Serum Osteocalcin, P1NP, Alkaline Phosphatase, 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 I 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 (electrochemiluminescence 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.

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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 considered at \(p<0.05\) (for student t test and linear regression).

The inclusion and exclusion criteria are introduced in figure 3.

### 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 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).

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...
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 propeptide, also called P1NP (which was studied in current sample) and also type I pro-collagen C-terminal pro-peptide (also named PICP) [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
- 25OH = 25-hydroxyvitamin D
- OC = osteocalcin
- CL = CrossLaps
- PTH = parathormone

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**Table 4**

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

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