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. 25OHD 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 secondary PTH 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 (circulating 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-methylidene-cyclohexylidene]ethylidene]-7a-methyl-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]

Ourpurpose 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-sectionalretrospective study of observational type. The study was conductedbetween 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 Absortiometry) performed at the following levels: lumbar spine (from first to fourth vertebra), 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).

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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 = [6xCaT (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) represent 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 (aminoterminalpropeptide of type I collagen), and alkaline phosphatase(AP; this is ahomodimeric protein enzyme serving as basic phosphatase which requires alkaline *p*H 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 25OHD and parathormone (PTH) assays [15,16]. 25OHD 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 ttest).

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). 25OHD was found deficient in both populations, irrespective of age

Parameter	Units	Normal ranges	Method of detection
CaT	mg/dL	8.5-10.2	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	1.6-2.55	Colorimetric
25OHD	ng/mL	30-100	Chemiluminescenece
PTH	pg/mL	15-65	Electrochemiluminescence
AP	U/L	38-105	Colorimetric
P1NP	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)

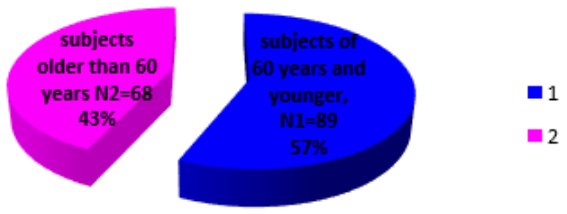

Fig. 1. The subjects included in the study (N=157)

Table 2
THE AGE DISTRIBUTION FOR THE ENROLLED PATIENTS (N=157)

		age (years)
N1-mean		54.11235955
N1-SD		4.42728678
N1-median		55
N2-mean		67.67647059
N2-SD		5.426417351
N2-median		66
p value		p<0.00005

	CaT	CaI	TP	P	Mg	24-h Ca
N1-mean	9.581264	4.134667	7.369286	3.723115	2.021746	0.164
N1-SD	0.396774	0.167679	0.497866	0.553509	0.14245	0.080498
N1-median	9.6	4.15	7.35	3.64	2	0.17
N2-mean	9.504848	4.125714	7.376731	3.680385	1.9418	0.15175
N2-SD	0.582922	0.379224	0.513606	0.604042	0.248224	0.129775
N2-median	9.5	4.19	7.35	3.595	2	0.115
p value	0.3367	0.890236	0.935903	0.695682	0.033704	0.854354

Table 3
THE VALUES OF BIOCHEMICAL PARAMETERS DETECTED IN BLOOD AND URINE (N=157)

Table 4

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

	AP	CrossLaps	Osteocalcin	P1NP	25OHD	PTH
N1-mean	82.42768116	1.719203704	24.59788462	57.92294118	20.195	49.39037
N1-SD	37.37943569	9.053578247	12.0948322	32.22332945	9.706634	18.01705
N1-median	75	0.46	23.665	48.29	18.55	47.69
N2-mean	77.23018519	0.452770833	23.84869565	52.47071429	18.48803	47.95021
N2-SD	21.46112928	0.211948031	13.40372446	24.84005337	8.105419	25.76521
N2-median	73.5	0.38	18.75	47.1	17.29	46.75
p value	0.364331839	0.335201921	0.771779707	0.466205853	0.280765	0.74304

	Lumbar BMD	Lumbar T-score	Lumbar Z-score
N1-mean	1.070026316	-0.892435897	-0.415789474
N1-SD	0.158110592	1.332986283	1.207206432
N1-median	1.058	-1.05	-0.45
N2-mean	0.970272727	-1.510606061	-0.177878788
N2-SD	0.204806892	1.379646419	1.199020774
N2-median	0.96	-1.55	-0.3
p value	0.001	0.007	0.241
	Femoral neck BMD	Femoral neck T-score	Femoral neck Z-score
N1-mean	0.908428571	-0.872857143	-0.024285714
N1-SD	0.135431096	0.901194285	0.796265871
N1-median	0.904	-1	-0.1
N2-mean	0.834403226	-1.256451613	0.044354839
N2-SD	0.14836745	1.122003619	0.905543259
N2-median	0.833	-1.45	-0.1
p value	0.003	0.03	0.643
	Hip BMD	Hip T-score	Hip Z-score
N1-mean	0.975193548	-0.266666667	0.240322581
N1-SD	0.160054073	1.24122728	1.015851363
N1-median	0.981	-0.2	0.1
N2-mean	0.913709091	-0.750909091	0.360727273
N2-SD	0.156344429	1.240351313	1.087238865
N2-median	0.884	-1	0.14
p value	0.03	0.03	0.537
	1/3 radius BMD	1/3 radius T-score	1/3 radius Z-score
N1-mean	0.6815	-0.592564103	-0.055263158
N1-SD	0.075516152	0.948432829	0.85002301
N1-median	0.6815	-0.5	0
N2-mean	0.552914286	-1.688571429	-0.065714286
N2-SD	0.232355632	1.295869326	1.191129681
N2-median	0.585	-1.8	0.1
p value	0.001	<0.00001	0.965

Table 5

DXA ANALYSIS BETWEEN GROUPS (N1-N2; A TOTAL OF 157 SUBJECTS)

(table 4). The BMD and T-score N1-N2 difference was statistical 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.

Abbreviations

AP = Alkaline Phosphatase
BMD = Bone Mineral Density
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
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