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Research Article

Influence of Polymorphic Options of the Vitamin D Receptor Gene on the Formation of Early of Osteoporotic Conditions with the Comorbidity of Osteoarthritis and Obesity in Persons of Young Age

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Abstract

Objective of the Study: The objective of the work was to establish the prognostic and diagnostic importance of the *G63980A* polymorphism of the vitamin D receptor gene, the reference SNP *(rs) 1544410 (VDR)* in the cumulative course of osteoarthritis and obesity in young age people and to determine the possibility of predicting the risk of developing bone mineral density in these patients.

Materials and Methods: 96 young age patients (35.5 ± 0.9) with comorbid OA and obesity and 96 practically healthy individuals identical in age and sex were examined. The control group consisted of 18 people with osteoarthritis and normal weight. Anthropometric criterion of obesity was considered the Quetelet index. The assessment of the functional state of the musculoskeletal system and the analysis of indicators of algo-functional activity were determined using the WOMAC test questionnaire. Measurements of bone mineral density were performed using dual energy X-ray absorptiometry (DEXA) on a HOLOGIC Explorer QDR W Series Bone Densitometer (USA). Gene polymorphism was investigated in the human genome by real-time polymerase chain reaction using the *VDR kit (Bsml c. IVS7 G> A, rs1544410)* "Vitamin D receptor mutation". Statistical data analysis was performed using the Stata 12.1 software package: statistical processing - using the W-criterion Shapiro-Wilkie, Pearson and the homogeneity of the dispersions - Leven criterion. The indicator of the relative risk of developing the disease, the odds ratio (OR) was calculated using the mathematical formula: OR = (Pr (c | A) / Pr (nc | A)) / (Pr (c | C)/Pr (nc | C)).

Results: It was established that, despite the short anamnesis of the disease, the course of osteoarthritis with obesity is accompanied by a prevalence of severe radiographic forms of joint damage (40.6%) versus 16.7% in the comparison group. The analysis of the frequency and nature of absorptiometrically verified disorders of the bone mineral density showed that in 59.4% of the patients of the main group osteopenic states developed. In patients of the comparison group, abnormalities of the BMD were observed in 50% of patients.

Conclusion: The presence of obesity in young age people with osteoarthritis worsens the clinical and instrumental manifestations of the disease, as evidenced by the predominance of more severe radiological stages of the disease and an increase in the indicators of the algo-functional index WOMAC. Despite the short anamnesis of the disease, with comorbidity of osteoarthritis and obesity in young age people, there are prerequisites for the formation of osteopenic complications. Among patients with osteoarthritis and obesity an unfavorable BB variant of the vitamin D receptor gene genotype is found more often compared with the control group, which is confirmed by pronounced X-ray progression of joint damage. The adverse effect of the B-allele of the *VDR* gene on the course of OA in patients with obesity is confirmed by the presence of impaired bone mineral density. It was also established that the variant of bb polymorphism of the *VDR* gene in patients with OA and obesity is most favorable for the course of the disease.

***Keywords**: Osteoarthritis; Obesity; Vitamin D Receptor Gene; Osteoporosis*

Introduction

One of the possible complications of osteoarthritis (OA), which runs with obesity, is secondary osteoporosis (OP). According to results obtained by Orwoll E. (Portland, USA), it was established that bone structure and body mass index (BMI) affect the amount of risk of osteoporotic fractures. Traditionally, patients with low body mass (BM) are in a high-risk fracture group due to reduced bone mineral density (BMD). On the other hand, among people with excessive BM and obesity, the prevalence of osteoporotic fractures, including fractures of the femoral neck, is quite high [1]. In addition, questions of gender dependence in the formation of OPs are considered. Thus, men with low BM, low BMDs have a higher risk of fractures compared with those ones with normal BM, although the causes of this dependence have not been established [2]. In addition, unlike women with obesity, thickening of soft tissues in the trochanter area in men is not a factor protecting the femoral bone from the impact force during the fall, with the greater the BM, the higher the impact force [3]. It was found that men with obesity have elevated levels of estradiol and sex-binding globulin, which promotes to suppressing the positive effects of estrogens on BMD. To some extent, the increased risk of fractures in men with obesity is affected by low physical activity and, as a consequence, muscle disorders, and also insulin resistance (RES) and low testosterone levels [4].

One of the factors in the formation of OPs in patients with OA and obesity is gene aberration, that is, the study of candidate genes related to bone metabolism [5]. In this case, the researchers' attention is focused on the mutations of the three candidate genes, namely, *G63980A*, *rs1544410* of the vitamin D receptor (*VDR*) gene, *C13910T, rs4988235* of the lactase gene (*LCT*) and the *T99G*, *rs2297480* of the farnesyl diphosphates synthase gene (*FDPS*) gene.

The polymorphism of the vitamin D receptor gene (*VDR*) is regarded as a "genetic control" of maintaining sufficient bone mass [6]. A total of eleven epidemiological studies on the association between *VDR* polymorphism and the risk of low traumatic fractures have been conducted. Six of them showed that the change in polymorphic variants of the *VDR* gene was associated with an increased risk of fractures, including the hip and spine [7,8].

According to the literature of recent years, there have been reports that vitamin D is involved in the metabolism of not only bone but also cartilage tissue. Vitamin D stimulates the synthesis of proteoglycan by chondrocytes, modulates the activity of matrix metalloproteinases, which have a destructive effect on the structure of cartilage. Thus, a decrease in the level of 24, 25- and 1.25-vitamin D is associated with an increase in the activity of matrix metalloproteinases, and its normal level reduces the activity of enzymes *in vitro* [9].

Objective of the Study

The objective of the work was to establish the prognostic and diagnostic importance of the *G63980A* polymorphism of the vitamin D receptor gene, the reference SNP *(rs) 1544410 (VDR)* in the cumulative course of OA and obesity in young age people and to determine the possibility of predicting the risk of developing decrease in bone mineral density in these patients.

Materials and Methods

The work involved 96 patients with OA and overweight or obesity (main group). In order to determine the effect of obesity on the flow of OA was allocated a comparison group - 18 patients with OA and normal weight. The average age of the patients in the groups was 35.5 ± 0.9 years and 34.8 ± 0.8 years respectively; among patients surveyed, women were prevailed (75% and 72.2% respectively). The control group was formed on the principle of "copy-pairs". It included 96 practically healthy individuals, identical in age and sex.

The Order of the Ministry of Health of Ukraine dated October 12, 2006 "On the provision of medical care to patients with osteoarthritis", unified diagnostic criteria of the Association of Rheumatologists of Ukraine (2004) and the criteria proposed by the American College of Rheumatologists (ACR 1986) and the EULAR 2010 revision were used during verification of OA along with the assessment of clinical manifestations of the disease, data of objective and instrumental methods of research [10-12].

An assessment of the functional state of the musculoskeletal system and the analysis of the indicators of algo-functional activity were performed using a WOMAC test questionnaire. The WOMAC questionnaire is a generally accepted questionnaire for evaluating symptoms of arthrosis (functionality) with 24 questions, which are divided into three sections. The first subclass has 5 questions that allowto evaluate the pain syndrome. The second subclass has 2 questions, which characterize the severity of rigidity of the joints: the third one - 17 questions about manifestations of physical activity and restriction of mobility of joints. To characterize the pain syndrome, the visual-analog scale of pain (VAS) is used [13].

The presence of obesity was determined by generally accepted anthropometric indices of the criteria of the International Diabetes Federation (IDF, 2005). Criteria for obesity were considered Quetelet Index, or Body Mass Index (BMI).

Research of *G63980A* polymorphism of vitamin D receptor (*VDR*) gene in serum was performed by ELISA using commercial test systems "Human VDR ELISA KIT" (Germany).

BMD measurements were performed using two-energy X-ray absorptiometry (DEXA) on the HOLOGIC Explorer QDR W Series Bone Densitometer (USA). This method makes it possible to evaluate the different state of bone tissue in norm, to identify the initial manifestations of the changes - osteopenia or severe violations - osteoporosis. BMD measurements were performed in three sections - the lumbar spine, the proximal femoral area and the forearm bones [14].

Statistical processing of the measurement results was carried out using the methods of variation statistics using the licensed software Stata 12.1. To assess the statistical validity of the differences between the mean values of parameters in the comparable groups, they were carried out by analyzing the distribution of the studied indices using the W-criterion of Shapiro-Wilkie and the homogeneity of the variances was a Leven criterion. Since the results obtained were normally distributed, the mean and standard deviation were calculated. The Pearson correction coefficient was used to evaluate the relationship between the pairs of independent qualitative characteristics. All methods of analysis and calculated indices were evaluated at a given marginal level of error of the first kind (α) not higher than 5% ($p < 0,05$). The t-test was used to evaluate the statistical validity of the differences between the mean values of the parameters under conditions of normal distribution. The level of statistical significance was taken not less than 95% ($p < 0,05$) [15].

To calculate the odds ratio (OR) relative risk, i.e. to determine if the absence or presence of a particular result is due to the presence or absence of a particular factor in a particular statistical group, the calculation was based on the mathematical formula: $OR = (Pr (c \mid A) / Pr (nc \mid A)) / (Pr (c \mid C) / Pr (nc \mid C))$ [16].

Results and Discussion

The localization of the inflammatory-dystrophic process in patients with OA was as follows: complaints of changes in knee joints were 53 patients (55.2%); goats - 29 people (30.2%); the combination of several groups of joints was determined by 14 patients (14,6%). In the group with OA and normal body mass (BM), this distribution corresponded to 55.6%, 22.2% and 22.2%.

Clinical evaluation of the functional state of the musculoskeletal system and analysis of indicators of algo-functional activity were performed using a test-questionnaire. To this end, the patients were divided into 3 groups: with moderate, medium and high levels of the algo-functional index WOMAC.

Thus, the duration of the disease in most cases (52 persons - 54.2%) corresponded to a term of up to 5 years. The second group was 31 persons (32,3%) with anamnesis from 6 to 10 years (31 - 32,3%); over 10 years of OA was detected in 13 patients (13.5%). Individuals with isolated OA to Subgroup I included 11 persons (61.1%), to II -7 persons (38.9%).

The average indicators of algo-functional activity for WOMAC allowed to characterize the main clinical manifestations (Table 1).

Patients with OA	Indices of algo-functional activity, %			
	Pain	Flexibility	FJD*	W**
Primary group (n = 96)	44,3 ± 1,8	54,5 ± 2,4	49,5 ± 1,9	48,8 ± 1,8
Comparison group (n = 18)	35,3 ± 1,8	46,5 ± 1,9	40,6 ± 2,2	39,9 ± 2,4

Table 1: *The generalized index of osteoarthritis severity and its components (by WOMAC).*
*Note - *: Functional Joint Disorders; ** - Wis a generalized WOMAC indicator.*

At the X-ray stage, the patients were divided into 4 groups (Table 2).

Patients with OA	X-ray stages of osteoarthritis							
	I		II		III		IV	
Number of patients	abs.	%	abs.	%	abs.	%	abs.	%
Primary group (n = 96)	18	18,8	39	40,6	23	24,0	16	16,6
Comparison group (n = 18)	6	33,3	9	50,0	3	16,7	0	0

Table 2: *Distribution of patients to osteoarthritis by radiological stages of the disease.*

Thus, joining obesity in patients with OA increases the number of patients with more severe X-ray stages of the disease: up to 40.6% vs 16.7% of the comparison group.

The analysis of the frequency and nature of BMD showed that there were 57 cases (59.4%) of the underlying group in the identified violations. In this case, 43 cases (44.8%) of the observations were evaluated for osteopenia and 14 (14.6%) - osteoporosis. According to DEXA data, parameters of the norm of BMD were recorded in 39 patients with OA and obesity, which was 40.6%.

In patients of the comparison group, the violation of BMD was observed in 50% of patients (compared with 59.4%), with only 3 (16,7%) cases of osteoporosis.

All patients were evaluated for the trophological status using commonly accepted clinical anthropometric indices. The anthropometric criteria for obesity were considered to be Quetelet index, or BMI greater than 30.0 kg/m^2 (Table 3).

Patients with OA	Intervals of the values of the height weighted Quetelet index							
	25 ÷ 30		30 ÷ 35		35 ÷ 40		> 40	
	abs.	%	abs.	%	abs.	%	abs.	%
Total	34	35,4	45	46,9	11	11,4	6	6,3

Table 3: *Distribution of patients with osteoarthritis by the Quetelet index.*

Thus, most people with OA had excessive BM and obesity of the 1st stage (82.3%).

In determining the polymorphism of the gene of the receptor of vitamin D, the following data were obtained (Table 4). Thus, among patients with OA with normal BMI an increase of 2.8 times the number of persons with unfavorable BB-genotype was determined. This

genotype in the main group exceeded the control indicators by almost 4 times. At the same time, allele B was determined in 80.2% of the main group, 70.6% of the comparison group compared with 59.4% of the control group.

Variants of the genotype *VDR*	Main group		Comparison group		Control group	
	abs.	%	abs.	%	abs.	%
bb	19	19,8	10	29,4	39	40,6
Bb	33	34,4	13	38,2	46	47,9
BB	44	45,8	11	32,4	11	11,5

Table 4: *Variants of polymorphism of the VDR gene in the examined groups.*

B-allele in the genotype of the *VDR* gene correlated with the radiological stage of the disease: the largest number of patients had 2 (33 persons - 34.4%) and 3rd (19-19.8%) X-ray stages.

When distributing BMD changes taking into account the polymorphism of the gene of the receptor of vitamin D, the following results were obtained (Table 5).

Gen*VDR* (variants of genotypes)	BMD (DEXA)					
	Osteopenia		Osteoporosis		Not disturbed	
	abs.	%	abs.	%	abs.	%
bb (n = 19)	3	15,8	0	-	14	84,2
Bb (n = 33)	14	42,4	5	15,5	14	42,4
BB (n = 44)	24	54,5	9	27,3	11	33,3

Table 5: *Changes in the vitamin D receptor gene polymorphism under different BMD variants in the patients of the main group.*

Thus, in patients with bb-genotype, a violation of BMD (osteopenia) was found in 15.8% of subjects, which, combined with clinical symptoms of the disease, makes it possible to determine that this variant of the polymorphism of the *VDR* gene in OA and obesity is most favorable for the course disease.

At the same time, the unfavorable B-allele of the *VDR* gene resulted in violations of BMD in 52 cases (54.2%), among which the manifestations of osteopenia were 14 with the genotype Bb and 24 with the genotype BB; osteoporosis was detected in 5 and 9 patients respectively.

That is, the combination of OA, overweight or obesity occurs on the background of changes in the polymorphism of the receptor gene of vitamin D, which negatively affects the clinical course of the disease, the results of additional research methods and indicators of structural and functional status of bone tissue. The prevalence of an unfavorable B-allele in the *VDR* gene increases the risk of osteopenic changes that requires early diagnosis of this complication and correction of therapy.

Conclusion

The presence of obesity in young age people with osteoarthritis worsens the clinical and instrumental manifestations of the disease, which is confirmed by the prevalence of more severe radiological stages of the disease (40.6%) and an increase in the indexes of the algofunctional index of WOMAC.

Despite the short anamnesis of the disease, with the comorbidity of osteoarthritis and obesity in young age people there are prerequisites for the formation of complications, namely, the development of secondary osteoporotic changes (59,4%).

Among patients with osteoarthritis and obesity, quite often (45.8%) of the control (11.5%) is found to be an unfavorable BB variant of the vitamin D receptor gene, which is confirmed by pronounced X-ray progression of joint damage.

The adverse effect of the B-allele of the *VDR* gene on the course of osteoarthritis in patients with obesity is confirmed by the presence of violations of BMD in 52 cases (54.2%), among which osteoporosis manifestations are recorded in 14.6% of the subjects.

Bibliography

1. Orwoll ES., *et al.* "The Limited Clinical Utility of Testosterone, Estradiol, and Sex Hormone Binding Globulin Measurements in the Prediction of Fracture Risk and Bone Loss in Older Men". *The Journal of Bone and Mineral Research* (2016): 633-640.

2. Kuprienko NV. "Osteoporoz i osteoartroz: sovremennyie tendentsii diagnostiki. Profilaktiki i lecheniya". *Biology Sustavyi. Pozvonochnik* 1.5 (2012): 140-145.

3. Valderrábano RJ., *et al*. "Bone Density Loss Is Associated with Blood Cell Counts". *The Journal of Bone and Mineral Metabolism* (2016): 212-220.

4. Povoroznyuk VV., *et al.* "Cuchasniy poglyad na problemu osteoporozu u cholovikiv v Ukrayini". *Biology Sustavi. Pozvonochnik* 2 (2012): 6.

5. Richards JB., *et al*. "Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture". *Annals of Internal Medicine* 151 (2009): 528-537.

6. Yang M., *et al*. "Anti-inflammatory effect of 1,25-dihydroxyvitamin D3 is associated with crosstalk between signal transducer and activator of transcription 5 and the vitamin D receptor in human monocytes". *Experimental and Therapeutic Medicine* 9.5 (2015): 1739-1744.

7. Uitterlinden AG. "The association between common vitamin D receptor gene variations and osteoporosis: a participant level meta-analysis". *Annals of Internal Medicine* 145 (2011): 255-264.

8. Gao J., *et al*. "Influence of BsmI polymorphism in vitamin D receptor gene on the risk of fracture in Caucasian populations: a meta-analysis". *International Journal of Clinical and Experimental Medicine* 8.1 (2015): 589-597.

9. Kim SH., *et al.* "Vitamin D Inhibits Expression and Activity of Matrix Metalloproteinase in Human Lung Fibroblasts (HFL-1) Cells". *Tuberculosis and Respiratory Diseases* 77.2 (2014): 73-80.

10. Hochberg MC., *et al.* "American College of Rheumatology 2012 recommendations for the use of non-pharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee". *Arthritis Care and Research* 64 (2012): 465-474.

11. Moiseyenko RO and Nakazu MOZ. "Pro nadannya medichnoyi dopomogi hvorim iz osteoartrozom". *Ukrayinskiy Revmatologichniy Zhurnal* 27.1 (2007): 74-75.

12. Altman R., *et al.* "Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology". *Drugs* 75.8 (2015): 859-877.

13. Blom AB. "Cytokine targeting in osteoarthritis the inflammatory hypothesis: any progress in risk stratification and therapeutic targets?". *Circulation* 114 (2009): 1557-1560.

14. Radchenko VA., *et al*. "Kostnaya densitometriya v klinicheskoy praktike". *Ortopediya, Travmatologiya I Protezirovanie* 2 (2015): 100-108.

15. Kobzar AI. "Prikladnaya matematicheskaya statistika dlya inzhenerov I nauchnyih rabotnikov – 2 izdaniei (ispravlennoe)". Moskva: FIZMATLIT (2012) 816.

16. Kartel NA., *et al.* "Genetika. Entsiklopedicheskiy slovar". Minsk: Belorusskaya nauka (2011).

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