SERUM DIHYDROTESTOSTERONE IS A MAJOR DETERMINANT OF BONE MINERAL DENSITY IN MEN

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SUMMARY

In the present investigation, 100 osteoporotic men with 100 age-matched normal were studied for serum sex steroids, parathyroid hormone (PTH), thyroid hormone (T_3), cytokines and bone turnover markers. Serum dihydrotestosterone (DHT) was significantly decreased in osteoporotic men compared with normal. In osteoporotic men, there was a significant increase in serum interleukin (IL)-1alpha. A marked increase in bone formation marker, serum osteocalcin (OCN) and bone resorption marker, serum tartrate-resistant acid phosphatase (TRAP) reflects high bone turnover in osteoporotic men. In conclusion, the present findings point out an important role of DHT in maintaining BMD in men. A strong positive correlation of serum DHT with BMD offers new perspectives in understanding the role of non-aromatizable androgen in the regulation of bone metabolism in men.

Key words: BMD, cytokines, DHT.

INTRODUCTION

Recent epidemiological studies have pointed out that male osteoporosis is a formidable health problem worldwide. Despite the considerable public health burden attributable to male osteoporotic fractures, the causative factors of this metabolic bone disease are largely unknown. Although androgens are crucial for both skeletal development and maintenance in men (1), several clinical and experimental studies have indicated a major role for estrogens in the regulation of male skeleton (2). Accumulating evidences suggest that the actions of androgens may be mediated primarily by conversion to estrogens. Therefore, the relative contributions of estrogens versus androgens in regulating bone mass in men and the relative roles of testosterone and DHT in regulating BMD are also yet to be identified.

The role of cytokines on bone metabolism has received much attention since bone cells could produce and respond to these factors (3). There is now an increasing body of evidence that bone-resorbing cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-alpha) and macrophage-colony stimulating factor (M-CSF) may be potential candidates for mediating the bone loss following estrogen deficiency (4). In contrast, IL-4, IFN-gamma and transforming growth factor (TGF)-beta inhibit both osteoclast formation and its activity (3). However, to the best of our knowledge, there is no study on systemic cytokine levels in osteoporotic men and their relation to BMD, especially in Indian men.

Therefore, the present study was aimed at correlating the serum levels of sex steroids, parathyroid hormone (PTH), thyroxine (T_3), cytokines and bone turnover markers with BMD in osteoporotic men in a cross-sectional sampling in and around the Chennai city, India.

MATERIALS AND METHODS

In this study, 100 osteoporotic men (mean age 62 ± 1.1 years, range 40-70 years) and 100 healthy volunteers who served as normal (mean age 60 ± 1.3 years, range 40-70 years), living in and around the city of Chennai, India were investigated. The study was approved by the local Human Ethical Committee and all participants gave written informed consent. A standardized questionnaire was completed, which included current cigarette smoking, alcohol use, physical activity and medication use. None of the subjects were receiving any treatments known to interfere with bone metabolism. BMD was measured at the calcaneum using peripheral dual energy X-ray absorptiometry (DEXA). The current WHO definition of osteoporosis was applied to identify osteoporotic men.

*Fasting blood samples were collected from the an*tecubital vein of the normal and osteoporotic men between 08:00 and 09:00 h. Samples were immediately centrifuged, sera separated and then stored at –86° C until assayed. Serum total DHT and E_2 were assayed using RIA kits (DSL, USA). Testosterone was quantified by liquid phase RIA adopting the WHO procedure (5). Intact human PTH (1-84) was measured with IRMA kit and total T_3 was determined using *RIA* kit (DiaSorin, Italy). Serum osteocalcin was measured using IRMA kit (DSL, USA) and

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tartrate-resistant acid phosphatase (TRAP) was determined adopting standard biochemical method. Serum concentrations of IL-1beta, TNF-alpha, IL-4 and IFN-gamma were measured using the sandwich ELISA kits, according to the manufacturer's instruction (R & D Systems, USA). Data were statistically analyzed adopting Students' *t* test.

RESULTS

Serum hormones and bone turnover markers in normal and osteoporotic men (Table 1)

Among the hormones measured, DHT alone showed a decrease (42%) in osteoporotic men compared to normal. There was a significant increase ($P<0.05$) in osteocalcin and TRAP, reflecting high bone turnover in osteoporotic men compared to normal.

Table 1. Serum hormone profiles and bone turnover markers in normal and osteoporotic men

Parameter	Normal men (n = 100)	Osteoporotic men (n = 100)
DHT (pg/ml)	493 ± 45	286 ± 22*
Testosterone (ng/ml)	5.4 ± 0.41	4.9 ± 0.4
Estradiol (pg/ml)	28.4 ± 2	26.9 ± 2.1
PTH (pg/ml)	19 ± 1.5	21 ± 1.6
Total T_3 (ng/ml)	0.68 ± 0.05	0.73 ± 0.05
OCN (ng/ml)	5.7 ± 0.4	7.1 ± 0.5*
TRAP (IU/ L)	9.96 ± 0.99	18.26 ± 1.33*

Each value is mean ± SEM. * Denotes statistical significance at $P < 0.05$.

Serum cytokines in normal and osteoporotic men (Table 2)

A 29% increase ($P<0.05$) in IL-1beta was observed in osteoporotic men compared to normal.

Table 2. Serum levels of cytokines in normal and osteoporotic men

Serum cytokines (pg/ml)	Normal men (n = 100)	Osteoporotic men (n = 100)
IL-1beta	11.75 ± 0.9	15.1 ± 0.9*
TNF-alpha	70 ± 6	81 ± 6
IL-4	97.0 ± 8	95.8 ± 7
IFN-r	38.8 ± 2	40.1 ± 2.5

Each value is mean ± SEM. * Denotes statistical significance at $P < 0.05$.

Relationship of hormonal and biochemical parameters with BMD

Among the sex steroids, serum DHT positively correlated with the BMD ($r = 0.473$, $P<0.01$). IL beta ($r = -0.537$, $P<0.01$) and TNF- alpha ($r = -0.389$, $P<0.05$) negatively correlated with BMD.

DISCUSSION

Our findings point out that serum level of DHT is positively correlated with BMD, which corroborates with the observation that administration of non-aromatizable androgens prevented castration- induced bone loss in adult rats (6). Vandenput et al. (1) pointed out that administration of testosterone prevented orchidectomy-induced bone loss in estrogen receptor-alpha knock-out (ER alpha KO) male mice, ruling out the possibility of ER alpha -mediated action of androgen. Thus, it appears that DHT plays an important role in bone metabolism and the decrease in DHT could have resulted in diminished BMD in osteoporotic men. The possible reason for the decrease in serum DHT may be the defective expression and/or activity of 5 alpha -reductase or increased metabolic clearance of DHT in osteoporotic men.

The increase in IL-1beta in osteoporotic men could be due to decreased DHT since our recent *in vitro* study demonstrated that DHT significantly decreased the secretion of IL-1beta in the conditioned media of SaOS-2 cells (unpublished data). IL-1-beta or PTH-stimulated PGE_2 production in mouse calvarial cells was also suppressed by 5alpha -DHT by 50% (7). Therefore, it is reasonable to suggest that the decrease in DHT could have increased IL-1beta in osteoporotic men. However, the decrease in DHT in osteoporotic men was not associated with any significant increase in TNF-alpha. The treatment of human leukemia T cell line, Jurkat, with 5 alpha-DHT resulted in a 50% decrease in the level of TNF- alpha mRNA (8) and our recent *in vitro* study demonstrated that DHT significantly decreased the secretion of TNF-alpha in the conditioned media of SaOS-2 cells (unpublished data). Pearson's correlation coefficients strongly support our results, since both IL-1beta and TNF–alpha are negatively associated with BMD. Thus, it appears that the availability of cytokines at the bone microenvironment than at systemic level may determine the bone function, and could regulate BMD. In conclusion, our study demonstrates that DHT is mandatory for maintaining BMD in men.

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