The aim of the research was to realize a clinical study on menopausal patients, focused on 25-hydroxyvitamin D (25OHD) assays versus Dual-Energy X-Ray Absorptiometry (DXA) categories. This transversal, observational, real-life study was effectuated on Caucasian Romanian females. A total of 60 subjects were grouped according to lumbar T-score: normal T-score (N=28), osteopenia (N=22), and osteoporosis (N=10). The lowest average value of 25OHD is found in patients with osteoporosis, which is statistically significant lower than in patients with osteopenia. The average values of PTH were within normal levels for each group. 25OHD did not correlate with PTH or lumbar BMD. Overall the mean values of 25OHD are in deficient ranges regardless osteoporosis, osteopenia or normal DXA.

Keywords: 25-hydroxycholecalciferol, vitamin D, bone, osteoporosis

Vitamin D represents a complex endocrine and biochemical system with multifunctional roles in humans (fig. 1) [1-3].

Vitamin D metabolism is a multiple enzymatic steps-based system; the most important molecule for clinical evaluation in daily practice is 25-hydroxyvitamin D (25OHD) 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-methylheptan-2-ol (also named calcifediol, calcidiol or 25-hydroxycholecalciferol) (fig. 2) [13].

The two major enzymes are hydroxylases and they act as follows: first cholecalciferol-25-hydroxylase converts cholecalciferol (vitamin D₃) into 25OHD at hepatic site. Then 25OHD becomes a substrate for renal 25-hydroxycholecalciferol-1α-hydroxylase which produces 1,25-dihydroxyvitamin D₃ or 1,25(OH)₂D₃ (also named calcitriol or 1,25-dihydroxycholecalciferol) [13]. The endocrine function of these biochemical structures is represented by the facts that 25OHD is a pro-hormone or pre-hormone meaning an inactive form, with a binding affinity for nuclear vitamin D receptor of 1000 times less potent than active hormone while calcitriol is the actual active hormone; the 1-α-hydroxylation of 25OHD is also possible outside the kidneys, for instance, at the level of
Blood levels of 25OHD represent a major tool for assessment of vitamin D status in humans and current assays are for total 25OHD (meaning free 25OHD and molecules attached to transporter binding proteins, both albumin and globulin called vitamin D binding protein or VDBP) [16-18]. New data point the importance of switching to free component assessment (based on an immunoassay method) but this did not become a routine test yet [19-21].

We aim to introduce a clinical study on menopausal patients focusing on levels of 25OHD in these patients depending on categories of risk indicated by DXA (Dual-Energy X-Ray Absorptiometry) categories.

**Experimental part**

**Material and method**

The research is a real-life study transversal observational study on Caucasian Romanian females. The study was conducted between 2016 and 2017. The parameters are analysed at the end of data collection.

The studied characteristics of the patients were focused on prior and current skeleton status including fracture risk evaluation. The tools used are: anamnesis, physical examination - body mass index was calculated based on formula weight/(height)^2, peripheral blood tests (fainting venous sampling) provided the results for 25OHD (chemiluminescence kit), biochemical parameters -total (colorimetric, VITROS VITROS FS5.1) and ionic (colorimetric, COBAS C 501) serum calcium, phosphorus (colorimetric assay COBAS C 501), bone turnover markers for formation: alkaline phosphatase (colorimetric, VITROS FS5.1), osteocalcin (electrochemiluminescence), P1NP (ELISA kit), for resorption: CrossLaps (electrochemiluminescence), hormonal assays for parathormon (PTH, electro-chemiluminescence immunoassay).

The patients were enrolled in three groups based on DXA - BMD using as surrogate T-score (WHO groups: normal, osteopenia, and osteoporosis) (fig. 4) [22]. DXA data were provided by a GE Lunar Prodigy machine. Romanian FRAX (Fracture Risk Assessment Tool) was used based on online calculator and 10-year probability of fracture was provided for four major osteoporotic fractures meaning clinical spine, forearm, hip, shoulder sites (R1), and also 10-year absolute risk of hip fracture (R2). The input parameters for R1 and R2 calculator are introduced in figure 5 [23,24].

Numeric parameters are introduced as mean and standard deviation (SD), median, minimum and maximum. The parameters' database were introduced through Excel and exported in SPSS 21; statistical significant was considered at p<0.05; linear regression with different adjustments was also used.

The subjects were enrolled based on following inclusion criteria: menopausal Caucasian Romanian subjects with adequate data at lumbar DXA scan, informed consent, and exclusion criteria like active cancers, bone metastases, Paget's disease, haematological malignancies, prior diagnosis of osteoporosis, previous or current medication against osteoporosis or medication to reduce the fragility fracture risk (vitamin D and calcium supplements are not included), incomplete panel of bone parameters according to protocol, age below 41 years old, primary hyperparathyroidism.

**Results and discussions**

A total of 60 subjects were grouped according to lumbar DXA T-score: normal T-score (N=28, Group NM), osteopenia (N=22, Group OE), and osteoporosis (N=10, Group OP) (table 1).

We aim to introduce a clinical study on menopausal patients focusing on levels of 25OHD in these patients depending on categories of risk indicated by DXA (Dual-Energy X-Ray Absorptiometry) categories.

**Table 1**

| THE STUDIED GROUPS OF MENOPAUSAL WOMEN WITHOUT PRIOR SPECIFIC THERAPY FOR OSTEOPOROSIS |
|-----------------------------------|
| **Group** | **Number of patients** | **T-score (DXA)** |
| NM       | 28                  | <+1 DS            |
| OE       | 22                  | < -1, > -2.5 DS   |
| OP       | 10                  | ≤ -2.5 DS         |

Fig. 4. DXA groups according to WHO classification [22]

Fig. 5. FRAX algorithm inputs and outputs [23]
markers are introduced in table 3. The three groups have similar values (a difference with borderline significance was found for CrossLaps values between GROUP OE and NM, respectively OP and NM).

The blood levels of bone hormones 25OHD and PTH are introduced in table 4. The lowest average value of 25OHD is found in patients with osteoporosis which is statistically significant lower than 25OHD on patients with osteopenia. Less than 10% of all subjects have secondary hyperparathyroidism. The average values of PTH were within normal levels for each group. 25OHD did not correlate with PTH or lumbar BMD (p>0.05).

BMD and T-scores as well as R1 and R2 are introduced in table 5. R1, respective R2 were similar between each combination of two groups' regardless statistical significant difference between lumbar values provided by DXA of BMD, respective T-score.

As limits of the study we mention non-longitudinal, non-interventional data, the lack of routine profile X-Ray of the spine as typical screening method for prevalent vertebral fracture, and relative young age of the menopausal females (also taking into account the mean age of menopause and average period of time since menopause) which associates a relative low fracture risk. Prior studies on similar Romanian population of 50 years and older showed a 10-year probability of major osteoporotic fracture of 5.3% and it increases to 13% in persons of 80 years old [25]. No particular pattern of risk regarding the combination of clinical risk factors was registered in studied population and it confirms the data from literature. [26,27] As collateral observation we also introduce the idea that the prevalent biochemical levels of 25OHD from this study represents a prior unselected population from the point of view of previous supplementation (and also similar observation is available for calcium supplements). We did

| Group          | Age (years) | Age of menopause (years) | Years since menopause | BMI (kg/m²) |
|----------------|-------------|--------------------------|-----------------------|-------------|
| Group OP (N=10) | 63.6        | 48                       | 15.6                  | 25.33       |
| mean           | 62          | 49.5                     | 13.5                  | 25          |
| median         | 6.91        | 4.05                     | 8.27                  | 5.09        |
| minimum        | 51          | 40                       | 2                     | 18          |
| maximum        | 75          | 52                       | 29                    | 33          |
| Group OE (N=22) | 60.27       | 48.01                    | 12.31                 | 26.9        |
| mean           | 58.5        | 49.5                     | 10.5                  | 26          |
| median         | 6.78        | 5.39                     | 9.44                  | 5.04        |
| minimum        | 51          | 41                       | 1                     | 18          |
| maximum        | 77          | 57                       | 35                    | 35          |
| Group NM (N=25) | 60.27       | 48.01                    | 12.31                 | 29.87       |
| mean           | 58.5        | 47                       | 11                    | 29          |
| median         | 6.56        | 4.85                     | 7.86                  | 6.28        |
| minimum        | 45          | 41                       | 2                     | 21          |
| maximum        | 74          | 55                       | 28                    | 45          |
| p value OP-OE  | 0.21        | 0.9                      | 0.35                  | 0.43        |
| p value OE-NM  | 0.37        | 0.54                     | 0.78                  | 0.07        |
| p value OP-NM  | 0.05        | 0.6                      | 0.18                  | 0.05        |

| Group          | Alkaline phosphatase (U/L) | CrossLaps (ng/mL) | Osteocalcin (ng/mL) | PINP (ng/mL) |
|----------------|---------------------------|-------------------|---------------------|--------------|
| Group OP (N=10) | 78.27                     | 0.57              | 25.49               | 58.28        |
| mean           | 76                        | 0.56              | 27.49               | 52.29        |
| median         | 22.45                     | 0.15              | 12.13               | 24.75        |
| minimum        | 44                        | 0.29              | 11.37               | 25.3         |
| maximum        | 119                       | 0.81              | 46.92               | 162.7        |
| Group OE (N=22) | 92.24                     | 0.54              | 27.74               | 63.6         |
| mean           | 76.2                      | 0.56              | 27.49               | 52.29        |
| median         | 22.45                     | 0.15              | 12.13               | 24.75        |
| minimum        | 45.28                     | 0.25              | 13.65               | 42.67        |
| maximum        | 222                       | 1.01              | 14.77               | 31.99        |
| Group NM (N=25) | 78.55                     | 0.42              | 24.33               | 56.24        |
| mean           | 78                        | 0.36              | 24.89               | 47.43        |
| median         | 18.57                     | 0.2               | 11.53               | 19.75        |
| minimum        | 49                        | 0.13              | 8.82                | 21.2         |
| maximum        | 112                       | 0.185             | 56.26               | 93           |
| p value OP-OE  | 0.11                      | 0.8               | 0.88                | 0.76         |
| p value OE-NM  | 0.22                      | 0.08              | 0.36                | 0.26         |
| p value OP-NM  | 0.98                      | 0.07              | 0.36                | 0.4          |

*normal levels for the following: alkaline phosphatase – 38-105 U/L, osteocalcin – 15-46 ng/mL, CrossLaps – 0.33-0.782 ng/mL
not measure the pharmacological intervention of supplements which cannot be distinguished based on 

A general high prevalence of low 25OHD in studied menopausal population which is characteristic for different European areas including for Romania confirmed the data from literature. [29-31] PTH levels were not correlated with PTH despite hypovitaminosis D despite the clear relationship of negative feedback between 25OHD and PTH [32].

A similar Croatian study on 194 postmenopausal unselected women of 50 years old or older (average age of 60.6 years, with a mean menopause duration of 11.4 years) included 13.9% females with DXA confirmation of osteoporosis based on T-score, and a mean 25OHD of almost 19 ng/mL; also a statistically significant difference between 25OHD values of menopausal group and normal DXA group was identified [33]. Our data on European population with similar climate suggested the same results. These observations lead to the practical point that 25OHD assays need to be carefully evaluated in osteoporotic patients who further need specific medication against osteoporosis in addition to vitamin D and calcium supplements. After the studies of Craciunescu et al [34], 25-OH-D level is an independent predictor of femoral neck BMD value and in cases with 25-OH-D values lower than 20 ng/mL, urgent DXA evaluation is needed. The researches of [35] revealed that the inclusion of 25(OH)D3 to an adipogenic differentiation cocktail significantly inhibited adipocyte differentiation at the concentrations of 25 and 2500 nmol/ L. The studies of Ene et al [36] consider that Vitamin D deficiency correction might serve as a prevention approach for the progression of alopecia areata. The studies of Stoian et al [37] showed that serum ferritin

| Group | 25-hydroxyvitamin D (25OHD) ng/mL | Parathormone (PTH) pg/mL |
|-------|----------------------------------|------------------------|
| Group OP (N=10) | | |
| mean | 15.99 | 63.93 |
| median | 15.1 | 50.99 |
| SD | 6.22 | 40.54 |
| minimum | 3.98 | 24.53 |
| maximum | 25.08 | 152.3 |
| Group OE (N=22) | | |
| mean | 22.46 | 49.11 |
| median | 22.95 | 51.01 |
| SD | 9.35 | 16.54 |
| minimum | 7.68 | 17.2 |
| maximum | 43 | 77.39 |
| Group NM (N=28) | | |
| mean | 19.34 | 51.07 |
| median | 18 | 48.07 |
| SD | 9.02 | 22.4 |
| minimum | 5.15 | 30.1 |
| maximum | 40.38 | 132.1 |

| p value OP-EO | 0.01 | 0.19 |
| p value OE-NM | 0.24 | 0.76 |
| p value OP-NM | 0.09 | 0.24 |

Table 4
THE THREE STUDIED GROUPS: BONE HORMONES VALUES 25OHD AND PTH

| Group | Lumbar BMD (g/sqcm) | Lumbar T-score (SD) | L1-FRAX (%) | L1-FRAX (%) |
|-------|-------------------|-------------------|-------------|-------------|
| Group OP (N=10) | | | | |
| mean | 0.821 | -2.89 | 2.92 | 0.49 |
| median | 0.856 | -2.7 | 2.3 | 0.25 |
| SD | 0.077 | 0.54 | 1.29 | 0.46 |
| minimum | 0.672 | -4.2 | 1.6 | 0.1 |
| maximum | 0.879 | -2.5 | 5.4 | 1.4 |
| Group OE (N=22) | | | | |
| mean | 0.985 | -1.57 | 3.83 | 0.9 |
| median | 0.994 | -1.5 | 3.5 | 0.6 |
| SD | 0.043 | 0.26 | 1.77 | 1.04 |
| minimum | 0.882 | -2.4 | 1.5 | 0.1 |
| maximum | 1.054 | -1.1 | 3.1 | 4 |
| Group NM (N=28) | | | | |
| mean | 1.2 | 0.162 | 5.35 | 1.62 |
| median | 1.156 | -0.15 | 3.6 | 0.75 |
| SD | 0.14 | 1.18 | 5.69 | 2.7 |
| minimum | 1.055 | -1 | 2.1 | 0.2 |
| maximum | 1.592 | 3.5 | 28 | 14 |

| p value OP-EO | 0.0001 | 0.0001 | 0.154 | 0.241 |
| p value OE-NM | 0.0001 | 0.0001 | 0.189 | 0.243 |
| p value OP-NM | 0.0001 | 0.0001 | 0.147 | 0.198 |

Table 5
DXA AND FRAX RESULTS FOR THE ENTIRE COHORT OF 3 MENOPAUSAL WOMEN GROUPS
levels were negatively associated with the presence of 25(OH) vitamin D deficiency in women and this association was independent of age, body composition. Large population studies are quoted in different guidelines of hypovitaminosis D and reveal the conclusion that more than one half of non-responders to bisphosphonates actually associate inadequate low levels of 25OHD as major cause of suboptimal response [38-40]. A secondary analysis from Aberdeen study (a randomized controlled trial) published in 2018 showed that in patients with low 25OHD the vitamin D supplementation directly improves BMD only in those adult subjects with baseline 25OHD below 30 nmol/L (meaning 74 ng/mL) [41]. When these data apply to our studied population all the subjects are below the mentioned threshold thus the become candidates to vitamin D supplementation. On the other hand, another study also published in 2018 showed that a direct correlation between long term serum 25OHD and hand, another study also published in 2018 showed that a direct correlation between long term serum 25OHD and 

Conclusions

Overall the mean values of 25OHD are in deficient ranges regardless osteoporosis, osteopenia or normal DXA. A statistical significant lower level of serum 25OHD is found in post-menopausal subjects with osteoporosis versus osteopenia.

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