Association of OPG genotypes with their serum levels in Iraqi type 2 diabetic patients

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Abstract:- Type 2 diabetes mellitus(T2DM) is an prevalent multifactorial disease that has both genetic and environmental risk factors, resulting in impaired glucose homeostasis. Osteoprotegerin (OPG) is a secretory glycoprotein that belongs to the Tumor Necrosis Factor (TNF) receptor family and involves in the regulation of bone metabolism, ectopic calcification including vascular calcification processes, and endothelium regeneration. OPG is also referred to by its acronym. It is involved in ectopic calcification, which includes vascular calcification and endothelium renewal, and affects bone metabolism. Common genetic variations that are functionally significant can be found in the OPG gene, which codes for the protein. Type 2 diabetics have higher osteoprotegerin concentrations than non-diabetic subjects. OPG has the potential to function as a biomarker for type 2 diabetes. This study aims to investigate the association of polymorphism of OPG gene T950>C (rs2073617) gene to their serum levels among Iraqi patients. Using a suitable sample procedure, 65 volunteers (40 verified type 2 diabetic patients and 25 healthy Genetic polymorphism of Osteoprotegerin *(OPG) gene T950>C* (rs2073617) was carried out using RFLP-PCR. Serum levels of OPG were performed using by enzyme-linked immunosorbent assay technique (ELISA) using a Human- OPG kit. Based on the polymorphism of *OPG gene T950>C*, serum levels of OPG were higher among Type2DM patients with TT and CC genotypes than that with TC genotypes (TT, 2.6764±0.64032, CC 3.4532±1.80736 and TC 1.6103±0.07901, $P < 0.05$. The three genotypes for type 2 diabetes also showed an increase in the level of OPG compared to these levels for the same genotypes of the control group, although there were no significant differences. Furthermore, patients with TC genotype had the lowest OPG level with an insignificant difference compared to controls1.6103±0.07901 vs. 1.5266±0.04768, $P > 0.05$. Polymorphism of the *Osteoprotegerin (OPG) gene T950>C* (rs2073617) can be associated with the serum levels of OPG among Type2DM patients.

Keywords:*OPG gene T950>C*, T2DM , PCR, ELISA

1.Introduction

Type 2 diabetes mellitus (T2DM) is an increasingly prevalent multifactorial disease that has both genetic and environmental risk factors, resulting in impaired glucose homeostasis [1].Osteoprotegerin, or OPG for short, is a glycoprotein that is secreted and belongs to the family of Tumor Necrosis Factor (TNF) receptors. It is involved in the regulation of bone metabolism as well as ectopic calcification, which encompasses the processes

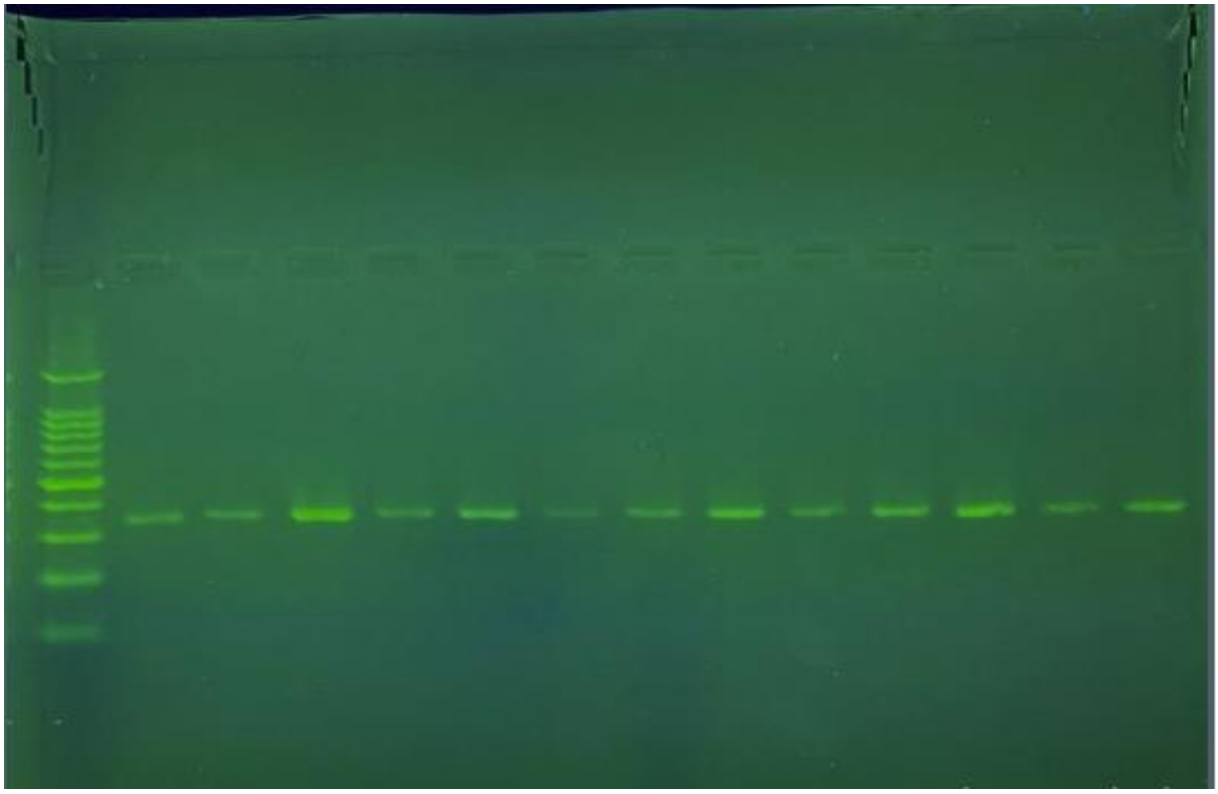
of vascular calcification, vascular tone augmentation, and endothelium regeneration [2]. It has been demonstrated that patients with type 2 diabetes have higher osteoprotegerin plasma levels, which are associated with microvascular issues [3] .In type 2 diabetic patients, osteoprotegerin levels were much higher than in non-diabetic controls, and these levels were significantly correlated with inflammation and arterial stiffness [4].In the OPG gene, common genetic variants of functional significance have been linked to osteoporosis and neuroarthropathy. In addition to this, these genetic variations are taken into consideration to be early warning signs of cardiovascular disease [5][6][7].Genome-wide analysis (GWA) studies have identified reproducible associations between certain single nucleotide polymorphisms (SNPs) and the incidence of T2DM [7]. OPG may serve as a possible biomarker for type 2 diabetes. At present, there are no studies of Iraqi population groups that investigated polymorphism of *OPG gene T950C* (rs2073617) among type2 DM patients or investigated the evaluation of its levels in the sera of those patients. This study aims to investigate the association of polymorphism of OPG gene T950C (rs2073617) gene to their serum levels among Iraqi patients.

2. Materials and Methods

This study included (40) patients with T2DM (20 males, 20 females) whose ages ranged from 40 to 70 years, and (25) apparently healthy individuals(controls) subjects (13 males and 12 females) healthy whose ages ranged from 40 to 70years
Samples were collected from November to February. They were chosen from different hospitals in Waist.

Five milliliters of blood were collected from all participants and placed in a tube without anticoagulant and placed in a centrifuge at a speed of 2000 rpm for 10 minutes. After that, the serum was withdrawn into an eppend or ff tube 2ml and preserved after being labeled with deep freezing until further processed.

using Restriction Fragment Length Polymorphism (RFLP) Polymerase Chain Reaction (PCR). the optimal conditions of OPG gene detection by PCR initial denaturation 95^{o}C, 5 min. 1 Cycle. Denaturation 94^{o}C ,40 sec. Annealing 60,^{o}C45 sec.. 35Cycle..Extension-1,72^{o}C, 40 sec. Extension -2 ,72^{o}C,10 min1 cycle . OPG concentrations in sera were measured by "enzyme-linked immunosorbent assay (ELISA)" technique using Human-Osteoprotegerin(Bioassay Technology Laboratory).



Figure(1): Electropherogram of *OPG gene*. The band size is 342 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1 hour. N: DNA ladder (100 bp).

3. Results

3.1 Serum levels of OPG according to *Osteoprotegerin (OPG) gene T950>C* genotypes

Serum levels of OPG according to *OPG gene T950>C* genotypes. T2DM patients with TT and CC genotypes showed higher levels of OPG than that with TC genotype (TT, 2.6764±0.64032, CC 3.4532±1.80736 and TC 1.6103±0.07901, $P < 0.05$. The three genotypes for type 2 diabetes also showed an increase in the level of OPG compared to these levels for the same genotypes of the control group, although there were no significant differences. These results confirm the previously mentioned results that demonstrate the association of the genotypes with the predisposition to disease, as these genotypes TT and CC increase the possibility of developing type 2 diabetes. On the other hand, the patients with the TC genotype showed the lowest level of this OPG among T2DM patients, and this supports the previous results, as this genotype does not affect the degree of predisposition to the disease.

Table (3-1): Serum levels of OPG according to *OPG gene T950>C* genotypes

Parameters / Groups	Pg/ml Mean+SE		
	TT	**TC**	**CC**
Control	1.6350±0.16856	1.5266±0.04768	1.7540±0.07700
T2DM p atients	2.6764±0.64032	1.6103±0.07901	3.4532±1.80736*
P-value	0.12835	0.432752	0.379136
Statistical Significant	NS	NS	NS

***P< 0.05**

NS : Non-significant P> 0.05

SD: Standard deviation

4.Discussion

Based on the polymorphism of ***OPG gene T950>C***, serum levels of OPG were higher in T2DM patients with CC and TT genotypes compared to healthy controls. Furthermore, patients with TC genotype had the lowest OPG level with an insignificant difference compared to controls. These results are in agreement with the results of association analysis which demonstrate that these genotypes represent the most ones predisposed to T2DM. The current study is the first report showing that both elevated serum OPG levels and variant genotypes of the OPG gene were associated with an increased risk of T2DM. Very few research have examined the association between the OPG gene T950>C polymorphism with blood levels in T2DM patients. Polymorphisms in the OPG gene have been linked to osteoporosis and vascular dysfunction. In addition, patients with a C allele in the promoter region at position 950 (TC and CC) have considerably greater circulating OPG blood levels, and genetic variants in the OPG gene give a higher risk of cardiovascular disease and carotid plaque vulnerability in Caucasians. Polymorphisms in the promoter region of the OPG gene were not related to aortic calcification or coronary artery disease in Koreans, according to Rhee et al [8].

5. Conclusion

Polymorphism of the *Osteoprotegerin (OPG) gene T950>C* (rs2073617) can be associated with the serum levels of OPG among Type2DM patients.

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