Biochemical Analysis of Mineral Metabolism and Central Bone Mineral Density in 157 Adult Women

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This is a cross-sectional retrospective study of observational type. 157 menopausal subjects were included. A number of N1=89 were younger of 60 years old (also included) and a number of N2=68 were older than 60 years old. Median of age was of 55 years, respective 66 years. The biochemical parameters like total and ionic serum calcium, serum magnesium, and phosphorus between the two groups N1-N2 were similar (p>0.05). The median values of mentioned chemical elements were within normal limits. The bone turnover markers were not statistically significant different between N1 and N2. 250HD was found deficient in both populations, irrespective of age. DXA- BMD and T-score N1-N2 difference was statistical significant for all the four central sites. Biochemical mineral parameters seem not to be influenced by the cut off of 60 years in menopausal women aged between 40 and 80 years. Yet, a large prevalence of hypovitaminosis D is identified regardless the age without secondaryPTH raise. The statistical significant results are for BMD and T-score for all the four central sites.

Keywords: serum calcium, urinary calcium, bone mineral density.

Mineral metabolism is assessed at biochemical level based on blood tests as calcium (circuiting and urinary), serum phosphorus, and magnesium but, also, a more complex analysis includes the blood bone turnover markers, vitamin D status evaluation, mostly using the levels of 25-hydroxyvitamin D[25-hydroxycholecalciferol or (6R)-6-[(1R,3aR,4E,7aR )-4-[(2Z)-2-[(5S)-5-Hydroxy-2,3,3a,5,6,7-hexahydro-1H-inden-1-yl]-2-methyl-heptan-2-ol][1-6].These assays are necessary at any age for skeletal assessment involving physiological and pathological conditions as primary hyperparathyroidism, hypovitaminosis D, osteomalacia and rickets, bone metastases, normal teeth development, and potential dental anomalies, etc.[1-6]. Studies as NHANES study evaluated the calcium and vitamin D system in relationship with different physiological parameters as dietary intake with an age- and gender-specific variation pattern, geographic areas or economic income of the population, etc. [7]. The levels of calcium intake thus of circulating calcium have direct implications on skeletal and oral health. [6,7]

Our purpose is to present a clinical study in adult females to reveal the biochemical aspects of mineral metabolism, an analysis based on adult women aged between 40 and 60 years versus a similar population but older than 60 years.

Experimental part

Method and subjects

Study design

This is a cross-sectional retrospective study of observational type. The study was conducted between 2016 and 2017. The patients were evaluated for different medical conditions but the population was not pre-selected for calcium anomalies presentation, neither for skeletal anomalies (apparently healthy regarding potential dysfunctions of mineral metabolism).

Material (patients)

The clinical evaluation of the subjects included the medical background in order to evaluate the inclusion and exclusion criteria, fasting morning blood assays and 24-hours urinary calcium assessment. Also, each patient had a central DXA (Dual-Energy X-Ray Absorbtometry) performed at the following levels: lumbar spine (from first to fourth vertebrae), left hip (for total hip and femoral neck areas), and third distal radius level at non-dominant arm. DXA analysis provided BMD (Bone Mineral Density) at the four central sites: lumbar, total hip, femoral neck, distal third radius and derived T-scores and Z-score (which are directly provided by the DXA machine, a GE Lunar Prodigy device) according to WHO criteria [8].

Inclusion criteria were: adult female, menopausal status for at least one year (without current or prior estrogens replacement therapy), age between 40 and 80 years, informed written consent.

Exclusion criteria are: confirmed cancers of any pattern, including primary or secondary bone neoplasia; lack of complete panel of investigations including central DXA at the four mentioned sites, specific medication for fracture risk reduction (previous supplements with vitamin D and calcium are not quantified and thus allowed for this study).
The blood biochemical assays

Calcium (an alkaline earth metal having the atomic number 20, situated at fourth period) is tested in daily human practice at blood and urinary level. [10] There are two types of blood-derived values for every day practice: total serum calcium and ionic serum calcium. Total calcium (CaT) is based on a correction (mg/dL) according to the formula: CaT = measured serum CaT (mg/dL) + 0.8[4-measured serum albumin (g/dL)]. [10] Serum ionic calcium (CaI) levels are calculated based on CaT and circulating total proteins (TP) based on formula: CaI = [6CaT (mg/dL) -TP (g/dL)/3]: [TP (g/dL) + 6]. [10] The normal serum values and the method of detection are displayed in table 1. 24-h urinary calcium (24-h Ca) is measured on a urinary sample covering an entire day (Table 1). Also, the mineral metabolism includes the assessment of serum phosphorus (P) representing the chemical element associating the 15 atomic number (third period) and clinically tested as introduced in table 1 [11]. Moreover, magnesium (Mg) is a chemical element (alkaline earth metal) having the atomic number 12 (third period) (table 1) [12].

The activity of bone cells is reflected by bone turnover markers: of formation - osteocalcin (also named G1 protein of the bone), P1NP (aminoterminal propeptide of type I collagen), and alkaline phosphatase (AP; this is a homodimeric protein enzyme serving as basic phosphatase which requires alkaline pH for optimal function. [13] The bone resorption marker is serum CrossLaps which is C-terminal telopeptide (a named derived from carboxy-terminal collagen crosslinks) [14](Table 1).

The endocrine control of mineral metabolism is reflected by calcifediol (25-hydroxycholecalciferol) or 25OH and parathormone (PTH) assays [15,16]. 25OH offers the best reflection of vitamin D status which is regulated based on a negative feedback with PTH (table 1) [15,16].

### Statistical tests

Statistical analysis introduced features as mean, standard deviation (SD), and median. The statistical significant results are considered at p<0.05 (for functions as test).

### Results and discussions

157 menopausal subjects were included in the study. A number of N1=89 were younger of 60 years old (also included) and a number of N2=68 were older than 60 years old (fig. 1). Median of age was of 55 years, respective 66 years (table 2). The biochemical parameters between the two groups N1-N2 were similar (p>0.05) including CaT, Ca I, Mg, P, 24-h Ca (table 3). The median values of mentioned chemical elements were within normal limits (table 3). The bone turnover markers were not statistically significant different between N1 and N2 (table 4). 25OH was found deficient in both populations, irrespective of age.

| Parameter       | Units       | Normal range  | Method of detection     |
|-----------------|-------------|---------------|-------------------------|
| CaT             | mg/dL       | 8.3-10.2      | Colorimetric            |
| Ca I            | mg/dL       | 3.9-4.9       | Colorimetric            |
| 24-h Ca         | g/24-h      | 0.1-0.3       | Spectrophotometric      |
| P               | mg/dL       | 2.5-4.5       | Colorimetric            |
| Mg              | mg/dL       | 1.6-2.5       | Colorimetric            |
| 25(OH)D         | ng/ml       | 30-100        | Chemiluminescence       |
| PTH             | pg/ml       | 15-65         | Electrochemiluminescence|
| AP              | U/L         | 30-105        | Colorimetric            |
| PINP            | ng/ml       | 15-65         | Immunometric            |
| Osteocalcin     | ng/ml       | 15-46         | Electrochemiluminescence|
| CrossLaps       | ng/ml       | 0.33-0.782    | Electrochemiluminescence|

**Table 1**

**NORMAL LEVELS OF BIOCHEMICAL AND HORMONAL PARAMETERS INCLUDED IN MINERAL AND BONE METABOLISM ASSESSMENT (UNITS, NORMAL LAB RANGES AND METHOD OF DETECTION)**

| N1 | N2 | p value | p value  |
|----|----|---------|----------|
| N1-mean | 54.11235955 | 4.42728678 |
| N1-SD | 55 | 67.67647059 | 5.42647351 |
| N1-median | 66 | p<0.0005 |

**Table 2**

**THE AGE DISTRIBUTION FOR THE ENROLLED PATIENTS (N=157)**

| Parameter       | Units       | Normal range  | Method of detection     |
|-----------------|-------------|---------------|-------------------------|
| CaT             | mg/dL       | 8.3-10.2      | Colorimetric            |
| Ca I            | mg/dL       | 3.9-4.9       | Colorimetric            |
| 24-h Ca         | g/24-h      | 0.1-0.3       | Spectrophotometric      |
| P               | mg/dL       | 2.5-4.5       | Colorimetric            |
| Mg              | mg/dL       | 1.6-2.5       | Colorimetric            |
| 25(OH)D         | ng/ml       | 30-100        | Chemiluminescence       |
| PTH             | pg/ml       | 15-65         | Electrochemiluminescence|
| AP              | U/L         | 30-105        | Colorimetric            |
| PINP            | ng/ml       | 15-65         | Immunometric            |
| Osteocalcin     | ng/ml       | 15-46         | Electrochemiluminescence|
| CrossLaps       | ng/ml       | 0.33-0.782    | Electrochemiluminescence|

**Table 3**

**THE VALUES OF BIOCHEMICAL PARAMETERS DETECTED IN BLOOD AND URINE (N=157)**
The BMD and T-score N1-N2 difference was statistically significant for all the four central sites (Table 5).

This is a study of chemical assays involving the mineral metabolism and central DXA in menopausal women (N=157) of above (N1) and over 60 years old (N2). The cut-off of 60 years is important in skeletal evaluation as well as others cardio-metabolic features [17].

Limits of the study are worth to be mentioned: lack of correlation data with calcium and vitamin D supplements; also the menopausal status might influence the bone profile as DXA and bone turnover markers, an effect that has not been quantified in the study.

Conclusions

Biochemical mineral parameters seem not to be influenced by the cutoff of 60 years in menopausal women aged between 40 and 80 years. Yet, a large prevalence of hypovitaminosis D is identified regardless the age without secondary PTH raise. The statistical significant results are for BMD and T-score for all the four central sites analysed at central DXA.

Table 4
THE BONE TURNOVER MARKERS AND HORMONAL PARAMETERS (N=157)

| Abbreviations |
|---------------|
| AP = Alkaline Phosphatase |
| BMD = Bone Mineral Density |
| CaT = serum total calcium |
| CaI = serum ionic calcium |
| DXA = Dual-Energy X-Ray Absortiometry |
| P = phosphorus |
| PTH = parathormone |
| TP = total proteins |
| SD = standard deviation |
| 25OHD = 25-hydroxyvitamin D |
| 24-h Ca = 24-hours urinary calcium |

References
1. POIANA, C, CARSOTE, M POPESCU, A, HORTOPAN, D, STANESCU, B, IOACHIM, D, ActaEndocrinologica, III, no.1, 2007, pp. 81
2. GHEMIGIAN, A, GHEMIGIAN, M, POPESCU, I, VIJA, L, PETROVA, E, DUMITRU, N, DUMITRU, I. Hormones (Athens). 12, no. 3, 2013, pp. 454
3. ANTONESCU, E, TOTAN, M, BOITOR, GC, SZAKACS, J, SILISTEANU, SC, FLEACA, SR, MITARIU, SC, SERB, BH, Rev.Chim (Bucharest), 68, no. 2, 2017, pp. 243
4. DASCALU, IT, TUCULINA, Mj, RAESCUC, M, POPESCU, SM, COREGA, C, VAIDA, L, BOLD, A, Rom. J. Morphol. Embryol., 54, no. 3 Suppl, 2013, pp. 857
5. PREDA, SA, MORARU, I, RAESCUC, M, BUNGET, A, NICOLA, A, GHEORGHITA, L, DASCALU, I, TUCULINA, M, ALBULESCU, DM, IANOJVICI, N, Journal of Dental and Medical Sciences (IOSR-JDMS), 17, no. 6 Ver. 3, 2018, pp. 89
6. TRAIISTARIU, MR, KAMAL, D, KAMAL, KC, ROGOVEANU, OC, POPESCU, M, BONDARI, S, ALEXANDRU, DR, IONOVICI, N, GRECU, DC., RJME, 56, nr. 4, 2015, pp. 1447
7. WALLACE, TC, REIDER, C, FULGONI, VL 3RD., J Am Coll Nutr., 32, no. 5. 2013, pp. 321
8. ***http://www.who.int/chp/topics/Osteoporosis.pdf
9. TIMOFTE, D, OCHIUZ, L, URSARU, M, CIUNTU, B, IONESCU, L, CALU, V, MOCANU, V, PUJIA, CI, Rev.Chim (Bucharest), 68, no. 10, 2017, p. 2341
10. SAVA, L, PILLAI, S, MORE, U, SONTAKKE, A, Indian J Clin Biochem. 20, no. 2, 2005, pp. 158
11. ***https://en.wikipedia.org/wiki/Phosphorus
12. ***https://en.wikipedia.org/wiki/Magnesium
13. ***https://en.wikipedia.org/wiki/Alkaline_phosphatase
14. ***https://en.wikipedia.org/wiki/C-terminal_telopeptide
15. MIHALACHE, L., GAVRIL, R.S., ARHIRE, LI, NITA, O., GHERASIM, A., OPRESCU, A.C., LAPUSTE, C., CONSTANTINESCU, D., PADUREANU, S.S., Rev. Chim. (Bucharest), 67, no. 12, 2016, p. 2413
16. COCOLOS, A.M., DUMITRU, N., PETROVA, E.N., COCOLOS, I., TIGLIS, M., DRAGOMIRESCU, R.F.I., OLARU, M., DUMITRU, A., GHEMIGIAN, A.M., Rev.Chim. (Bucharest), 69, no. 1, 2018, p. 134
17. POIANA, C, RADOI, V, CARSOCTE, M, BILEZEKIAN, J, Bone Research, 1, no. 3, 2013, pp. 260

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