Investigation the Role of IL-17 rs2275913 Gene Polymorphism with the Risk of Osteoporosis in a Sample of Iraqi Pre and Postmenopausal Women

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Abstract

Objective: This study aimed to investigate the relationship between the genetic polymorphism and alleles frequencies of IL-17 rs2275913 G/A with the risk of low bone mineral density (osteoporosis and osteopenia). Furthermore studying the relation between some immunological and biochemical parameters with this disease in a sample of Iraqi pre and postmenopausal women. **Methodology:** In this study, we investigated about the IL-17 rs2275913 G/A polymorphisms and the risk of low bone mineral density (BMD) among 30 patients with osteopenia, 30 patients with osteoporosis and 30 healthy controls. Serum IL-17 level and its correlation with the IL-17 rs2275913 G/A genotypes were analyzed. The study was carried out from November 2020- January 2021 in Baghdad Teaching Hospital/ Bone density examination unit/ in Baghdad-Iraq. **Conclusion:** It is concluded that the IL-17 rs2275913 G/A genotype was not associated with increased risk for development of low (BMD) in Iraqi pre and postmenopausal women. **Recommendation:** Based on study conclusion, during menopause, it is important for women to have a DEXA scan to detect low BMD and receive appropriate care to prevent degradation of the micro-architecture of bone tissue, which increases the risk of bone fractures.

Keywords: *Low Bone Mineral Density, IL-17, Single-nucleotide polymorphism Susceptibility, Baghdad Teaching Hospital.*

Introduction

Bone mineral density (BMD) is a measure of the inorganic mineral content in bone, and is one of the more informative assessments of bone quality in both clinical studies and forensic investigations. Several factors, such as age, sex, disease, genetics, and lifestyle, affect BMD measurements, and normative standards must be applied for specific groups and individuals. One of the most common disorders associated with low BMD is osteoporosis and increased fracture risk, due to a decrease in bone strength and an increase in bone fragility [(1)].

Osteopenia is a clinical term used to describe a decrease in BMD below normal reference values, yet not low enough to meet the diagnostic criteria to be considered osteoporotic. It is, as defined by the World Health Organization (WHO), is a t-score between -1 to -2.5 [(2)]. This condition happens when the body disposes of more bone than it is making. It is transform into osteoporosis so far as that is concerned isn't unavoidable. Diet, work out, and in some cases prescription can help keep the bones thick and solid for quite a long time. Furthermore Osteopenia as a rule doesn't have any side effects. This makes it difficult to analyze except by a bone mineral thickness test [(3)].

Postmenopausal osteoporosis is a chronic disease associated with age-related declines in bone mass, changes in bone microarchitecture, and skeletal fragility. These changes place postmenopausal women at increased risk of fragility fractures, which are linked to significant morbidity, economic cost, and negative impact on health-related quality of life [(4)].

Interleukin-17 (IL-17) is characteristic cytokines of CD4+ cells and the group of Th17 cells secrete, showed that IL-17 plays an important role in the formation of osteoclast. IL-17 is secrete factors that can work together with tumor necrosis factor alpha (TNF-α) enhance the process of the development of inflammation and bone transformation [5]. IL-17 is a recently discovered family of cytokines composed of six members. Additional isoforms homologous to IL-17A designated as IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F were discovered afterwards [6].

Material and Method

Study population: This study was conducted with 30 osteopenia patients and 30 osteoporosis patients with pre and postmenopausal Iraqi women who attended Baghdad Teaching hospital in Baghdad/Iraq between November 2020- January 2021.Osteopenia and Osteoporosis was defined according to the conventional World Health Organization (WHO) definition. Subjects with a history of bone disease, metabolic or endocrine disorders such as diabetes mellitus, hyperthyroidism, hyperparathyroidism, renal disease, liver disease, medications known to affect bone metabolism (e.g.anticonvulsants, corticosteroids, heparin sodium) were excluded. The control group comprised 30 healthy volunteers for the general health checkup in our hospital during the same period. After obtaining written informed consent, 5 mL of peripheral blood was collected for DNA extraction and ELSA test. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect demographic characteristics and medical histories. All the specimens we recruited were of Arabic/ Iraq ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study.

Bone mineral density measurements: Area BMD (g/ cm2) was measured by dual energy X-ray absorptiometry (DEXA). Densitometers were calibrated daily. Left hip and posterior–anterior lumbar spine (L2–L3–L4) scans were performed with the patient lying supine on the imaging table using the protocols recommended by the manufacturer.

DNA extraction and genotyping: Genomic DNA was isolated from EDTA anticoagulated peripheral blood with a commercially available extraction kit (Geneaid/ Taiwan) according to the manufacturer's instructions. Genotype determination for one SNP in the IL-17 gene (rs2275913 G/A) was performed by real-time polymerase chain reaction (RT-PCR). The polymorphisms within IL17 rs2275913 G/A were genotyped with TaqMan genotyping assays using the Roto-Gene Q apparatus Real-Time (Roto-Gene Q, Italy). Probes and primers designed for RT-PCR shown in Table 1.

Table 1: Details of RT-PCR primer and probe sequences and conditions in our study.

Polymorphism	Primer sequence	Product	RT-PCR conditions
rs2275913 G/A	F: 5`-CGTGTCGCAGTGGGTTCA-3` R: 5`-TTCTGCCCTTCCCATTTTCC-3`	18 mer	40 cycles: 95°C for 10s, 95°C for 15s, 60°C for 1m.
	Probe sequence		
	P/G: 5`-AGAATCTCTCCTTCTGAA-3` P/A: 5`-AAGAATCTCTTCTTCTGAA-3`	22 mer	

Serum level measurement of IL-17: The interleukin-17 (IL-17) level were determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available ELISA quantitative kit (Shanghai Yehua/ China) according to manufacturer's instructions.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) [7].

Result

This study included 30 0steopenia, 30 osteoporosis patients and 30 healthy controls, the mean ages of osteopenia, osteoporosis patients and healthy controls were 36.46 ±1.36, 55.80 ±1.48 and 41.33 ±1.81 years, respectively. The genotype and allele frequencies of the IL-17 rs2275913 G/A polymorphisms for all the studied variations are shown in Table2.

Table 2: Genotype of IL-17 rs2275913 G/A gene polymorphism with Allele frequency.

genotype	Osteoporosis No (%)	Osteopenia No (%)	Control No (%)	P –value
GG	15 (50.00%)	10 (33.33%)	10 (33.33%)	00092 **
GA	11 (36.67%)	10 (33.33%)	13 (43.33%)	0.0452 *
AA	4 (13.33%)	10 (33.33%)	7 (23.33%)	0.0076 **
Allele frequency				
G	0.68	0.50	0.55	-
A	0.32	0.50	0.45	-
* (P≤0.05), ** (P≤0.01).				

There were no association in the genotypes between patients and controls, and the allele frequencies of IL-17 rs2275913 G/A for the three study groups appeared that there were no significant different between patients and controls. Compared GA genotype between control and patients, heterozygous GA genotype was associated with significantly increased in control (43.33%) and decreased in osteoporosis (36.67%) and osteopenia (33.33%). AA genotype was higher in osteopenia (33.33%) and control (23.33%) then decreased in osteoporosis (13.33%). In addition, GG genotype was higher in osteoporosis (50.00%) and less in osteopenia (33.33%) and control (33.33%). While in the allele frequencies G and A there were no significant different between patients and controls.

Serum IL-17 level and its correlation with the IL-17 rs2275913 G/A genotypes. There were no statistically significant associations between IL-17A gene polymorphisms and Serum IL-17 Level in osteoporosis and osteopenia, while there were statistically significant differences between IL-17A gene polymorphisms and Serum IL-17 Level in control group p-value (0.038). In

contrast IL-17A plasma levels were significantly higher in patients (31.72-16.37)) comparatively to controls (15.05), and this result due to our study and another studies that the serum IL-17 level related to osteoporosis but the IL-17 G/A (rs2275913) genotypes has no related to osteoporosis (Table 3).

Table 3: Serum IL-17 level and its association with the IL-17 G/A (rs2275913) genotypes.

Group	Genotype	Mean ± SE
		IL-17
Osteoporosis	GG	25.53 ±1.90
	GA	42.69 ±12.73
	AA	24.81 ±1.48
	P-value	0.238 NS
Osteopenia	GG	18.32 ±1.25
	GA	15.59 ±1.86
	AA	14.99 ±0.69
	P-value	0.364 NS
Control	GG	16.82 ±1.62 a
	GA	15.88 ±1.40 a
	AA	10.98 ±0.79 b
	P-value	0.038 *
Means having with the different letters in same column differed significantly. * (P≤0.05).		

Discussion

In the present study, we selected, one SNP IL-17 rs2275913 G/A to evaluate their association in patients with Iraqi pre and postmenopausal women with osteopenia, osteoporosis and healthy controls. Inflammatory processes and cytokines play essential roles in the pathogenesis of women osteoporosis. Variations in cytokine levels among individuals are a plausible explanation for differences in disease susceptibility and severity, and are principally attributable to single nucleotide polymorphisms (SNPs) in cytokine-encoding genes [8].

Interleukin-17 (IL-17) is a proinflammatory cytokine produced by the memory CD4 + T cells after activation and has been shown to be involved in amplifying inflammatory response by recruiting immune

cells such as neutrophils and monocytes and inducing other proinflammatory molecules [9]. IL-17 is essential to both the adaptive and innate immune systems. It has five confirmed receptors (IL-17RA-RD and SEF) and six members (IL-17A-F). Moreover, IL-17, as a pro-inflammatory cytokine, can trigger the release of chemokines and cytokines [10].

Conclusion

It is concluded that there were no significant differences in the allele frequencies of IL-17 rs2275913 G/A for the three study groups osteopenia patients, osteoporosis patients, and controls. So IL-17 rs2275913 G/A genotype and A allele, was not associated with increased risk for development of low BMD in Iraqi pre and postmenopausal women and there were no association between Serums calcium, alkaline phosphatase (ALP), BMD and IL-17 rs2275913 G/A genotype. Also it is concluded increase IL-17 level play an important role in the development risk of osteoporosis and osteopenia in pre and postmenopausal women (11) (12).

Recommendations

During menopause, it is important for women to have a DEXA scan to detect low bone mineral density and receive appropriate care to prevent degradation of the micro-architecture of bone tissue, which increases the risk of bone fractures. a.To increase bone density and reduce the risk of osteoporosis, it is critical to consume calcium and vitamin D3-rich foods, as well as get enough sunlight for a sufficient period of time to promote production of vitamin D3. It is also critical to exercise regularly to maintain bone density and reduce the risk of low bone mineral density.

Conflict of Interest: None

Funding: Self

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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