Serum Osteocalcin, P1NP, Alkaline Phosphase, and CrossLaps in Humans

The relationship with body mass index

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This is a clinical study on 56 subjects included in normal weight (NW) group (N=17), overweight (OW) group (N=19) and grade I obese (O) group (N=20), based on BMI (Body Mass Index) values: NW group had a mean BMI of 22.2 ± 2.14 kg/sqm, OW group had a BMI of 25.89 ± 1.04 kg/sqm, and O group had an average BMI of 32.2 ± 2.09 kg/sqm (p-value NW-OW, NW-O, respective OW-O groups was p<0.0005). The 3 groups were similar as age (p-value NW-OW groups = 0.7, between NW- O groups = 0.8, respective between OW - O group = 0.7). The circulating bone formation (osteocalcin, P1NP alkaline phosphatase) and resorption profile (CrossLaps) indicated no statistical significant difference between groups while the coefficient of regression r between each biochemical bone marker and BMI in every BMI group exceeded the value of p>0.05. All the 3 groups had a mean value of 25-hydroxycholecalciferol in deficiency ranges (< 30 ng/mL, normal recommended values are above 30 ng/mL) without significant differences regarding BMI groups, except for obese group when compare to the other two groups. No secondary hyperparathyroidism was associated in any group despite low vitamin D levels. Based on our observation, bone turnover biochemical markers are not influenced by BMI.

Keywords: osteocalcin, P1NP, alkaline phosphatase, menopause, bone turnover marker

Skeleton is a dynamic complex organ with a permanent balance between bone formation and bone resorption [1,2]. Some pathological conditions like Paget's disease, primary and secondary osteoporosis, primary hyperparathyroidism first affect the bone turnover in different ways [3-6]. The best reflection of bone dynamics is done through biochemical bone turnover markers which are chemical products of bone cells with blood and/or urinary release thus they are easy to assess in daily practice [7]. Their chemical structure varies but most of them are related to matrix proteins like collagen in relationship with different enzymes [8,9].

Recently, bone status was showed to be damaged in clinical circumstances priory unknown as negative targeting the skeleton by type 2 diabetes mellitus or obesity [10,11]. These conditions also affects the turnover of the bone cells thus the chemical molecules exported by them might reflect skeleton biology; moreover, the fat-derived hormones or adipokines control the bone function [10-12]. Alkaline phosphatase (AP) which is a basic phosphatase (a protein enzyme of 86 kD, containing 5 cysteines per monomer, 2 zinc atoms and 1 magnesium atom to achieve the catalytic role, usually the optimum active pH is alkaline), osteocalcin (OC) which is a gamma-carboxyglutamic acid-containing protein (having a vitamin K dependent gla domains synthesis), also associating a hormone role, with non-collagenous bone origin, and P1NP (also known as total procollagen type 1 N-terminal propeptide, with a trimeric structure and monomeric degradation currently considered the most relevant bone formation marker) represent bone formation markers [13-15].

Experimental part
The aim of the study
Our aim is to introduce a study in human adults to highlight the bone turnover markers profile in relationship with body mass index (BMI) groups.

Material and method
This is a transversal non-interventional study on female subjects. The study was done between 2016 and 2018.

BMI was calculated based on weight, and height using the classical formula of weight (kg) / (height)^2 (sqm). We used the traditional groups based on BMI cut-offs: normal weight (NW) with BMI between 18.5 kg/sqm and 24.9 kg/sqm, over weight (OW) group with BMI between 25 and 29.9 kg/sqm, and obesity (first grade) group (O) including patients with a BMI of 30 kg/sqm up to 24.9 kg/sqm. Also the age of the patients at the moment of study is registered.

The assessments include venous blood bone turnover markers as following: bone formation alkaline phosphatase (colorimetric assay of VITROS type), osteocalcin (electro-chemiluminescence method), P1NP (ECLIA method of assessment), and bone resorption marker CrossLaps (electrochemiluminescence assay). Figure 1 introduces the biochemical markers reflecting the bone turnover metabolism which were assessed in the current study.
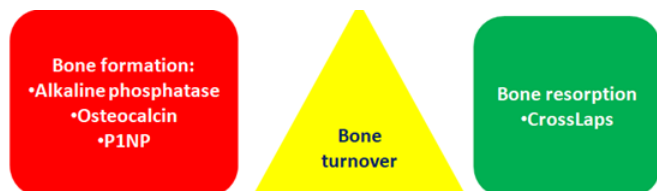
* email: adycamen@icloud.com

Fig. 1. Panel of investigations regarding bone turnover assessment

Also, circulating pro-hormone 25-hydroxyvitamin D ((*6R*)-6-[(*1R,3aR,4E,7aR*)-4-[(*2Z*)-2-[(*5S*)-5-Hydroxy-2-methylidene-cyclohexylidene]ethylidene]-7a-methyl-2,3,3a,5,6,7-hexahydro-*1H*-inden-1-yl]-2-methyl-heptan-2-ol) or 25OHD was measured using chemiluminescence technique. [16] Moreover, PTH (parathormone) was also assessed (electro-chemiluminescence technique). Normal values are introduced in figure 2.

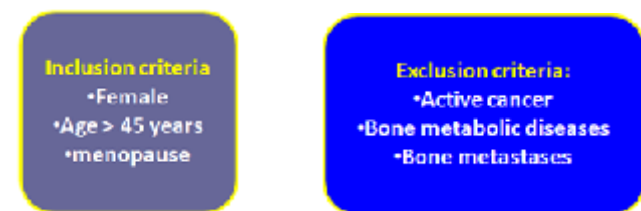Statistical analysis used the calculation of mean and standard deviation (SD); statistical significant was

Fig. 2. Inclusion and exclusion criteria

considered at $p<0.05$ (for student ttest and linear regression).

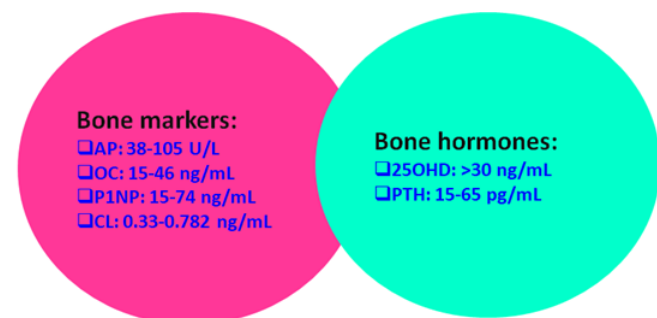The inclusion and exclusion criteria are introduced in figure 3.

Fig. 3. Normal values of bone turnover markers and also of 25-hydroxyvitamin D and parathormon

Results and discussions

56 subjects were included in NW group (N=17), OW group (N=19) and O group (N=20) (fig. 4) The groups were formed based on BMI values thus BMI was statistically significant different between the groups: NW group had a
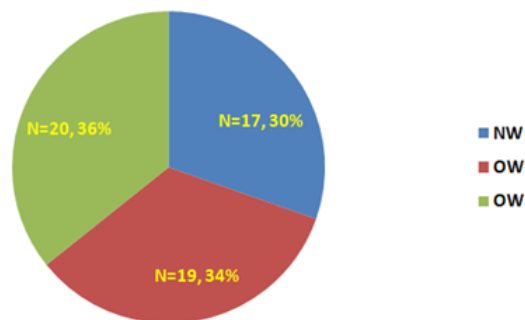

Fig. 4. The three studied groups based on BMI values (NW=normal weight, OW=overweight, O=obese)

mean BMI of 22.2 ± 2.14 kg/sqm, OW group had a mean BMI of 25.89 ± 1.04 kg/sqm, and O group had an average BMI of 32.2 ± 2.09 kg/sqm (p-value between NW and OW, NW and O group, respective OW and O group was $p<0.0005$). The three groups were similar as age (p-value between NW and OW group was 0.7, between NW and O group was 0.8, respective between OW and O group was 0.7) (table 1).

Table 1
PARAMETERS OF THE STUDIED POPULATION (N=56). THE PATIENTS WERE MENOPAUSAL WOMEN WHO WERE GROUPED BASED ON BMI VALUES IN NW (NORMAL WEIGHT), OW (OVER WEIGHT), AND FEMALES WITH FIRST GRADE OF OBESITY (O GROUP)

Group	Number of patients	Age (mean) years	Age (SD) years
NW	17	47.52	5.03
OW	19	48.00	5.47
O	20	47.40	4.51

The circulating bone formation and resorption profile indicated no statistical significant difference between groups while the coefficient of regression r between AP, OC, P1NP, respective CL and BMI in each BMI group was $p>0.05$ (table 2).

All the three groups had a mean value of 25OHD in deficiency ranges without significant differences regarding BMI groups, except for obese group when compare to te others two groups, p=0.04 for NW-O groups, respective p=0.02 for OW-O groups (table 3). No secondary hyperparathyroidism was associated despite low vitamin D levels (table 3).

The limits of the study are the general limits of bone turnover studies meaning their high variability from one

Table 2
BONE TURNOVER MARKERS STUDIED IN 3 GROUPS OF MENOPAUSAL SUBJECTS (AP = ALKALINE PHOSPHATASE, OC = OSTEOCALCIN, CL = CROSSLAPS)

Group	Number of patients	AP (U/L)	OC (ng/mL)	P1NP (ng/mL)	CL (ng/mL)
NW	17	73.31±25.14	29.49±11.23	53.89±23.22	0.55±0.19
OW	19	82.35±31.11	26.41±13.74	63.38±45.35	0.51±0.25
O	20	92.02±39.00	23.34±12.23	57.23±24.54	0.44±0.23

Table 3
BONE HORMONE VALUES IN MENOPAUSAL WOMEN WHO WERE GROUPED BASED ON THEIR BMI VALUES
(25OHD = 25-HYDROXYVITAMIN D, PTH = PARATHORMONE); THE STATISTICAL SIGNIFICANCE IS CONSIDERED AT P<0.05

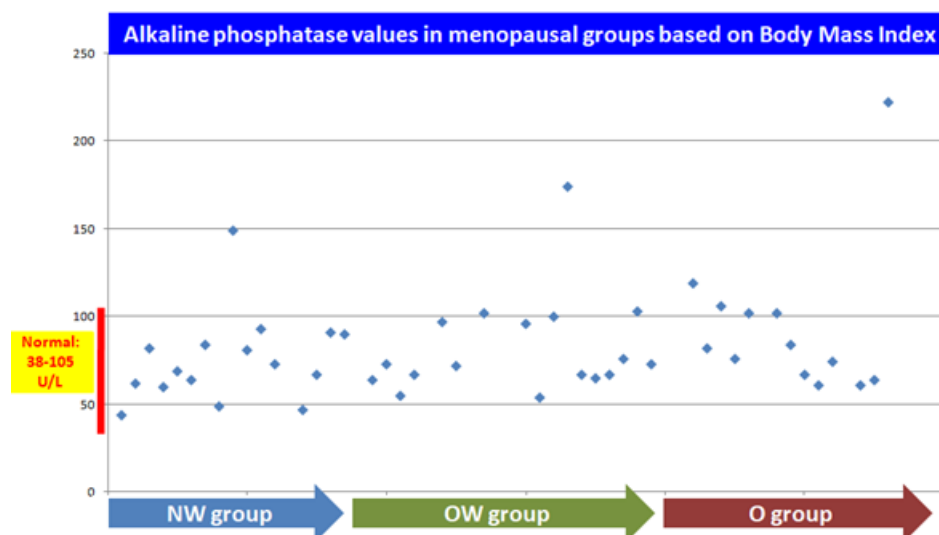| Group	Number of patients	25OHD (ng/mL)	PTH (pg/mL)
NW	17	23.04±11.36	48.49±23.39
OW	19	21.43±6.96	51.16±28.37
O	20	16.22±7.09	50.19±13.24
p-values			
NW-OW groups		0.6	0.7
NW-O groups		0.04	0.5
OW-O groups		0.02	0.8

Fig. 5. The distribution of alkaline phosphatase values in menopausal groups according the baseline body mass index of each patient

Table 4

BONE RESORPTION (OR OSTEOCLASTIC OR BREAKDOWN) AND BONE FORMATION (OR OSTEOBLASTIC OR BUILDUP) MARKERS (ADAPTED FROM 17, 22, 26, 27, 28)

Bone process	Molecule	Chemical data	Abbreviation
Resorption	C-telopeptide	Cross linking telopeptide of type I collagen	CTx
	Cross-links Cross Laps	Collagen cross-linking telopeptide of type I collagen	PYD or DPD
	TRAP	Tartrate-resistant acid phosphatase	TRAP
	Hydroxyproline		OHP
	Bone sialoprotein		BSP
	Gamma carboxyglutamin acid		GLA
Formation	P1NP	Pro-collagen type I N-terminal propeptide	
	Alkaline phosphatase	Bone-specific alkaline phosphatase	AP or ALP or BSAP
	Serum osteocalcin	Bone gla protein	OC or S-OC

person to another and the need of larger population study. Moreover, we did not include all the potential categories of BMI (obesity of higher grades except first grade, also under weighted persons).

As strength of the study we mention the collateral observation regarding the high prevalence of hipoyitaminosis D in menopausal studied population.

This particular study used a combination of 4 biochemical molecules that are easy to asses in daily clinical practice through blood tests. The panel of bone turnover markers varies and their use is currently limited by a large inter- and intra-individual variation that is why a classic frame of use according to practical guidelines does not exist [17].

As mentioned in our study a large heterogeneity of the serum molecules was found (fig. 5). However, the use of chemical indices for bone metabolism is largely applied in population studies in order to point out the trend line of a particular condition or therapy regarding skeleton status, for instance, hypogonadism-related bone loss, bone metastases, primary hyperparathyroidism [18-25]. Bone formation is due to osteoblasts while bone resorption is based on osteoclasts functions [17]. Formation requires collagen I pro-peptide (P1NP) while resorption involves collagen I fragments as NTX or CTX [17]. Generally, bone collagen synthesis starts from type I pro-collagen which is later converted to type I collagen which contains a pro-

collagen terminal propetide, also called P1NP (which was studied in current sample) and also type I pro-collagen C-terminal pro-peptide (also named P1CP) [17].

One of the most challenging aspects in the field of bone turnover markers is the fact that many chemical products of bone cells are available and their results from studies show different results [16]. We introduced in Table 4 a glimpse of these markers indicating remodelling and demineralization.

Conclusions

Based on present study, the biochemical bone turnover markers are not distinctive between the groups of different BMI cut-offs. Large intra-individual variations do not allow a predictive model which represents one of the reason of directly applying the use of this molecules in daily practice.

Abbreviations

AP = Alkaline Phosphatase
BMI = Body Mass Index
SD = standard deviation
25OHD = 25-hydroxyvitamin D
OC = osteocalcin
CL = CrossLaps
PTH = parathormone

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