Vitamin D Receptor Genotypes, Bone Mineral Density and Biochemical Markers of Bone Turnover in Egyptian Premenopausal Women with Graves ' Disease

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Abstract: ***Objective:*** To find out the correlation of vitamin D receptor (VDR) gene polymorphisms *(BsmI, TaqI and ApaI)* with bone mineral density (BMD) and biochemical markers of bone remodeling in premenopausal female patients with Graves' disease (GD). ***Methodology:*** The study included 65 premenopausal Egyptian female patients with GD, aged 27-45 years and 30 healthy women with matched age. The genotyping was performed by the use of the restriction fragment length polymorphism analysis. Also, BMD at lumbar spine and femoral neck by dual energy X-ray absorptiometry and biochemical markers of bone turnover (as serum calcium, phosphorus, total alkaline phosphtase, carboxy terminal telopeptide of type l collagen and osteocalcin as well as urinary deoxypyridinoline/urinary creatinine ratio) were evaluated in patients and controls. ***Results:*** The distribution of genotype frequencies differs between GD and controls (*BsmI*: X^2=7.57, P= 0.022; *ApaI* X^2=7.88, P= 0.020; *TaqI* X^2=6.23, P= 0.044). We found over representation of the VDR *BsmI* "bb" (odds ratio 2.48; 95% CI 0.93-6.61), *ApaI* "aa" (odds ratio 2.68; 95% CI 0.82-8.74) and *TaqI* "TT" (odds ratio 3.21; 95% CI 1.24-8.25) genotypes in GD patients compared with controls. However, no significant association was seen between BMD and VDR (*BsmI, TaqI* and *ApaI*) genotypes in GD patients. Moreover, the VDR genotypes did not differ in serum concentrations of carboxy terminal telopeptide of type l collagen (ß-CTx) and osteocalcin as well as urinary deoxypyridinoline/urinary creatinine ratio. ***Conclusion:*** Although there was an over representation of VDR *BsmI, TaqI* and *ApaI* risk alleles in Egyptian women with GD, these were not associated with BMD or biochemical markers of bone turnover.

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Key Words: Graves' disease, VDR polymorphism, bone mineral density, bone turnover markers.

Introduction:

Hyperthyroidism concerns 2% of adult people with women's predominance. One of its common forms is Graves' disease (GD) that tends to affects women between the second and fourth decade of life. The etiology of this autoimmune disorder results from the presence of thyroid stimulating antibodies which binds to the TSH receptor (TSH-R) [(1)]. The genetic background of GD was investigated in several studies that showed increased disease susceptibility associated with polymorphisms in the HLA and CTLA-4 (cytotoxic T lymphocytes associated-4) genes [(2)].

Hyperthyroidism is characterized by accelerated bone turnover, which is caused from direct stimulation of bone cells by the high thyroid hormone concentrations [(3)]. Bone histomorphometry is consistent with preponderant osteoclastic resorption in cortical bone leading to increased porosity, whereas a reduction of the absolute bone volume occurs less often in the cancellous bone [(1)]. The biochemical markers of bone formation and bone resorption, such as osteocalcin (OC), carboxy terminal telopeptide of type l collagen (ß-CTx), alkaline phosphatase (ALP), bone-specific ALP (B-ALP), and urinary collagen pyridinoline (Upyr) or urinary deoxypyridinoline cross-links (UDXP) were elevated in hyperthyroid patients, indicating increased bone turnover in favor of osteoclastic bone resorption [(4)].

Activation of bone resorption results in the elevated level of calcium that inhibits parathyroid hormone (PTH) and1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] synthesis and then calcium absorption[(5)]. In consequence these processes lead to calcium deficit and secondarily stimulate PTH synthesis to restore calcium balance. Mineralization time is also reduced, which additionally decreases bone quality [(6)]. The changes in bones caused by hyperthyroidism are mostly reversible and depend on the hormonal state of the thyroid [(7)]. Normalization of thyroid function requires time to restore normal bone quality [(8)].

The vitamin D receptor (VDR) mediates the action of the vitamin D endocrine system in calcium homeostasis and bone metabolism [(9)]. VDR

is a nuclear hormone receptor that acts as a transcriptional regulator in response to circulating $1,25(OH)_2D_3$, the active hormonal form of vitamin D, that found to be significantly lower in autoimmune than in non-autoimmune hyperthyroidism[10,11]. 1, $25(OH)_2D_3$ exerts its immuno-modulatory effects by down-regulating the expression of HLA class II molecules on thyrocytes and inhibiting lymphocyte proliferation as well as secretion of inflammatory cytokines[12].

VDR gene polymorphism (VDRGP) has been extensively studied in different diseases. In the etiology of GD association of the following VDR polymorphisms: Bsm1, ApaI, TaqI and FokI with disease development have been studied [13]. Also VDR gene is contributed to the genetic background of the disturbances observed in bone metabolism [9]. However, there is still no answer which polymorphic variants can predispose GD patients to decreased bone density [1]. The main problem is the large number of metabolic processes connected with bone quality that are difficult to identify. There are limited studies on the associations of the VDR gene allelic variations with bone metabolism in GD [14], and non-have yet been performed in Egyptian populations?

So the aim of this study is to find out the potential relationship between VDR gene polymorphisms (BsmI, TaqI and ApaI) with bone mineral density and biochemical markers of bone remodeling in Egyptian female premenopausal patients with Graves' disease.

Subjects and Methods:

The present study comprised 65 premenopausal Egyptian female patients with Graves' disease their age ranged from 27 to 45 years (mean age 33±5.0 years). They were selected from attendants of Endocrinology Outpatient Clinics of Specialized Medical Hospital, Mansoura University, Egypt. The diagnosis of Graves' disease was made on clinical grounds, together with high free triiodothyronine (FT_3), high free thyroxine (FT_4) and low TSH concentrations in the serum, also elevated levels of anti-peroxidase antibodies and diffuse thyroid enlargement with increased activity on isotopic scanning.

The studied patients were divided into two subgroups on the basis of thyroid function; subgroup A comprised 30 patients with active hyperthyroidism or with normalized TSH levels lasting less than 12 months and subgroup B comprised 35 patients, whose serum TSH level remained within normal range for longer than 12 months through using the appropriate antithyroid drugs such as carbimazole.

Exclusion criteria included post-menopausal women to nullify the effect of menopause on the bone status, patients with co-morbidity (hypo-and hyper parathyroidism, vitamin D deficiency, Cushing's disease, diabetic nephropathy, inflammatory bowel disease, malabsorptive disease or renal diseases) or on medication (steroid, bisphosphonates, calcium or vitamin D) influencing bone turnover. Also patients with history of previous surgery or radiotherapy to the thyroid gland were excluded.

Also the present study included 30 healthy euthyroid female subjects, presented for routine medical examination as a control group with no personal or family history of GD, their age ranged from 26 to 43 years (mean age 32±6.0 years).

All participants provided written informed consent after receiving oral and written information concerning the study. The study protocol was approved by local ethics committee of the hospital. All patients and controls were subjected to: through history, full clinical examination, thyroid function tests (serum free T_3, free T_4 and TSH), radioactive isotope scanning and uptake of the thyroid gland with Technicium 99.

In all patients and controls, bone mineral density (BMD) was determined by dual X-ray absorptiometry (DEXA) using DPX-IQ-Lunar System, USA. Assessment was performed in posterior-anterior position at lumbar spine and proximal femur. BMD was expressed as areal density in g/m².

Sampling: 10 ml peripheral venous blood samples were obtained from all patients and controls in the morning after 12 hours overnight fasting. Each blood sample was divided into 2 tubes, one tube is left for coagulation for 30 minutes then centrifuged at 3000 rpm for 15minutes to separate serum which was immediately aliquoted and stored at -20°C until performing the biochemical markers of bone turnover. The other part was put in EDTA-K_3 tubes and stored at -20 °C until DNA extraction.

At the same time, urine were collected for twenty four hours, urine volume measured then, 10 ml was stored in Falcon tubes at -20°C until assay of urine creatinine and deoxypyridinoline.

Biochemical markers of bone turnover:

- Serum calcium, phosphorus and total alkaline phosphatase (ALP) as well as urine creatinine were determined, using Coobas Integra 400 plus, Roche Diagnostic, Germany.
- Thyroid hormones (free T_3, free T_4, TSH), antiperoxidase antibodies, Serum carboxy terminal telopeptide of type l collagen (ß-CTx) and serum osteocalcin (OC) were measured by electro-chemiluminescent immunoassay, using Elecsys 2010, Roche Diagnostic, Germany [15,16].
- Urine deoxypyridinoline (UDXP) was analyzed by Immulite Pyrilinks-D chemiluminscent enzyme-labeled immunoassay using Immulite-1000 DPC, Los Angeles,

according to the method of Reid et al (2004) [17]. Urinary deoxypyridinoline was corrected for urinary creatinine and a ratio of DXP in nmol/l/urinary creatinine in mg/dl was calculated.

Genotype analysis:

The human VDR gene has eight coding exons and three alternative 5'- noncoding exons spanning over 75 kb of DNA on chromosome 12q12-12q14 [10]. VDR belongs to the nuclear hormone receptor superfamily and modulates the transcription of target genes in response to $1,25(OH)_2D_3$, a potent immunomodulatory hormone [1]. There are five known polymorphisms in the VDR locus: the exon 2 initiation codon polymorphism, detected by the FokI restriction enzyme [11], a cluster of polymorphisms in the 3'-end of the VDR gene, defined by the restriction enzymes BsmI, ApaI, and TaqI [12] and the polyadenylase polymorphism further down the VDR3'-untranslated region [1].

DNA isolation:

Genomic DNA was extracted using QIAamp® DNA Mini Kit from Qiagen (Hilden, Germany), according to the manufacturer's instructions. DNA yield and purity were determined by measuring absorbance at 260/280 nm.

Materials:

Primers were obtained from Invitrogen Life Technologies (Breda, The Netherlands). Restriction enzymes BsmI, TaqI and ApaI were Sigma products (Taufkirchen, Germany).

- Primer 1 (5'-GGGAGACGTAGCAAAGG-3')
- Primer 2 (5'-AGAGGTCAAGGGTCACTG-3')
- Primer 3 (5'-CAGAGCATGGACAGGGAGCAAG-3')
-Primer 4 (5'-GCAACTCCTCATGGCTGAGGTCTCA-3')

Genotyping:

DNA was amplified with a standard PCR technique using thermal cycler (Biometra, USA). Mastermixes were optimized and contained the following: 2 mmol/L $MgCl_2$, 0.5 umol/L of the primer 1 and 2 or 0.8 umol/L of the primer 3 and 4; and 2 ul of mixture that contained Taq polymerase and deoxyribonucleoside triphosphates. The restriction fragment lengh polymorphisms (RFLPs) were coded as Bb (BsmI), Aa (ApaI) or Tt (TaqI), the uppercase letter signifying the absence of the site and lowercase letters signify the presence of the restriction site.

Detection of the BsmI restriction site was achieved by amplifying a region spanning the site with one primer originating in exon 7 (primer 1) and the other originating in interon 8 of VDR gene (primer 2). Amplification was started with an initial denaturation at 95°C for 10 min followed by 40 amplification cycles of denaturation at 95°C for 30s, annealing at 69°C for 30s and extension at 72°C for 30s. PCR products were 359 bp long. After amplification, PCR products were digested with 5 U of BsmI restriction enzyme for 2 hours at 65°C and then electrophoresed on 3% agarose gel containing 0.4 mg/l ethidium bromide and visualized under UV illumination. The presence of the BsmI restriction site on both alleles (defined as bb) generated 182 and 177 bp fragments, whereas the absence (BB) yielded one undigested 359 bp fragment.

Region of VDR gene containing ApaI and TaqI restriction site was obtained in PCR reaction using primer 3 originating in intron 8 and primer 4 originating in exon 9 of VDR gene. Amplification was started with an initial denaturation at 95°C for 10 min followed by 40 amplification cycles of denaturation at 95°C for 30s, annealing at 69°C for 30s and extension at 72°C for 30s. PCR products were 740 bp long. For detection of TaqI and ApaI restriction sites, each PCR products were digested with 3 U of TaqI for 2 hours at 65°C or with ApaI enzyme using 10 U for 2 hours at 30°C. After digestion with ApaI according to the presence or absence of restriction site genotypes were identified as AA (one undigested PCR fragment of 740bp), aa (two fragments of 515 and 225bp) and heterozygous Aa. The TaqI digestion revealed one obligatory restriction site, the homozygous TT (absence of the specific TaqI restriction site) yielding fragments of 490bp and 245bp. The homozygous tt exhibited fragments of 290, 245, and 205bp and the heterozygous Tt provided 490, 290, 245 and 205bp fragments.

Statistical Methods:

Statistical analysis was done using the SPSS Version 10 (1999) (SPSS Inc, Chicago, Illinois). Student "t" test was used for comparison of quantitative data of two groups. The frequency distribution of VDR genotypes in patients and controls was determined and evaluated using the X^2 test. The strength of association was estimated by crude odds ratio, with 95% confidence interval (95% CI). The relationship between various VDR genotypes with BMD and biochemical markers of bone turnover was evaluated by analysis of variance (ANOVA). Significance was considered when P value <0.05.

Results:

Clinical data and thyroid functions of patients with hyperthyroidism and GD versus euthyroid control subjects are compared in ***Table (1)***.

Our results showed that the BMD were significantly lower, while biochemical markers of bone turnover were significantly higher in GD patients (subgroup A & B) compared to controls ***(Table 2)***.

Our results showed the prevalence of VDR gene polymorphisms in patients with Graves' disease and their matched controls ***Table (3)***. Analysis of VDR-BsmI genotype polymorphism revealed a significant few number of patients carried BB genotype. Furthermore, the B allele was also significantly under represented among GD patients (32.3%).

Similarly comparing the distribution of ApaI polymorphisms in the patients and the controls, revealed a highly significant difference (X^2=7.88, P= 0.020). The VDR "aa" occurred more frequently in the patients than in the controls; whereas "AA" genotype was significantly under transmitted to the patients. Also the A allele was more frequent among controls (63.3%).

While, The distribution of TaqI genotype frequencies differed significantly between the patients and the controls. The "TT" genotype occurred more frequently in the patients, with the T allele significantly over presented among the patients (71.5%).

BsmI, ApaI and TaqI genotype polymorphisms were not associated with serum free T_3 (p=0.27, 0.33, 0.37 respectively), free T_4 (p=0.60, 0.65, 0.34 respectively) and TSH levels (p=0.81,0.68,0.25 respectively) measured in the patients of Graves' disease . Also, all of the biochemical markers of bone turnover (serum calcium, phosphorus, ALP, B-CTx, OC and urinary DXP/urinary creatinine ratio) and BMD (lumbar spine and femur neck) did not differ in BsmI, ApaI and TaqI genotypes of Graves' disease patients ***(Table 4-6)***.

Table 1: Compare clinical and thyroid function tests of hyperthyroid Graves' disease patients and euthyroid controls.

Parameters	*Graves' Disease (N=65)*		*Controls*	*Significance*	
	Subgroup A (N=30)	*Subgroup B (N=35)*	*(N=30)*	P1	P2
Age (years)	33.0 ± 6.0	34.0± 4.0	32.0 ± 6.0	NS	NS
Duration of GD (years)	4.6 ± 1.8	5.4 ± 2.1	-	-	-
Body mass index (kg/m^2)	25.8 ± 1.2	26.1 ± 1.2	27.3 ± 0.9	NS	NS
Waist to hip ratio (cm)	0.79 ± 0.30	0.82 ± 0.11	0.81 ± 0.10	NS	NS
Free T_3 (pmol/l)	5.92 ± 1.34	5.31 ± 0.92	4.95 ± 0.72	<0.001	<0.01
Free T_4 (pmol/l)	21.3 ± 4.96	18.72 ± 2.13	17.93 ± 1.83	<0.01	NS
TSH (uU/ml)	0.143 ± 0.072	1.74 ± 0.64	2.12 ± 0.62	<0.0001	NS

Significant P<0.05, P1 subgroup A versus controls, P2 subgroup B versus controls

Table 2: Compare biochemical markers of bone turnover and BMD in Graves' disease patients and controls.

Parameters	*Graves' Disease (N=65)*		*Controls*	*Significance*	
	Subgroup A (N=30)	*Subgroup B (N=35)*	*(N=30)*	P1	P2
Serum Ca. (mg/dl)	9.42 ± 0.56	9.39 ± 0.56	9.37 ± 0.52	NS	NS
Serum Ph. (mg/dl)	4.12 ± 0.49	3.83 ± 0.60	3.55 ± 0.53	<0.0001	NS
ALP (U/L)	246.0 ± 52.0	202.0 ± 64.0	67.5 ± 24.0	<0.0001	<0.0001
B-CTx (ug/l)	4.32 ± 0.70	4.04 ± 0.79	3.21 ± 0.66	<0.0001	<0.0001
OC (ng/ml)	19.8 ± 7.2	11.7 ± 5.6	8.8 ± 1.8	<0.0001	<0.05
UDXP/Ur.creatinine	12.4 ± 4.17	10.71 ± 3.49	6.18 ± 1.60	<0.0001	<0.0001
Lumbar BMD (g/cm²)	1.01 ± 0.144	1.06 ± 0.172	1.19 ±0. 107	<0.0001	<0.001
Femur BMD (g/cm²)	0.877± 0.079	0.937 ± 0.107	0.995 ± 0.135	<0.001	<0.05

Significant P<0.05, P1 subgroup A versus controls, P2 subgroup B versus controls

Table 3: Prevalence of VDR gene polymorphisms in patients with Graves' disease and their matched controls

	GD(N=65)	*Controls (N=30)*	*X^2*	*P*	*Odds ratio (95%CI)*
VDR Bsml genotype					
BB	5 (7.7%)	8 (26.7%)			0.23 (0.06-0.77)
Bb	32 (49.2%)	15 (50%)	7.575	0.022	0.96 (0.41-2.30)
bb	28 (43.1%)	7 (23.3%)			2.48 (0.93-6.61)
VDR Apal genotype					
AA	10 (15.4%)	12 (40%)			0.27 (0.10-0.73)
Aa	36 (55.3%)	14 (46.7%)	7.88	0.020	1.41 (0.59-3.38)
aa	19 (29.3%)	4 (13.3%)			2.68 (0.82-8.74)
VDR Taql genotype					
TT	35 (53.8%)	8 (26.7%)			3.21 (1.24-8.25)
Tt	23 (35.3%)	16 (53.3%)	6.239	0.044	0.48 (0.19-1.15)
tt	7 (10.9%)	6 (20%)			0.48 (0.14-1.58)

CI= confidence interval, Significant P<0.05

Table 4: Bone mineral density and biochemical markers of bone turnover in relation to VDR Bsml genotype in the Graves' disease patients

	BB (N=5)	*Bb (N=32)*	*bb (N=28)*	*P (Anova)*
Serum Ca. (mg/dl)	9.12 ± 0.52	9.38 ± 0.55	9.48 ± 0.57	0.40
Serum Ph. (mg/dl)	4.20 ± 0.48	3.96 ± 0.56	3.91 ± 0.54	0.56
Serum ALP (U/L)	255.4 ± 83.4	215.0 ± 60.1	225.2 ± 61.5	0.40
Serum B-CTx (ug/l)	4.21 ± 0.70	4.31 ± 0.79	3.98 ± 0.72	0.25
Serum OC (ng/ml)	18.12± 9.8	15.19 ± 7.45	15.66 ± 7.97	0.74
UDXP/Ur.creatinine	10.66 ± 2.50	11.08 ± 3.81	12.10 ± 4.18	0.54
Lumbar BMD (g/cm²)	0.975 ± 0.210	1.02 ± 0.156	1.05 ± 0.156	0.51
Femur BMD (g/cm²)	0.879 ± 0.126	0.910 ± 0.114	0.909 ± 0.107	0.83

Significant P<0.05

Table 5: Bone mineral density and biochemical markers of bone turnover in relation to VDR Apal genotype in the Graves' disease patients

	AA (N=10)	*Aa (N=36)*	*aa (N=19)*	*P (Anova)*
Serum Ca. (mg/dl)	9.67 ± 0.53	9.35 ± 0.53	9.55 ± 0.57	0.052
Serum Ph. (mg/dl)	3.92 ± 0.66	3.95 ± 0.54	3.98 ± 0.49	0.97
Serum ALP (U/L)	242.3 ± 86.2	224.5 ± 60.7	208.1 ± 49.7	0.36
Serum B-CTx (ug/l)	3.90 ± 0.53	4.23 ± 0.82	4.19 ± 0.75	0.49
Serum OC (ng/ml)	18.64 ± 9.11	15.57 ± 7.22	13.74 ± 8.28	0.28
UDXP/Ur.creatinine	10.42 ± 3.39	11.26 ± 3.75	12.53 ± 4.34	0.33
Lumbar BMD (g/cm²)	1.02 ± 0.194	1.02 ± 0.151	1.07 ± 0.151	0.38
Femur BMD (g/cm²)	0.894 ± 0.101	0.901 ± 0.101	0.927 ± 0.123	0.62

Significant P<0.05

Table 6: Bone mineral density and biochemical markers of bone turnover in relation to VDR Taql genotype in the Graves' disease patients

	TT (N=35)	*Tt (N=23)*	*tt (N=7)*	*P (Anova)*
Serum Ca. (mg/dl)	9.48 ± 0.57	9.39 ± 0.58	9.16 ± 0.35	0.36
Serum Ph. (mg/dl)	4.05 ± 0.54	3.87 ± 0.58	3.76 ± 0.34	0.27
Serum ALP (U/L)	225.0 ± 62.0	211.7 ± 67.2	245.1± 43.3	0.44
Serum B-CTx (ug/l)	4.18 ± 0.75	4.20 ± 0.82	3.96 ± 0.66	0.75
Serum OC (ng/ml)	16.04 ± 8.20	14.20 ± 7.90	18.17 ± 4.0	0.45
UDXP/Ur.creatinine	11.94 ± 3.57	10.84 ± 4.25	11.32 ± 4.43	0.58
Lumbar BMD (g/cm²)	1.02 ± 0.155	1.07 ± 0.176	0.955 ± 0.120	0.20
Femur BMD (g/cm²)	0.904± 0.121	0.925 ± 0.123	0.867 ± 0.091	0.43

Significant P<0.05

Discussion:

Several VDR gene polymorphisms are among the most important and the most frequently analyzed genetic risk factors for primary osteoporosis [18,19]. Morrison et al. (1994; 1997) [10,20] observed an association of allele B of Bsml polymorphism with changes of BMD in the lumbar spine and femoral neck as well as serum osteocalcin levels. Also, according to Langdahl et al. (2000) [21] BB and Bb genotypes were also more frequent in patients with osteoporotic fractures. While, investigation of the VDR polymorphisms in relation to osteoporosis induced by hyperthyroidism was less common [5].

The present study was concerned for 65 young premenopausal, regularly menstruating Egyptian women with diagnosed Graves' disease who had similar lifestyles and did not suffer from other diseases affecting bone metabolism. In this way estrogens deficit was likely eliminated and an excess of thyroid hormones was the most probable reason for changes in BMD and biochemical markers of bone turnover. Association of VDR gene polymorphisms (BsmI, TaqI and ApaI) with bone mineral density and biochemical markers of bone turnover was studied.

Hyperthyroidism results in accelerated bone loss by leading to a higher bone turnover. Osteoporosis induced by hyperthyroidism is usually reversible as long as there are no osteoporotic fractures [5]. Trabecular bone is more metabolically active and more sensitive to unfavorable factors, so BMD changes in lumbar spine appear rapidly after hyperthyroidism manifestations [6-8]. In the present study we found significantly lower BMD values in both lumbar spine and femoral neck in patients with hyperthyroidism and those with normalized TSH level lasting less than 12 months. At the same time, the significantly higher bone formation markers (serum ALP and osteocalcin) and bone resorption markers (B-CTx and urinary DXP/ur.creatinine ratio) in patients with GD found in the present work, confirms the higher turnover state in the skeleton of patients with hyperthyroidism. Also we found that, BMD was higher and biochemical markers of bone turnover were lower in women with euthyroid lasting over 12 months, confirms the hypothesis that the rate of bone repair process after elimination of an unfavorable factor is high, after 1 year the BMD reached values corresponding to peak bone mass.

We observed the association between allelic variants of the VDR gene and phonotype of Graves' disease. The Apal genotype aa and Taql genotype TT appeared to be associated with increased susceptibility to Graves' disease, whereas genotypes AA and Bsml genotype BB appeared to confer a decreased risk of Graves' disease in the Egyptian population. The same results obtained by Stefanic et al (2005) [22] during studying the association of VDR gene polymorphism with Graves' disease in Eastern Croatian population. However, an important discrepancy in our finding is the direction of disease risk conferred by given alleles in our patients when compared with those observed in other studies [1,23].Whereas the Apal A allele seemed to confer increased risk in Japanese cohort, it appeared protective in Egyptian and Croatian population [22]. Furthermore, the B allele of Bsml genotype being overrepresented in Japanese patients sample, the reverse association was suggested by our data [1,13]. Likewise, study in Caucasian patients failed to produce any association of GD with VDR gene polymorphism [24].

Our study did not demonstrate a statistically significant association between the analyzed polymorphisms of VDR gene and BMD in young women with Graves' disease. This contrast with previous reports [10,12,25] showing that "B" and "t" alleles are associated with low BMD [20,21].

Results of similar studies for different populations are discrepant [1,13,26]. Obermayer-Pietsch et al. (2000) [26] showed an association between BB genotype of VDR Bsml and low bone mass in Austrian patients with diagnosed hyperthyroidism [26]. There are also a few studies not confirming any associations that are related to our results [5,27]. No significant correlation of VDR gene allelic variations with BMD in Japanese Graves' disease women was found [13]. However, those authors noticed that Japanese female homozygous for allele F of VDR Fok1 polymorphisms had higher risk of osteoporosis when remission lasted less than 5 years.

Also, we failed to demonstrate that Bsml, Apal and Taql polymorphic variants in the VDR locus have a major impact on the biochemical markers of bone turnover (serum calcium, phosphorus, ALP, B-CTx, OC and urinary DXP/urinary creatinine ratio) in our study group of Graves' disease female patients. This contrast with previous reports showing that B and t alleles are associated with changes in these markers [10,26]. Our results are however consistent with other finding [28,29,30].

Zajickova et al. (2002) found a relation between heterozygous "Aa" genotype and B-CTx or alkaline phosphtase levels. This documents a

regulatory role of the gene in bone formation although Apal polymorphism was not related to BMD at any skeletal site. Also, Bsml and Taql genotypes were not related to markers of bone remodeling. The relation between VDR polymorphisms and biochemical markers of bone resorption was also investigated. Neither circulating B_2 microglobulin nor urinary DXP differ in the VDR genotype subgroups [28].

Several hypotheses have been reported to explain conflicting results between VDR genotypes with BMD and biochemical markers of bone turnover in different population, such as linkage disequilibrium with functionally relevant genetic variants and environmental factors modifying the genotype effect on BMD. Interaction with other candidate genes and with the environment might bring further insights into the complex pathophysiology of a polygenic disease such as osteoporosis [28].

The present study has several limitations. First, it was conducted on a limited sample of Graves' disease patients of Egyptian origin, so that our findings cannot be generalized to other races, gender or different age subgroup. Second, although the onset of Graves' disease was comparable, the adjustment of BMD according to the duration of disease would be appropriate for ANOVA analysis.

The fact that genotypic differences have no relation to biochemical markers or BMD does not contradict the pathogenic role of these polymorphisms, since these alterations could serve as the genetic basis that initiates processes leading to the disease[29-30]. Although an association found between certain alleles and a phenotype does not prove that the presence of these alleles has functional implications, further studies in an independent sample might be of value to investigate the role of these polymorphisms.

Conflict of Interest:

No conflict of interest to declare.

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