

Genetic Polymorphism in TNF Receptor and Osteoprotegerin Genes associated with the Incidence of Diabetic Foot

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Abstract

A diabetic foot is a group of pathological changes affecting the lower extremities caused by a diabetic patient with mechanical or thermal bruises that cannot be cured for the availability of pathogens associated with complications of diabetes. This work was conducted to evaluate the role of two SNPs rs2073618 and rs3134069 present in TNF receptor and Osteoprotegerin genes respectively in etiology of diabetic foot patients in a sample of the Iraqi population by ARMS PCR.

The result indicates that both SNPs significantly increased in the diabetic foot to compare with type 2 diabetic mellitus patients and control volunteers.

Keywords: Diabetic foot, T2DM, TNF receptor, Osteoprotegerin, ARMS.

Introduction

One of the common causes of death and disability among human is type 2 diabetes mellitus and its complication.⁵ The complication of diabetic may lead to the diabetic foot which varies in its etiology form wound healing delay to cut the lower leg. The differentiation between diabetic foot was made according to its etiology to three type: "first, neuropathic diabetic foot (sensorimotor neuropathy), second, diabetic foot related to angiopathy (characterized predominantly by atherosclerotic lesions in the lower limb vessels) and third, mixed diabetic foot in which both sensorimotor neuropathy and atherosclerotic changes are present". The widest spread one is a neuropathic diabetic foot.¹²

The genetic polymorphisms could play a key role in the risk of T2DM and according to published data the individual polymorphisms play the most important role.¹¹

Single nucleotide polymorphisms (SNPs) may affect the expression of pro and anti-inflammatory cytokines and these SNPs are found in regulatory sequence upstream to the gene.¹⁴ There were many

studies showing the correlation between TNF- α , IL-6 and IL-10 gene variation with diseases related with metabolism.^{2,3} Other investigations studied the relation of SNPs in genes of cytokine with inflammation markers, a lot

of questions still not answered to explain their role in diabetes prevalence.^{1,3,5,8}

The activator of the receptor of nuclear factor NF- κ B ligand (RANKL protein) is expressed by cell line of osteoblastic (the precursors osteoblasts and mature one), chondrocytes, activated T lymphocytes and fibroblasts.⁶ Osteoclastogenesis main step is RANKL binding to its receptor RANK, which coagulates in the preosteoclasts cell membrane. On the other hand, activated osteoblasts produce and secrete the cytokine osteoprotegerin (OPG), which play role to interact with the RANKL receptor and block the interaction of RANKL to RANK.

The OPG Binding to RANKL leads to bone re-sorption inhibition and induce mass building of bone. The balance of RANKL and OPG level is critical for normal metabolism of bone. In return, unbalanced RANKL/OPG ratio could be a possible cause of an uncontrolled decrease in the mass of bone.⁴ This work aimed to evaluate the role of two SNPs in TNF promoter region and osteoprotegerin gene as a risk factor in the incidence of diabetic foot among Iraqi type 2 diabetic mellitus patients

Material and Methods

Subjected groups: This study involved 50 patients with T2DM, 50 diabetic foot and 50 healthy volunteers as a control group. The patients were recruited from the Specialized Center for Endocrinology and Diabetes in Al Basra and Maysan Governorates from January to June 2018.

Blood collection and laboratory investigation: The blood was withdrawn from fasting patients and the controls from an antecubital vein by venipuncture (5ml) and put in EDTA containing tubes. Samples were centrifuged at 1000 rpm for 15 min at normal room temperature and resulting buffy coat was used for DNA isolation using the specific kit (supplied by Genaid company) by following the instructions of the manufactured company. The resulting DNA was checked for its purity and quantity by nanodrop.

Glycosylated hemoglobin (HbA1c) was estimated by hemolysate colorimetric kit as described by manufacturer company Elabscience, Spain.

Genotyping: The SNP of Osteoprotegerin Gene and TNF receptor superfamily member were genotyped by Allele-Specific PCR. The pairs of rs2073618 Osteoprotegerin Gene primers used for the amplification are; Wildtype Forward

Primer 5': GGGACCACAATGAACAGC, Mutant Forward Primer 5': GGGACCACAATGAACAGG, Common Reverse Primer 5': AAGTCAGCAGGAACCTTTC. Also, the pairs of rs3134069 TNF receptor Gene primers are:

Wildtype Forward Primer 5': CGCTGAACTTCTGGAGCA, Mutant Forward Primer 5': CGCTGAACTTCTGGAGCC, Common Reverse Primer 5': CTGTAATCATGAATGGGAC.

Amplification was carried out in an 8800 thermal Cycler, (Agilent Technologies, USA) using 20 µL reaction volume containing 5 µL (50-100 ng) of template DNA, 0.5 µL Nuclease-free water, 1 µL for each primer (10 pmol/mL) and 11.5 µL master mix,

The conditions of thermocycler were 2 min denaturation at 95°C and 35 cycles of 30-sec denaturation at 94°C, 45-sec annealing at 60°C and 1 min extension at 72°C. and the last step was final extension at 72°C for 10 min.

Results

The clinical and demographic data of 100 patients diagnosed with T2DM and foot diabetes involved in this study and compared with 50 healthy controls are shown in table 1. There were differences with significance between studied groups in relation to sex, age, body mass index (BMI), duration of diabetes, FBG, HbA1c. As shown in table 2, the frequencies of the CC, CG and GG allele genotypes of the rs2073618 polymorphism were calculated as 18%, 44% and

38% in T2DM and 26%, 46% and 28% in foot diabetes patients. It was revealed that genotype of CG in rs2073618 (P=0.01) and AC in rs3134069 (P=0.001) for both SNPs differed significantly between T2DM and foot diabetic patients.

In addition, the average frequency of the familial alleles in the study - the C and G allele for rs2073618 was 40% and 60% for T2DM, 48% and 52% for foot diabetes respectively; there was significantly difference in G allele when compared between patients groups (P= 0.003). For the rs3134069 genotype, the frequency C allele was 5% and 11% in T2DM and foot diabetic; A allele was 95% in T2DM and 89% in the second patient group. There was a significant difference (P=0.002) in A allele when compared between both groups.

Discussion

The present work was conducted to evaluate the role of polymorphism, the genotypes and allele frequency in TNF receptor and osteoprotegerin genes and their association with T2DM, foot diabetic diseases. In diabetic patients, a physically powerful association between elevated Osteoprotegerin concentrations and microvascular complications was identified.⁷ Recent studies like an Italian study conducted by Pitocco et al¹³ investigated the relationship between the genetic variability of TNFRSF11B for osteoprotegerin gene and diabetic complication and suggested a protective role of the C A alleles of the rs2073618 and rs3134069.

Table 1
General laboratory investigations of the participants

| Parameter | T2DM (n=50) | diabetic foot patients (n=50) | Healthy volunteers (n=50) | P-value |
|--------------------------|-------------|-------------------------------|---------------------------|---------|
| Age (years) | 57.5 ± 3.2 | 54 ± 4.8 | 56±1.5 | >0.05 |
| Male/ Female | 30/20 | 35/15 | 25/25 | >0.05 |
| Duration (years) | 14 ± 3 | 12 ± 5 | | >0.05 |
| BMI (kg/m ²) | 30.4 ± 2.6 | 28 ± 3.4 | 26.4 | >0.05 |
| FBG (mg/dL) | 210.2 ± 16 | 190.5 ± 12.7 | 72±6.3 | >0.05 |
| HbA1c (%) | 11.4 ± 0.5 | 9.4 ± 0.8 | 4.2±0.3 | >0.05 |

Table 2
The frequencies of rs2073618 and rs3134069 alleles and genotypes in DNA of patients with T2DM and foot diabetes.

| Rs | Allele type | T2DM | Foot diabetes | P value |
|-----------|-------------|---------|---------------|---------|
| rs2073618 | CC | 9 (18) | 13 (26) | 0.01 |
| | CG | 22 (44) | 23 (46) | |
| | GG | 19 (38) | 14 (28) | |
| | C allele % | 40 | 48 | 0.003 |
| | G allele % | 60 | 52 | |
| rs3134069 | CC | 1 (2) | 3 (6) | 0.001 |
| | AC | 5 (10) | 6 (12) | |
| | AA | 44 (88) | 41 (82) | |
| | C allele % | 5 | 11 | 0.002 |
| | A allele % | 95 | 89 | |

The study by Mrozikiewicz-Rakowska et al⁹ detected the C allele of rs3134069, found to have a protective role in T2DM patients with chronic kidney disease and foot diabetes. In the meantime, another study published by the same authors showed a correlation between diabetic foot patients and rs2073618 in patients with diabetes, regardless of the type of diabetic foot, but failed to show any association with the rs3134069 frequency.¹⁰

Ramus et al¹⁵ have shown that polymorphisms of the Osteoprotegerin genes in the specific region contain rs2073618 (part of exon I) and receptor of TNF contain rs3134069 (found in promoter sequence) might relate to retinopathy diabetic in patients from Slovakia suffering from T2DM. Osteoprotegerin gene has a critical role in the pathogenesis of target organs damage in T2DM, while the predictive value of osteoprotegerin gene polymorphisms requires more investigations.

Therefore, studies must focus on clinical characteristics such as physical activities needed to expand our knowledge for the role of SNPs T2DM and diabetic foot.

Conclusion

In conclusion, there is a moral correlation between the rs2073618, rs3134069 and T2DM in diabetic foot patients. Indeed, this work may be the pioneer study in this method to show the effect of genotypes and alleles, as risk factors for diabetic complications patients in southern Iraq. The main results of this work showed that individual polymorphisms in the Osteoprotegerin and TNF receptor gene had a critical role in the complication of diabetic diseases.

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