Small heat-shock protein Hsp20 attenuates -agonist-mediated cardiac remodeling through apoptosis signal-regulating kinase 1. *Circulation Research*, 99(11):1233–1242, 2006. URL https://doi.org/10.1161/01.RES.0000252323.93948.6c.

[62] H. N. Qiu, J. B. Li, and J. N. Lin. Muscle mass and central obesity on cognitive impairment in aging populations. In *Tissue Crosstalk in Obesity and Diabetes: A Focus on Skeletal Muscle*, pages 54–68. 2025. URL https://scholar.google.com/scholar?q=Muscle+mass+and+central+obesity+on+cognitive+impairment+in+aging+populations.

[63] Z. Han, X. He, Y. Feng, W. Jiang, N. Zhou, and X. Huang. Hsp20 promotes endothelial progenitor cell angiogenesis via activation of PI3K/Akt signaling pathway under hypoxia. *Tissue Engineering and Regenerative Medicine*, 19(6):1251–1266, 2022. URL https://doi.org/10.1007/s13770-022-00470-8.