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Original Research

Transforming Growth Factor-β1 gene polymorphism and osteoporosis in postmenopausal egyptian women

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Abstract: Transforming growth factor-β1 (TGF-β1) is a wide spread bone matrix protein that affect the function, formation and cell-cell interactions of osteoclasts and osteoblasts to regulate bone remodeling and sustain adequate bone mass. The aim of this study is to evaluate the role of the two polymorphism of transforming growth factor-β1 T869C and C-509T in developing osteoporosis in postmenopausal Egyptian women. This study was performed on 138 postmenopausal osteoporosis/osteopenic women and 128 postmenopausal female control group. There was a significant statistical difference in the CC, CT and TT (T869C) genotype frequencies between the osteopenia/osteoporosis and control subjects (p value <0.001). There was a non-significant statistical difference in the CC, CT and TT (T-509C) genotype frequencies between the osteopenia/osteoporosis and control subjects (p value <0.082). There was a significant statistical difference between TT,CT and CC of (T869C) and T score, Z score and calcium of osteopenia/osteoporosis group (p value <0.001). There was a non-significant statistical difference between TT, CT and CC of (T-509C) and T score, Z score of osteopenia/osteoporosis group (p value 0.32,0.31),but there was a statistically significant difference between the three genotyping and serum calcium and creatinine (p value 0.04). Multivariate regression analysis showed that T869C polymorphism is a significant risk factor for osteopenia/ osteoporosis (OR 3.57, 95% CI= 1.56-5.67). We concluded that T869C polymorphism of the TGF-β1 gene has an impact on bone mineral density and enhancement of the susceptibility to osteopenia/osteoporosis in Egyptian women.

Key words: Osteopenia/osteoporosis; Transforming growth factor β1; Bone mineral density; Polymorphism.

Introduction

Postmenopausal osteoporosis is the most commonbone disease (1). Bone loss in postmenopausal osteoporosis is caused by an imbalance between bone resorption and bone formation. It is unclear, however, whether this imbalance is due to an increase in bone resorption, a decrease in bone formation, or a combination of both mechanisms (2).

The majority of osteoporotic fractures happen in individuals with low BMD (3). The T-score is the relevant measure when screening for osteoporosis. It is the bone mineral density (BMD) at the site when compared to the young standardvalues. It is a plotting of a patient's BMD to that of afit thirty yearsold individuals. The US standard is to use data for a thirty-year-old of the same sex and ethnicity, but the WHO recommends using data for a thirty-year-old white female for everyone. Values for thirty-year-olds are used in post-menopausal women and men over age 50 because they better predict risk of future bone break. The standards of the World Health Organization are; individuals with a T-score of −1.0 or higher are considered normal, Osteopenia isconsidered between -1.0 and -2.5 and Osteoporosis is considered when T-score is −2.5 or lower (4).

Transforming growth factor-β1(TGF-β1) , TGF-β2 and osteoprotegerin (OPG), are cytokines closely associated with bone metabolism (5) . The TGF-β family contains three closely related mammalian isoforms TGF-β1, -β2, and -β3, yet TGF-β1constitutes the largest sources of TGF-β in bone (6).TGF-β1is very rich in bone tissue.The ratio of TGF-β1 and TGF-β2 is about 4:1, and there is 70% sequence homology between the two forms (7).

TGF-β1 has avariety of widely recognized roles in bone formation. It blocks apoptosis of osteoblasts, and also recruits osteoblastic precursors or matrix-producing osteoblaststo the site through chemotactic attraction (8), soTGF-β may act as a bone-coupling factor linking bone resorption to bone formation (9).

The transforming growth factor β1 (TGF-β1) gene is located on chromosome 19q13. The important role in bone turnover makes the TGF-β1 gene a candidate for mediating the genetic influence on BMD and risk of fracture (10) (11).

Molecular biological evidence showed that changes in the TGF-βgene can cause a Leu - Pro exchange at amino acid number 10, which comprises a T- C transition at nucleotide 29 and a T- C transition at nucleotide 869 in the region encoding the signal sequence (10).

Early studies demonstrated that TGF-β1 is a downstream factor of estrogen (12) and that TGF-β1 is also involved in the vitamin D signaling pathway (13) which plays an important role in the local regulation of bone metabolism.Mori.,et al 2010 (14) suggests that TGF-β1 genetic polymorphism isassociated with vita-

min D and has an important effect on theincidence of osteoporotic vertebral fracture after menopause.

Materials and Methods

This study was performed on 266 postmenopausal women with age range 44- 65 years. They wereselected from out clinic of Internal Medicine Department,Menufyia University Hospital. All subjects were apparently in good health and gave informed consent to participate in the study.This study was approved by the Ethics Committee of the Faculty of medicine Menoufyia University. All women were subjected to mineral density measurements and according to its results they were classified into 2 groups; group 1 (control) individual (128 women) with T score > 1.0 and group 2 (osteopenia / osteoporosis)that were 138 women with T score < 1.0.

Exclusion criteria were diabetes mellitus, high blood pressure, patients with cardiac diseases, andmetabolic bone diseases.

Measurement of BMD

Bone mineral density measurement was done on one-leg of all studied participants, with the subject in the sitting position, using a water-based Achilles Express ultrasonometer ''Lunar Achilles Express''QUS consideration. The patient's outcome is stated as a T-score and Z-score as a percentage plotted to the standard population. The diagnostic criteria for osteopenia/ osteoporosis in the studied individuals werecategorizedin consistent with WHO classification (1994) via T-score (>−1: normal, −1 to −2.5: osteopenia and −2.5 or less: osteoporosis).

Measurement of biochemical markers

Ten ml of venous blood were collected at the morning with overnight fasting. Blood samples were divided in two tubes plain tube and EDTA tube for genotyping. Blood in the plain tube were separated, and serum were stored in aliquots at -20°C until used. Calcium, and creatinine were measured by using cobasintegra 400 (ROCH diagnostics).

Genotyping of the polymorphisms

PCR reaction was done using 2 ml EDTA blood by the QIAamp DNA Mini Kit (Qiagen, Germany). Extracted DNA was quantified by spectrophotometry (MaestroNano; MaestroGen, Las Vegas, USA). The samples were kept at -20°C until usage. Genotyping were done by the duplex quantitative TaqMan 5' Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) using established protocols as directed by the manufacturer. The assay ID for rs1800469 was C_8708473_10 and for rs1800470 was C_22272997_10. Briefly, assay was performed in a final volume of 10 µl (consisting ofTaqman Genotyping Master Mix, 40x SNP Genotyping Assay, DNase-free water, and 10ng DNA) in 96-well plates using the following amplification protocol: 95°C for 10 min followed by 50 cycles at 95°C for 15 sec and 60°C for 1 min (annealing/extension). Fluorescence detection took place at 60°C. Non template controls were included in each run. The genotype call rate was more than 99%. Duplicate genotyping of 10% of samples were chosenrandomly for quality control. Assay was performed using StepOnePlus system and the automated sequence detection software (SDS) v2.3 was used for auto-calling (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The results were statistically analyzed using the Statistical Package for the Social Sciences software (SPSS version 13.0). (SPSS Inc., Chicago, IL, USA).Hardy-Weinberg equilibrium was computed to exclude any bias of results.Chi-square tests were used to study the differences between groups in genotypic frequencies. P value <0.05 was considered significant.Confidence interval (CI) 95% and Odds ratio (OR) wereconsidered to evaluate the association between TGF-b1 T869Cand TGF-b1 T905C polymorphisms and PMOP risk. Multiple linear regressions was used to determine the independent risk factor for osteoporosis.

Results

The data in table (1) showed the comparison between groups as regards the clinical data there was significant decrease in calcium, T-score and Z score in osteopenic \ osteoporotic group

The frequency of TGF-β1 gene polymorphism in osteopenic \osteoporotic and control groups show the following: C869Tgenotype CC 48.6%, CT 38.4% and TT 13% among the osteopenic \osteoporotic group, while control group CC 3.9%, CT 43.8% and TT 52.3% which is statistically significant. The number of alleles C 187 and T 89 in osteopenic \osteoporotic group,while in control group the number of alleles C 66 and T 190. These results shows significant statistical variation (table 2).

As regards the T509C genotype; TT 56.5%, CC 20.3% and CT 23.2% among osteopenic \osteoporo-

Table 1: Comparison between osteopenia / osteoporosis group and control group as regards different clinical and laboratory data.

	The studied cases n=266		Test of sig	P value
	Cases N = 138 (X ±SD)	Control N = 128 (X ±SD)		
Age (years)	53.52±2.88	53.71±3.36	t=2.33	0.11
BMI (kg/m^2)	20.83±1.88	21.23±2.14	t=1.66	0.10
T score	-2.46±0.48	1.16±0.99	U=14.10	<0.001
Z score	-2.55±0.46	1.16±0.98	U=14.10	<0.001
Calcium (mg/dl)	7.08±0.52	9.16±0.39	t=36.62	<0.001
Creatinine (mg/dl)	0.72±0.11	0.59±0.14	t=7.66	<0.001

X = mean, SD = Standard deviation, -t- = student t test, U= Mann Whitney U.

Table 2. Comparison between osteopenia / osteoporosis group and control group as regards C509T &T869C polymorphism.

	The studied cases				X^2	P value
	Cases N = 138		Control N =128			
	No	%	No	%		
C869T genotypes						
CC	67	48.6	5	3.9		
CT	53	38.4	56	43.8	81.46	<0.001
TT	18	13.0	67	52.3		
C869T allele	N=276		N=256			
C	187	67.8	66	25.8	93.81	<0.001
T	89	32.2	190	74.2		
T509C genotypes						
CC	28	20.3	27	21.2		
CT	32	23.2	30	23.4	29.34	<0.082
TT	78	56.5	71	55.4		
T509C allele	N=276		N=256			
C	88	31.9	84	32.8	41.0	<0.071
T	188	68.1	172	67.2		

X^2= Chi squared test, P value < 0.001 = highly significant.

Table 3. Statistical relation between different clinical and laboratory parameters and C869T genotypes among osteopenia / osteoporosis group.

	C869T genotypes among cases			Test of sig	P value
	TT N = 18 (X ±SD)	CT N =53 (X ±SD)	CC N =67 (X ±SD)		
Age (years)	53.83±2.26	53.26±3.21	53.64±2.78	F=0.37	0.69
BMI (kg/m^2)	20.61±1.82	20.75±1.96	20.94±1.84	F=0.28	0.76
T score	-2.00±0.44	-2.28±0.49	-2.73±0.26	F=33.79	<0.001
Z score	-2.10±0.42	-2.38±0.48	-2.81±0.25	F=33.85	<0.001
Calcium (mg/dl)	7.67±0.62	7.16±0.44	6.86±0.39	F=24.32	0.04
Creatinine (mg/dl)	0.72±0.11	0.73±0.11	0.71±0.12	F=0.53	0.59

F = F test of ANOVA .

Table 4. Statistical relation between different clinical and laboratory parameters and T509C genotypesamong osteopenia / osteoporosisgroups.

	T509C genotypes among cases			Test of sig	P value
	TT N = 78 (X ±SD)	CT N =32 (X ±SD)	CC N =28 (X ±SD)		
Age (years)	53.80±3.21	53.45±3.08	53.44±2.65	F=0.18	0.84
BMI (Kg/m^2)	21.20±1.81	20.92±1.96	20.61±1.85	F=1.09	0.34
T score	-2.41±0.52	-2.52±0.47	-2.52±0.46	F=1.15	0.32
Z score	-2.47±0.44	-2.61±0.45	-2.61±0.45	F=1.14	0.31
Calcium (mg/dl)	7.2 ±0.58	7.06±0.48	7.00±0.49	F=3.24	0.09
Creatinine (mg/dl)	0.73±0.09	0.75±0.11	0.72±0.12	F=3.23	0.14

F = F test of ANOVA .

tic group, while the control group TT 55% ,CC 21% and CT 23.4%, which is statistically non-significant P<0.001 (table 2) . The number of alleles in osteopenic \ osteoporotic group; C 88 and T 188 while in control C 84 and T172, which are statistically significant.

There was significant statistical relation between C869T genotypes and T score, Z score and calcium levelamong osteopenic \osteoporotic groupin table (3).

There was non-significantstatistical relation between T509C genotypes amongosteopenic \osteoporotic group regarding all studied parameters in table (4).

The Study of multivariate regression analysis for possible association between osteopenia\ osteoporosis and TGFβ1 gene polymorphism at position T869C and variables age, Tscore, Z score, calcium and creatinine were shown in table (5).

The variable C869T with Odds ratio 3.57 and CI; confidence interval 1.56– 5.67 was considered as a risk factor for osteopenia\ osteoporosis. On the other hand calcium, creatinine and BMD are considered as protective factors for osteopenia\ osteoporosis.

Discussion

Osteoporosis and BMD are both complex traits with astrong genetic component. Among the genes that have

Table 5: Multivariate regression analysis for independent risk factors of osteoporosis.

	SE	Odds ratio	95% CI
T score	2.34	0.98	0.55 – 2.56
Z score	1.12	0.73	0.78 – 3.67
Calcium	0.65	0.12	0.05 – 0.70
Creatinine	0.98	0.91	0.45 – 2.77
C869T	3.44	3.57	1.56 – 5.67

been associated with BMD are those encoding the vitamin D receptor, the estrogen receptors, collagen Iα1, apolipoprotein E, and TGFβ1. Secreted TGFβ1 is an important regulator of osteoblast proliferation and differentiation and directly affects bone formation *in vivo*. Homozygous knock-out of the TGFB1 gene in mice is associated with an osteopenic phenotype (15).

It is not surprising, that the TGFβ1 locus has emerged as a strong candidate gene in the genetic study of osteoporosis. The TGF-β1 amino acid sequence is well-preservedthrough mammalian classes, indicating a strong selection against variant forms of the protein. Variable expression or stimulation of TGF-β1 might be associated with bone turn over (16).

The transforming growth factor β1 (TGF-β1) gene is located on chromosome 19q13. TGF-β1 is the most common amongst the three genotypes of TGF-β in blood and bone. It is produced by osteoblasts as an inactive pro-peptide and incorporated into newly synthesized bone matrix. It is excreted during bone resorption and stimulated by the acidic microenvironment caused by the osteoclast, TGF-β1 inhibits the activity of the osteoclasts and stimulates proliferation and diversity of preosteoblasts. The complete effect on bone transformation of TGF-β1 showsa serious role in bone formation. This important role in bone turnover makes the TGF-β1 gene a candidate for mediating the genetic influence on BMD and risk of fracture (17).

Many studies were conducted to clear the relations between different polymorphism of TGFβ and BMD of postmenopausal women. In our study we found that genotype C869T subtype CC represent 48.6% which is significantly higher than control(3.9%), while TT show significant decrease than control. As regards to T509C genotype, there was no significant statistical difference between the osteopenia / osteoporosis group and the control group.

The results were in consistent with D. Utennam et al,(17) who found only the CT+CC genotype of the T869C polymorphism associated with low serum TGF-β1 levels and increased risk of osteopenic and osteoporotic fracture at lumbar spine and femoral neck. In their study, lower blood TGF-β1 levels were discovered in osteopenia/osteoporosis individualsrelatively than control subjects having the CT+CC genotype of the T869C polymorphism. Therefore, they concluded that the CT+CC genotype of the T869C polymorphism was the reason of lower blood TGF-β1 in osteopenia and osteoporosis among postmenopausal Thai women evaluated in that study. The T869C polymorphism of the TGF-β1 gene codes for the replacement of the amino acid leucine for proline at position 10 of the protein in the signal peptide sequence (18).

The T869C polymorphism associated with the serum level of TGF-β1 suggests that the Leu10→Pro substitution may affect the function of the signal peptide, possibly influencing intracellular trafficking or export efficiency of the preprotein. Though, it is stillvague whether the changes in the circulating levels of TGF-β1 among individuals with different TGF-β1 genotypes are reflected in the concentrations of this cytokine in the microenvironment of bone (19).

The results of reported byHinke et al., (20) were showed the TT genotype of C869T polymorphism was associated with a higher serum TGF-β1 concentration, high BMD of the lumbar spine, and the femoral neck in postmenopausal German women. Our results are also in consistent with Yamada et al., (19) and Lau et al. (21).

On the other hand the results of Langdahl et al., (22) showed that the CC genotype of the T869C polymorphism was associated with higher bone mass at the total hip in Danish women which is disagree with our results.

Tural et al., (23) also investigated the association between osteoporosis and TGF-β1 polymorphism in 146 osteoporotic and 97 healthy control women. However, they did not discover any changesamong the groups concerning TGF-β1 genotype spreading and allele occurrences.McGuigan et al.,(24) has shownthat there is no association between the -509C>T polymorphism and BMD or bone loss in a British population.

In our study, there was a significant statistical relationship between T allele of C869T and calcium level so we can conclude that T allele is protective and associated with relatively normal calcium level.

In our opinion, these controversial results may be due to the different sampling methods, environmental factors and the ethnicity, as genetic factors may vary from different ethnicities.Therefore, larger-scale and better-designed studies are necessary to determine the association between TGF-β1 gene polymorphisms (TGF-β1 T869C and TGF-β1 T509C) and the risk of postmenopausal osteoporosis.

To our knowledge, this study is the first to assess whether the T509C and T869C polymorphism is associated with genetic susceptibility to postmenopausal osteoporosis in Egyptian women.

Conclusion:We concluded that CC genotype of T869C polymorphism of the TGF-β1 gene has an impact on bone mineral density and enhancement of the susceptibility to osteopenia/osteoporosis in Egyptian women. We recommend further study in this issue with large number of participantsand include serum level of TGFβ1 and confirm its association with these two polymorphisms.

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