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## Brief Report

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# Reference Database of CrossLaps and Osteocalcin for a Healthy Iranian Population

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Markers of bone turnover are becoming an important tool for practitioners in the management of osteoporosis. Therefore, it is essential to establish a reference database of the markers before using them in various clinical settings.

A total of 785 individuals (37% males, 63% females) without apparent or suggested abnormalities affecting bone mass were randomly selected from 13 clusters in Bushehr Port in southern Iran. The serum CrossLaps ELISA and the N-MID Osteocalcin ELISA were used for the quantitative measurement of CrossLaps and osteocalcin in sera. Bone mineral density was determined for the lumbar spines (L2-L4), proximal femur (neck), and forearm (the distal part) using dual-energy X-ray absorptiometry.

Men had higher biochemical serum bone markers ( $P<0.0001$ ). In women, there were progressive increases in serum CrossLaps after 30 years of age, peaking at >60 years. In men, serum CrossLaps levels were decreased progressively by increases in age, with the peak at 20 – 29 years. In women, there was a significant decrease in serum osteocalcin from 20 – 29 years to 30–49 years, followed by a progressive increases during 50 – 59 years, with the peak at >60 years. In men, the highest concentrations for serum osteocalcin occurred at 20 – 29 years. At all sites checked for bone mineral densities, women in the high osteocalcin quartile had the lowest mean bone mineral densities values, but women in the high CrossLaps quartile had the lowest mean bone mineral densities at lumbar and radial sites. However, in men, bone mineral densities values at neither site differed between the lowest and the highest quartiles of serum biochemical bone markers.

We presented a five- year age-specific mean values of bone markers in a general healthy Iranian population. Only women in the high osteocalcin and CrossLaps quartiles had the lowest mean bone mineral densities values at the lumbar and radial sites. Our results suggest that the significance of osteoclastic bone resorption or bone formation as a determinant of bone mineral densities may depend on sex.

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**Keywords:** Biochemical markers of bone turnover • bone mineral density • CrossLaps • osteocalcin • osteoporosis

## Introduction

Markers of bone turnover are becoming an important tool for practitioners in the management of osteoporosis. Changes in bone density can generally be seen after about two years of treatment, whereas biochemical markers can

provide dynamic information about the effectiveness of therapeutic agents such as bisphosphonates, estrogens, selective estrogen receptor modulators (SERMS), and calcitonins within three to six months.<sup>1</sup>

In addition, markers of bone turnover are expected to predict how much bone an individual will lose and how great a risk for fragility fractures they gain.<sup>2,3</sup>

These potential uses may increase the prediction accuracy of bone densitometry and assist practitioners to successfully prevent osteoporosis or osteoporotic fractures in high- risk patients. Therefore, it is essential to establish a

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reference database of the markers before using them in various clinical settings. The objectives of this study were firstly to find the age-specific value of serum CrossLaps (degradation products of C-terminal telopeptides of type-1 collagen) which is a new biochemical marker of bone turnover and osteocalcin as a bone formation marker for a healthy Iranian population, and secondly to find the differences in bone mineral density (BMD) at various body sites between individuals with and without high biochemical markers of bone turnover.

## Materials and Methods

### Subjects

This study was performed as a part of a larger epidemiologic study—the Iranian Multicenter Osteoporosis Study (IMOS)—where 6,000 normal subjects were randomly selected from five major cities throughout Iran. Details of the study can be found elsewhere.<sup>4</sup> The study goal was to recruit 120 healthy men and women, aged 20 – 69 years in each age decade in every center. The subjects of the present report were randomly selected from 13 clusters in Bushehr Port, south of Iran.

### Measurements

Bone mineral density was determined for lumbar spines (L2-L4), proximal femur (neck), and the distal forearm using dual-energy X-ray absorptiometry (DXA) (Osteocore II Osteodensitometer, Medilink, France). The accuracy of the measurements was proved by scanning phantoms and analyzing the scans.

The serum CrossLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) was used for the quantification of degradation products of C-terminal telopeptides of type -1 collagen in sera. The intra-assay coefficient of variations for low (0.242 ng/mL), medium (0.375 ng/mL), and high (0.476 ng/mL) values were

5.4%, 5.0%, and 5.1%, respectively.

The N-MID® Osteocalcin ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) was used for the quantitative measurement of osteocalcin in sera. The intra-assay coefficient of variations for low (7.0 ng/mL), medium (21.8 ng/mL), and high (43.2 ng/mL) values were 3.4%, 2.0%, and 2.4%, respectively.

### Statistics

We found that log transformation of CrossLaps and osteocalcin gave a better fit to a Gaussian distribution. The 95%<sup>4</sup> interval for CrossLaps and osteocalcin was defined as the arithmetic mean of the log-transformed data  $\pm 2$  standard deviation (SD), raised to the power of 10.

ANOVA was used to analyze the effects of bone biochemical marker quartiles on BMD at different body sites. The Bonferroni pairwise multiple comparisons test was used as the post hoc test to evaluate the differences in bone parameters between a single quartile and each of the other quartiles. Statistical analysis was performed by an IBM computer using the SPSS software version 9.5 (SPSS Inc., Chicago, IL, USA).

## Results

A total of 785 individuals (37% males, 63% females) were evaluated. Of the studied population, 137 (17.5%) were 20 – 29 years, 176 (22.4%) were 30 – 39 years, 222 (28.3%) were 40 – 49 years, 163 (20.8%) were 50 – 59 years, and 87 (11.1%) were  $\geq 60$  years. Shown in Table 1 are the logarithmic means (and SD), as well as 95% confidence intervals for serum bone markers in the above age groups. Men had higher serum levels of biochemical bone markers ( $P < 0.0001$ ).

CrossLaps showed a significant variation with age in both sexes (ANOVA,  $P < 0.0001$  for women and  $P = 0.001$  for men). In women, there were progressive increases in serum Crosslaps after the

**Table 1.** Age- and sex-specific reference data for serum CrossLaps and osteocalcin in a healthy Iranian population.

	CrossLaps				Osteocalcin			
	Men		Women		Men		Women	
	Median ng/mL	95% CI * ng/mL	Median ng/mL	95% CI * ng/mL	Median ng/mL	95% CI * ng/mL	Median ng/mL	95% CI * ng/mL
20 – 30	550	204 – 1548	310	109 – 912	15.98	5.01 – 41.68	12.44	4.89 – 28.18
30 – 40	460	158 – 1318	260	100 – 691	11.96	5.24 – 27.54	8.11	3.46 – 19.95
40 – 50	405	147 – 1122	240	87 – 870	11.30	4.57 – 23.98	8.93	2.88 – 26.30
50 – 60	385	112 – 1348	420	123 – 1348	9.83	3.16 – 31.62	11.53	3.71 – 30.90
>60	375	87 – 1148	470	144 – 1584	12.35	5.12 – 24.59	13.10	4.26 – 38.90
<b>Total</b>	<b>430</b>	<b>134 – 1348</b>	<b>297</b>	<b>100 – 1513</b>	<b>11.95</b>	<b>4.36 – 30.19</b>	<b>9.82</b>	<b>3.38 – 28.18</b>

\*Confidence interval=arithmetic mean of log-transformed data ( $\pm$ SD), raised to the power of 10.

age of 30, peaking at >60 years. In men, serum CrossLaps levels were at their peak in the 20 – 29 years age group, and decreased progressively with age.

Osteocalcin also showed a significant variation with age in both sexes (ANOVA,  $P < 0.0001$ ). In women, there was a significant decrease in serum osteocalcin from 20 – 29 years to 30 – 49 years ( $P < 0.0001$ ). There were then progressive increases in serum osteocalcin from 50 – 59 years, peaking at >60 years ( $P < 0.0001$ ). In men, the highest concentrations for serum osteocalcin occurred at 20 - 29 years ( $P < 0.0001$ ).

In women, mean differences between the highest and lowest quartiles of osteocalcin were 7.4% ( $P = 0.004$ ) for femoral neck BMD, 10.6% ( $P < 0.00001$ ) for radial BMD, and 7.4% ( $P < 0.0001$ ) for lumbar BMD, which shows that women in the highest quartile of osteocalcin had lower BMDs. These values for CrossLaps were 5.5% ( $P = \text{NS}$ ) for femoral neck BMD, 8.2% ( $P = 0.02$ ) for radial BMD, and 10.0% ( $P < 0.0001$ ) for lumbar BMD, which shows that women in the highest quartile of CrossLaps had lower BMDs at lumbar and radial sites. In men, however, analysis by quartiles of biochemical markers of bone turnover revealed no significant association with BMD values at any studied site.

## Discussion

The present study established the reference values of two biochemical markers of bone turnover, serum CrossLaps and osteocalcin, in a group of healthy individuals of various ages. The subjects were randomly selected in an urban area to avoid sampling bias. Therefore, this population-based database covering a wide range of ages would give the researchers the opportunity to make international or national comparisons of bone turnover markers.

A steep elevation in the biochemical markers of bone turnover after menopause is widely accepted. Garnero et al.<sup>5</sup> reported 37%, 52%, and 79% higher levels of osteocalcin, bone alkaline phosphatase, and CrossLaps, respectively in women <10 years after menopause compared to premenopausal women. The corresponding differences for CrossLaps and osteocalcin in the present study were 40% and 24%.

The effects of aging on biochemical markers of bone turnover in men are poorly defined and are

controversial. A fall in biochemical markers of bone turnover with age has been reported,<sup>6</sup> while others have suggested that there is no change or an increase.<sup>7</sup> We did not observe a uniform pattern for bone turnover markers in men.

To evaluate the association between biochemical bone markers and BMD parameters, we compared average BMDs in both men and women, according to osteocalcin and CrossLaps quartiles. At all sites checked for BMD, women in the high osteocalcin quartile had the lowest mean BMD values but women in the high CrossLaps quartile had the lowest mean BMDs at lumbar and radial sites. One study also found a highly significant correlation between serum osteocalcin concentrations and BMD of the spine.<sup>8</sup> Another report divided subjects into quartiles according to the urinary excretion of cross-linked N-telopeptides (NTX) of type-1 collagen, a marker of bone resorption.<sup>9</sup> There was an inverse relationship between the quartile of urinary NTX excretion and the mean BMD. These findings are consistent with the concept that osteoporosis is characterized by an increase in both bone formation and resorption.

Despite these general trends, biochemical markers are not useful in making the diagnosis of osteoporosis because the values in normal subjects and patients with osteoporosis overlap substantially. In one report, for example, patients with low serum osteocalcin concentrations had bone densities that ranged from low to very high.<sup>1</sup> Using a population-based data set, it has been shown that none of the major biochemical markers of bone turnover provide sufficient diagnostic information useful for screening vertebral osteopenia or osteoporosis.<sup>10</sup>

In men, most measurements of biochemical markers of bone turnover have been performed in small cohorts with limited age ranges, and results obtained in large cohorts are scanty. In elderly men, biochemical bone markers were negatively correlated with BMD.<sup>11</sup> In the present study, BMD values at neither site in men differed between the lowest and highest quartiles of serum biochemical bone markers.

Our results suggest that the significance of osteoclastic bone resorption or bone formation as a determinant of BMD may depend on sex.

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