ORIGINAL ARTICLE

OSTEOPOROSIS AND ITS ASSOCIATION WITH TESTOSTERONE LEVELS IN MALES IN PAKISTANI POPULATION

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ABSTRACT

Background: Men may also experience bone loss due to declining testosterone levels with aging which might be associated with osteoblastic activity. The objective of the study was to find out the association of osteoporosis with testosterone levels amongst young males.

Methods: Participants (1000) were enrolled, age +45 years, from free orthopedics camps in different towns of Karachi after an informed consent. Bonemineral density was assessed by heel scan device. Blood samples were taken to assess Testosterone through Elisa. Information on demographics, general and medical history was obtained by an interviewer-administered questionnaire. This cross-sectional study was conducted from February 2014 to April 2015. The study protocol was duly approved by Ziauddin University Ethics Review Committee.Data was analyzed using SPSS version 20. P value less than 0.05 was taken as significant.

Results: A total of 987 subjects were finalized. Testosterone levels were low in 359(36.4%) subjects and normal in 628(63.6%). 113 had T-score ≥-2.5 on Single x-ray absorptiometry (SXA), and were considered to be osteoporotic according to WHO criteria and rest 874 were taken as controls. Out of 113 subjects, 82(72.6%) had low,whereas,31(27.4%) had normal levels of testosterone. Odds ratio for osteoporosis verses testosterone deficiency came out to be 6.65 (2.70-21.63), p-value was 0.0001 (win pepi version 11.39).When education was seen with the frequency of osteoporosis it was observed that the prevalence was seen higher in those who had attained matriculation or intermediate/ graduation (p-value 0.01).

Conclusion: Osteoporosis was found associated with testosterone deficiency with an odds ratio of 6.65 (2.70-21.63) and P- value of 0.0001 (win pepi version 11.39).

KEYWORDS: Osteoporosis; Testosterone; Osteoblasts.

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INTRODUCTION

Osteoporosis, earlier considered as a disease of old men, is beingincreasingly reported in younger men during the past few years[1]. Bone injuries and bone illnesses and perioperative complications including delayed fracture-healing are also frequently being reported in younger men. Low testosterone levels may lead to negative effects on the musculoskeletal system, they are associated with decrease in bone mineral density, leading to bone fragilityand its complications, especially in elderly men[2].

Testosterone, secreted by the Leydig cells of the testis, is the primary circulating androgen. It is eventually converted to Estrogen by aromatase activity. Testosterone and dihydrotestosterone the more potent converted form of testosterone, are the biologically active forms which circulate in the blood bound to SHBG, albumin, or other proteins. The availability and circulation of these hormones is controlled by hypothalamic-pituitary feedback through FSH and LH hormones.[3]

Osteoporosis has massive impact on community health and quality of life.[4] The health of the bones depends on physical activity, appropriate diet, lifestyle and the level of hormones in the body. Osteoporosis may be localized to certain bones or may involve the entire skeleton as a sign of metabolic bone disease. It is characterized by increased porosity of the skeleton due to reduced bone mineral density. Clinically, patients are generally unaware of their disease until they get a fracture of vertebra, hip or any other bone, hence the term 'the silent thief' was coined. [5]

Currently, it is now being recognized as a disease of both young and old men, with a prevalence of 4% to 6%.[6] The difference in fracture incidence observed between men and women is due not only to a difference in their bone strength but also to the type and frequency of trauma experienced by men compared to women due to difference in life style.[7]

Testosterone, the major circulating androgen in men, is essential for the appropriate development and function of muscular tissue, bones, etc. Testosterone is a C19 steroid synthesized from cholesterol and secreted primarily from the testes. It binds with albumin and SHBG in the circulation and is irreversibly converted into 5-dihydrotestosterone (DHT) in peripheral tissues by the action of enzyme 5-reductase. Both DHT and Testosterone can activate the androgen receptor.[8] The testosterone maintains cancellous bone mass and integrity by activating androgen receptor (AR), increases cortical bone size via stimulation of both longitudinal and radial growth. It has a biphasic effect on endochondral bone formation, stimulates endochondral bone formation at the start of puberty and induces epiphyseal closure at the end of puberty.[9]

This study was designed to determine the relationship between osteoporosis and Testosterone levels amongst young males.

METHODS

This study was designed as a cross sectional study, based on multistage sampling technique. The study site selected was Ziauddin Hospital Karachi. Patients were recruited from the free orthopedics camps arranged in different towns of Karachi. This cross-sectional study duration was February 2014 to April 2015. The study protocol was duly approved by Ziauddin University Ethics Review Committee.

Free orthopedic camps in different towns of Karachi during 2014 were set up where males gathered to get free bone scan and free testosterone levels. A total of 1000 males, ±45 years old, were recruited after an informed signed consent. The subjects' information regarding diets, habits, medical & surgical history was obtained through interviewer administered questionnaire.

The bone mineral density through Single x-ray absorptiometry was done on right foot heel of all 987 subjects. The reason for selection bone density through heel scan device is that it is cost effective and secondly risk of bone loss or fracture in future can be established by this method. For maximum exposure, the participant placed his right foot on the moulded support plate. The BMD was recorded and expressed in grams per square centimeter (g/cm2).Out of 130 cases having T scores less than -2.5,

Testosterone test was done by using ELIZA (Solid phase, ligand–labeled competitive chemiluminescent enzyme immunoassay). Since there is diurnal variation in serum testosterone levels with peak levels seen in the morning following sleep, 3 samples at 0,15 and 30 minutes' interval were collected and test was performed with Pooled serum.(0.5 serum,stable for 7 days at 28^{0}C). The patient serum and ligand labeled testosterone are added to the test unit containing a bead coated with polyclonal rabbit anti-testosterone and incubated for 30 minutes at 37∘C. Testosterone in the sample compete with ligand labeled testosterone for antibody binding sites on the bead. Unbound material is then removed by a centrifugal wash. An Alkaline Phosphatase – labeled anti–ligand is added which is again incubated for 30 minutes. The unbound enzyme conjugate is removed by a centrifugal wash, substrate is added and incubated for 10 minutes. The chemiluminescent substrate a phosphatase ester of adamantly dioxetane, undergoes hydrolysis in the presence of Alkaline Phosphatase to yield an unstable anion intermediate, which emits-light-photon which are measured by the luminometer is inversely proportional to the concentration of testosterone.

Data was analyzed using SPSS version 20. Frequencies and percentages were taken out for categorical variables. For Numerical variables Mean and standard deviation were calculated. Chi Square test was used to find out association between categorical data. Independent t- test and ANOVA was applied to assess difference of means among groups. P value less than 0.05 was taken as significant.

RESULTS

Out of 1000 recruited subjects a total of 987 subjects were finalized. Testosterone levels were measured in all osteoporotic and non-osteoporotic patients, as shown in Table 1.

TABLE 1: COMPARISON OF TESTOSTERONE LEVELS IN OSTEOPOROTIC AND NON-OSTEOPOROTIC SUBJECTS

TESTOSTERONE LEVELS						
BONE STATUS	NORMAL (270-1070NG/DL)[25]	LOW (< 270 NG/DL)[25]	TOTAL	ODDS RATIO	CI AT 95%	P-VALUE
OSTEOPOROSIS N (%)	31 (27.4%)	82 (72.6%)	113	5.70	2.70-	0.00001
NON-OSTEOPOROTIC N (%)	597 (68.3%)	277 (31.7%)	874		21.63	
TOTAL	628 (63.6%)	359 (36.4%)	987			

Testosterone levels were low in 359(36.4%) subjects normal levels in 628(63.6%).

When osteoporosis was compared with testosterone deficiency, odds ratio came out to be 5.70 (2.70-21.63). p-value was 0.00001 (win pepi version 11.39).

A heel bone density scan was also conducted on each individual, patients with T-score ≥-2.5 on single x-ray absorptiometry (SXA), were selected as osteoporotic according to WHO criteria.

Out of the 987 participants, 656 (65.6%) had a habit of walking or cycling as a part of daily routine whereas, 344 (34.4%) did not. Participants at younger age (less than 40 yrs.) were found active and less osteoporotic. The incidence of osteoporosis was observed in participants on the average at 45 years of age.

Table 2: COMPARISON OF VARIABLES WITH OSTEOPOROSIS

VARIABLE		OSTEOPOROTIC N(%)	NON OSTEOPOROTIC N(%)	TOTAL
DIET	APPROPRIATE	8 (7.1%)	237 (27.1%)	245 (24.8%)
	INAPPROPRIATE	105 (92.9%)	637 (72.9%)	742 (75.2%)
TOTAL		113	874	**987**
EXERCISE	ACTIVE	24 (21.2%)	81 (9.3%)	105 (10.6%)
	SEDENTARY	89 (78.8%)	793 (90.7%)	882 (89.4%)
TOTAL		113	874	**987**

When the relationship of diet was assessed in association with osteoporosis, it was found that out of a total 742 who were taking inappropriate diet, osteoporosis was found in 92.9% and out of the total 245 taking appropriate diet only 7.1% were found to be osteoporotic. Physical activity was also found associated with osteoporosis, those who had high daily activity out of them only 21.2% had osteoporosis compared to 78.8% cases of osteoporosis in people who had low physical activity.

DISCUSSION

There have been considerable advances in the understanding of male osteoporosis, but still a number of unresolved issues are left. The hormonal level, states of mobility, lifestyle and diet are relatively concerned in the imbalances of bone turnover. Our study demonstrates a strong association of low testosterone level with osteoporosis. It has been found that participants having low testosterone level 5.70 (CI-2.70-21.63) times more prone to develop osteoporosis at an early age compared to controls. Also observe that BMD is decreased, as from our data out of total number n=359 having low testosterone level, n=82 (22.8%) were osteoporotic.

The most important circulating androgen in men is testosterone, it is necessary for the suitable development and function of muscular tissue, bones, etc. Testosterone (T) is a C19 steroid synthesized from cholesterol and released primarily from the testes. It binds with albumin and sex hormone-binding globulin (SHBG) in the circulation and is irreversibly converted into 5_-dihydrotestosterone (DHT) in peripheral tissues by the action of enzyme 5_reductase. Both DHT and T can activate the androgen receptor (AR).[8]

A comprehensive review on the function of androgens, especially testosterone in maintaining bone health was done through compilation of data from studies at human, animal, and cellular level. The reviewers observed many important aspects. Testosterone is the major gonadal androgen in men, 95% of which is secreted by the testes. The measurement of Bone Mineral Density and fracture risk can determine the effects of testosterone on bone health in men. This effect of testosterone is accomplished through its direct or indirect action, preserving trabecular bone predominantly by decreasing osteoclastogenesis.[2] The testosterone keep up cancellous bone mass, integrity by activating AR, increases cortical bone size via stimulation of both longitudinal and radial growth. It has a biphasic effect on endochondral bone formation. It stimulates endochondral bone formation at the start of puberty whereas induce epiphyseal closure at the end of puberty. The age-related decreased testosterone level contributes in degenerative disorders disease of bone[10] and raise the rate of bone remodeling by removing restraining effects on osteoblastogenesis and osteoclastogenesis.[11] It produces a focal imbalance between resorption and formation by prolonging the lifespan of osteoclasts and shorten the lifespan of osteoblasts. Serum osteocalcin levels also decreased significantly in the men having low testosterone level. Osteocalcin

is produced primarily by mature osteoblastic cells and osteocytes during bone formation and binds with the mineralized bone matrix.[12,13] The RANKL activation is essential for the generation, survival and function of osteoclasts. Due to deficiency of sex steroid hormones RANKL is implicated in increased bone resorption.[14]

Unlike women, aging in men, is associated with strikingly rise in fracture threat. Men, especially are at higher risk of fractures when their testosterone is low and SHBG is high in the presence of low serum estrogen, yet the mechanism is not clear. However, researchers propose that the effect of estrogen is direct on the bone whereas, androgens indirectly exert anti-fracture influencethrough strengthening the skeletal infrastructure such as supporting muscle mass for better maneuverability[15].

Osteoporotic fractures are a very major global health problem with an expanding prevalence which is associated with significant morbidity and mortality[16]. In men, the incidence of osteoporotic fractures increases exponentially later in their life and lead to serious life threatening disability and death episodes[17]. This is paralleled by decrease in their serum levels of testosterone[18]. In a previous study, low trauma fractures were observed in 155 (15.5%) participants. Out of the 71% participants who were leading a non-activelife, a total of 41(37%) of them were osteoporotic, however, among the rest of the 45 participants who were involved in walking, running or cycling only 8(13%) were osteoporotic[19]. Another study from Scotland observed a high rate of overall fractures in men from 15 to 49 years of age, showing males of this age range to be 2.9 times more at risk to sustain a fracture than females[20]. Other studies also highlighted that before the age of 50 years, males are more susceptible to get a fracture than females[21]. Of these fractures, hip fractures in men have 31 percent mortality rate compared to a rate of 17 percent in women[26]. A study conducted in Lahore, Pakistan in 2011 reported that the overall incidence of osteoporotic fractures has shown a generally rising trendin direct relation with the increase in life expectancy. Osteoporotic fractures have been seen almost 10 or even 20 years earlier in Indian and Pakistani men and women as compared to their Caucasian counterparts[22]. The total disability-adjusted life years (DALYs) lost is 5.8 million annually due to osteoporosis. Of this number, 51% fractures occur in Europe and America alone[23]. Studies around the world suggest that androgens after aromatization in adipose or different tissues, influence bone either by direct interactions with androgen receptors (ARs), or through indirect binding to estrogen receptors (ERa and ERβ)[2].

In a recent Indian study, 8.5% patients had osteoporosis whereas 31.5% had osteopenia. The serum testosterone level was found low (<300 ng/dl) in 39 (19.5%) subjects. Researchers observed a positive correlation between serum testosterone and baseline BMD as well as a beneficial effect of androgen replacement therapy on BMD in patients with hypogonadism[24].

CONCLUSION

Osteoporosis was found to be significantly (p 0.0001) associated with testosterone deficiency in the Pakistani population. For prevention of osteoporosis and its complications it is essential that the optimal level of testosterone should be monitored and maintained by young and old men.

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