Association between the Bone Turnover Markers and The Gynecologic Factors Among Moroccan Women

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ABSTRACT

Background: The action of bone cells on the bone mineral density can be highlighted from their enzymatic activities, by the bone alkaline phosphates (BAP) and the Cross Laps-telopeptide terminal of the collagen type 1 (β -CTX). They are reliable to explore the activity of bone turnover. We aim to put in perspective the association between gynecological factors and the concentration of bone turnover markers (BTMs) among Moroccan women.

Methods: This is a cross-sectional study of 125 women from February to April 2015. A randomized sample was made. The biochemical parameters of bone metabolism concerned serum calcium, 25 hydroxyvitamin D (25OHD), parathyroid hormone (PTH), phosphorus, BAP and β -CTX. All data related to gynecologic, sociodemographic and anamnesis parameters was collected by standardized questionnaire.

Result: Among 125 women, 64(51.42) were menopausal, the mean BAP was 22.1 ± 7.6 ng/L, the median of the interval between successive births "IBSB" and of β -CTX were 36.0[24.0-58.0] months, 368.5 [237.0-504.8]ng/L. A positive correlation was observed between the β -CTX and IBSB ($r=0.2, p=0.05$). The BAP concentration was higher in menopausal women ($p<0.001$). The linear regression showed that 12% of BAP concentration was associated to the menopause ($R^2=0.12, p=0.001$) but no associated factors was founded with concentration of β -CTX.

Conclusion: Our study shows that the menopause is associated factor with concentration of BAP and IBSB is correlated with the concentration of β -CTX.

Keywords: Bone Turnover, Markers, Gynecologic, Women, Morocco.

Introduction

The bone remodeling is a complex physiological process during which the osteoclasts, cells of bone resorption, and the osteoblasts, cells of bone formation, act in sequential and coupled frequencies to destruct and renew the bone matrix. The objective of this physiological process is to lead an adequate bone mineral density (BMD) depending on the age and the sex (1). This bone remodeling process is under the action of several factors such as biological mediators, cited the vitamin D and the calcium, also under the action of sexual hormones and under the action of some external parameters related to the lifestyle of the person (1, 2).

The action of these cells on the bone can be highlighted from their enzymatic activities by the biochemical markers of bone turnover: Bone Alkaline Phosphatase (BAP) and

C-telopeptide Terminal Collagen type 1 (β -CTX). The BAP is an enzyme of the bone formation specific to the osteoblasts whereas the β -CTX is an enzyme of bone resorption specific to the osteoclasts (2). The use of these enzymes are reliable to their non invasive exploration of bone turnover (3), they allow in association with the bone densitometry to estimate the loss of bone and to monitor the response of therapeutic effect of several bone diseases (4).

The trend to establish a link between markers and BMD was also subject to research among Moroccan researchers (5, 6). Yet, to our knowledge, no study so far in Morocco has been undertaken to study the associated factors with the concentration of biochemical of bone turnover markers (BTMs).

Related to this background, we conducted this present study in Meknes region of Morocco, with the aim to put into

perspective the association between gynecological factors and the concentration of BMTs among women in the general population.

Materials and Methods

Subjects: The present cross-sectional study was conducted from February to April 2015 among 125 women of Meknes. The participants were recruited from a variety of sources, including workers in private and public companies, patients consulting at ambulatory medicine, medical staff of a laboratory and from “word of mouth”.

This research included Caucasian women, aged 18 years old and older, not presenting any diseases affecting the bone metabolism, such as thyroid and parathyroid disease, kidney and liver disease, chronic intestine inflammatory and tumors.

Our institutional committee approved this study. The ethical procedures of study were in accordance with the Declaration of Helsinki. The 125 women recruited gave their oral consent to participate in this investigation and were formed in advance about its objective and conditions of study. Any subject didn't want to be included or didn't want to continue answering could leave the study without any effect on her treatment process.

The investigation was conducted in the medical laboratory, each participant came to the laboratory to have a fasting blood test and to complete questionnaire. The standardized questionnaire was used to collect the data and was divided in three sections related to sociodemographic, anamnesis, anthropometric parameters, gynecological parameters and biological parameters.

Sociodemographic, Anamnesis, Anthropometric Parameters: The first component of the questionnaire concerned sociodemographic variables e.g. date of birth, socio-professional status, marital status and level of instruction. The anamnesis variables “personal and family history of fractures, the feeling of diffuse pain, defines as unpleasant sensory at the level of the bones and muscles (7)” and the anthropometric parameters “weight and the waist-hip ratio “WHR” calculated by dividing the waist by hip in meter”.

Gynecologic Parameters : The second component related to the gynecological characteristics including the age at menarche, parity, interval between successive births “IBSB” defined as the time separated two successive births (8), and the menopause defines as the ending of menstrual periods in a natural or surgical way due to the stop production of ovarian hormones(9).

Biological Parameters: The third component referred to the biochemical parameters of bone metabolism which

are serum calcium, phosphorus, 25 hydroxyvitamin D (25OHD), parathyroid hormone (PTH), β -CTX and BAP.

The biological analysis of calcium serum levels (reference range: 2.1- 2.6 mm) and phosphorus (reference range: 0.74 to 1.51 mm) were carried out by Cobas 8000. The biological analysis of 25OHD (reference range of >20 ng/ml), the BAP (reference range: 6.1-22.2 μ g/L), the PTHi (reference range: 4 to 26ng/L) and of the β -CTX (reference range <700ng/L) were carried out by CLIA.

The quantification method of 25OHD recognizes 25 (OH) D₂ and D₃. The threshold of hypovitaminosis D was fixed at 20 ng/ml as recommended by the American Institute of Medicine (10).

Statistical Analysis: The normality of the variables has been tested by Shapiro-Wilk. The Gaussian variables were reported as means with standard deviation (SD), the no Gaussian ones as median with interquartile ranges [IQR]. The categorical variables were expressed as absolute (n) and relative frequencies (%). The Spearman coefficient was used to determinate the correlation. Student's t test and Mann-Whitney U test were used to compare the average of BAP and the median of β -CTX in qualitative variables. The multiple regression models, we introduced the variables which were statistically significant at the univariate analysis, was used to determinate the associated factors with the concentration of BTMs.

Result

In this study of 125 women, 64(51.42) of them were menopausal, the mean $\pm$ (SD) age and BAP were 50.3(15.3) years, 22.1 $\pm$ 7.6 ng/L, the median with interquartile ranges [IQR] of the IBSB and β -CTX were 36.0[24.0-58.0] months, 368.5 [237.0-504.8]ng/L. The serum concentrations of calcium (2.3 $\pm$ 0.08 mm) and phosphorus(1.0 $\pm$ 0.1 mm) were within normal range whereas for the 25-OHDconcentration (8.8 $\pm$ 3.7 ng/ml) was in the low range. The characteristics of our sample in the univariate analysis are shown in Table 1.

For the threshold less than 20ng/ml, the prevalence of hypovitaminosis D was 98.4% (n=123). Among the deficient vitamin D women, 87 (70.7%) of them had a hyperparathyroidism (PTH=35.4 $\pm$ 13.9 ng/L), 14 (11.4%) a high bone resorption (β -CTX= 376.6 [239.6-513.0] ng/L), and 56 (45.5 %) a high bone formation (BAP =22.2 $\pm$ 7.7 μ g/L).

Correlation BTMs with Biological and Gynecological Parameters: A significant positive correlations between BAP and the age(r=0.3, p<0.001) and the PTH (r=0.1,

$p=0.04$) were observed there was a positive correlation between the β -CTX and IBSB ($r=0.2, p=0.05$). In addition, we noted significant negatives correlations between the PTH and the 25OHD ($r=0.2, p=0.02$), serum calcium ($r=-0.2, p=0.002$) and phosphorus ($r=-0.1, p=0.02$) but no correlations were found between 25OHD and BAP or β -CTX.

Comparison of BTMs with Amnesic and Gynecological Parameters : The BAP concentration was higher in women according to the menopause ($p<0.001$) and the diffuse pain ($p<0.001$). However, no difference in the BAP concentration was found according to the personal

and familial history of fractures ($p=0.6$ and $p=0.7$) (Table 2). The β -CTX median concentrations didn't show any differences according to the qualitative variables mentioned above (Table3).

Linear Regression Model for Btms: The significant variables of the study in the univariate analysis were introduced in descending linear regression model for the BAP and β -CTX. The results showed that 12% of BAP concentration has been associated to the menopause ($R^2=0.12$; $\beta=-0.29$, $p=0.001$), whereas the β -CTX concentration appeared to not be associated with any variable of this model ($p=0.7$).

Table 1: Database of the sample

	Total N	Characteristics
Age	125	50.32±15.4
Socio-demographic characteristics		
Marital status	125	
Married		57 (45.6)
Not married		68(54.4)
Socioprofessional status	125	
With the employment		57(45.6)
Without employment		68(54.4)
Level of Education	125	
Illiterate		38(30.4)
Primary school		27(21.6)
Secondary education		34(27.2)
Superior		26(20.8)
Anamnesis characteristics		
Widespread pain	125	
Yes		83(66.4)
Non		42(33.6)
Personal history fractures	125	
Yes		34(27.2)
Non		91(72.8)
Familial History fractures	125	
Yes		29(23.2)
Non		96(76.8)
Anthropometric characteristics		
Weight (kg)	123	72.9±14.2
WHR (m)	123	0.96±0.13
Gynecologic characteristics		
Age at menarche	106	12.2±1.6
Parity	79	3.0[2.0-5.0]*

	Total N	Characteristics
Menopause	125	
Yes		64(51.42)
No		61(48.8)
Space btween births	67	36.0[24.0-58.0]*
Biological parameters		
Calcium (mm)	125	2.3±0.08
Phosphorus (mm)	125	1.0±0.1
25OHD (ng/ml)	125	8.8±3.7
PTHi (ng/L)	125	35.3±13.8
β-CTX (ng/L)	125	368.5[237.0-504.8]*
BAP (µg/L)	125	22.1±7.6

* Mediane [IQR]

Table 2: Comparison of concentrations of BAP with amnesic and gynecologic parameters using Student t test

	BAP	P Value
Menopause		
Yes	(64)24.5±6.9	<0.001
Non	(61)19.7±7.6	
Widespread pain		
Yes	(83)23.2±8.0	0.016
No	(42)20.0±6.4	
Personal history of fractures		
Yes	(34)21.9±7.6	0.8 (NS)
No	(91)22.2±7.7	
Familial history of fractures		
Yes	(29) 23.0±7.9	0.5 (NS)
No	(96)21.9±7.6	

Table 3: Comparison of β-CTX with amnesic and gynecologic parameters using Mann-Whitney test

	B-CTX	P Value	U
Menopause			1860.0
Yes	(64)382.6[266.5-521.9]	0.6(NS)	
Non	(61)354.6[219.3-493.1]		
Widespread pain			
Yes	(83)367.8[248.9-496.6]	0.9 (NS)	1732.0
No	(42)408.7[216.8-534.3]		
Personal history of fractures			1515.0
Yes	(34) 364.3[212.5-488.5]	0.8 (NS)	
No	(91) 376.6[239.6-513.0]		
Familial history of fractures			
Yes	(29) 360.1 [213.4-617.2]	0.9 (NS)	1388.0
No	(96)385.4 [241.9-474.3]		

Discussion

The objective of this study was to determine the associated gynecological factors with the concentration of BTMs among Moroccan woman. The results showed that the menopause was an associated factor with the BAP and the IBSB was correlated with the β -CTX but wasn't considered as an associated factor with its concentration.

The literature reported that the BAP is a sensitive marker of bone remodeling during menopause (11,12) including secondary hyperparathyroidism, aluminum-related low-turnover bone disease, osteomalacia, adynamic osteopathy, osteoporosis, and skeletal beta2-microglobulin amyloid deposits. In spite of the enormous progress made during the last few years in the search of noninvasive methods to assess bone metabolism, the distinction between high- and low-turnover bone diseases in these patients still frequently requires invasive and/or costly procedures such as bone biopsy after double tetracycline labeling, scintigraphic-scan studies, computed tomography, and densitometry. This review is focused on the diagnostic value of several new serum markers of bone metabolism, including bone-specific alkaline phosphatase (bAP). Some researchers find similar results to ours, their studies have highlighted the correlation between the BAP and the sexual hormones during menopause; they showed that the concentration of BAP is related to the concentration of the follicle stimulating hormone (FSH) among menopausal women (13-15). A Chinese investigation reported that the concentration of FSH explained 1.9-11.8% of BAP concentration among postmenopausal women (13). A Moroccan study, among menopausal women found an correlation between ovarian hormones Estradiol and the Osteocalcin but the authors didn't consider the BAP in their study (5).

In the contrast to others studies (16) we didn't find the same ascertainment. The variation in outcomes can be attributable to the technical's methods and the genetic variety of population's studies which must be taken into account during BTMs interpretation (17).

The positive correlation between the BAP and PTH allows to suggest that the increase of BAP is associated to hyperparathyroidism leading the hyperactivity of bone and predict the risk of osteomalacia and secondary osteoporosis among women (12,18).

Our results showed that the IBSB was correlated with the β -CTX concentration. In fact, there is a physiological change in calcium metabolism during pregnancy which allows a mineral transfer from the mother to the fetal

skeleton to ensure its growth and its mineralization following a hormonal adjustment (19,20). So after the birth, the maternal bone needs time to be restored but the successive pregnancies affect the bone mineralization due to increase a process of bone resorption (21). To our background, no Moroccan study has been performed among mothers population addressing the influence of IBSB on BTMs.

We can suggest that the IBSB affects the bone by taking into account the hypothesis of the strong parity of the Moroccan population, with average of fertility equal to 7.2 children per woman in 1962 which are considered as menopausal women in our sample (22), and the psychosocial dimensions of the Moroccan society which impose the status of procreation for the women (23).

The results reported high prevalence of hypovitaminosis D in our population such as the reported by the study of El Allali et al. among Moroccan women where the prevalence of hypovitaminosis D was 91% (6). The 25-OHD deficiency can be related to many causes such as the individual factors regarding to the lifestyle of women, to the geography factors taking into account the position of the city, to the technical parameters and finally to the political factors due to the lack of vitamin D supplementation for foodstuffs.

Our investigation didn't demonstrate any link between the 25OHD and BTMs along with our study some authors didn't find any relationship (24) and thus to increased bone loss. We investigated 72 elderly subjects (16 men and 56 women) but others demonstrated that a deficiency in 25OHD leads to an increase in the serum concentration of BTMs (25). Those differences could be due to the season and geographical place of the study, the age and ethnic of the subjects and interlaboratory variation for the measurement of BTMs and 25OHD.

Our study presents strengths and limitations. The main limitations lie to the procedure to select the subjects, we cannot exclude that some subjects did not reported remote traumas, and the lack of the bone densitometry measurement. By contrast, the strengths of our research consisted to control the analytical part of our study using standard procedures undertaken by an experienced medical staff to manage and conduct the study also in standardization of the biological analysis among all subjects.

Conclusion

This study allows to determinate the gynecological factors associated with the concentration of biochemical

BTMs parameter which is the menopause. We also demonstrated that the IBSB was a correlated factor with the β -CTX. Longitudinal perspectives studies could be initiated to establish a link between these factors and the BTMs.

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