THE EFFECT OF RS2274907A>T ON INCIDENCE OF DIABETIC FOOT ULCERATION IN BABYLON PROVINCE

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ABSTRACT

Background: A diabetic foot (DF) is every pathology that effects directly from peripheral arterial disease (PAD) and sensory neuropathy disturbing the feet in diabetes mellitus.

Aims: this study expected to investigate relationship between (rs2274907A>T) single nucleotide polymorphism (SNP) the Omentin-1 gene and occurrence of DFU.

Subjects and Methods: 60 DFU patients and 60 healthy controls were enrolled in study. DNA was extracted from blood samples reserved from these members. Omentin-1 gene was amplified by conventional polymerase chain reaction (PCR) via particular primers. Genotyping of the SNPs of importance was done via Tetra Amplification Refractory Mutation System (T-ARMS)

Results: Results of (rs2274907A>T) SNP of Omentin-1gene indicate significant (p<0.05) association with the occurrence of DFU under genotype distribution, inheritance models and minor allele frequency analyses.

Conclusion: The Omentin-1gene SNP (rs2274907A>T) associated with the development of DFU in Babylon province.

Key words: Diabetic foot ulcer, single nucleotide polymorphism, Omentin-1 gene.

1. INTRODUCTION

Diabetic Foot Ulcers (DFUs) main complications related with diabetes and frequently associate with peripheral neuropathy, trauma and peripheral vascular disease. essential to recognize the molecular and genetic basis of diabetic foot ulcers in directive to adapt patient centered maintenance near individual patient groups (1).

wound healing a process that emerges next rupture of the skin barrier and commonly facilitated by cytokines and growth factors out by specialized cells stimulated by immune response, including fibroblasts, endothelial cells, keratinocytes and platelets. Cytokines and Growth factors are fundamental the organization of the molecular methods complicated in making cutaneous wound healing likely (2).

There is a significant part for single nucleotide polymorphism (SNPs) the variation of these cytokines and GF in DFU (3).

Genetic factors and origin also production significant part in the growth of diabetic neuropathy leading to DFU. Genes that applicants for study in DF have involved genes influencing oxidative stress, insulin resistance, immune regulation and fibrosis growth (4). Various genetic single-nucleotide polymorphisms have been studied in DF with single-nucleotide polymorphisms in the Omentin (5), VEGF polymorphisms (6) MCP-1 polymorphisms (7).

The current study was designated potential relationship among DFU and Omentin-1 polymorphism in Babylon province.
2. SUBJECTS AND METHODS

Subjects
The study population involved 60 subjects with DFU, and 60 healthy controls with age range (38-79 years old).

This study was carried out on patients attended to Babylon center in Merjan Medical City, Hilla city, Babylon province, Iraq.

Five milliliters of venous blood was reserved after every member in EDTA tubes.

genotyping of Omentin-1 gene
The DNA was extracted from the venous by genomic DNA mini kit (Favorgen, Taiwan). The SNP (rs2274907A>T) was amplified by specific, as shown in table 1.

Table (1) : The forward and reverse primers for Omentin-1 gene SNP with their product size.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence (5’-3’)</th>
<th>Ta (°C)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>outer F</td>
<td>5’-ACCCCTACCTTCCAGCCATCCC-3’</td>
<td>56</td>
<td>403 bp</td>
</tr>
<tr>
<td>R</td>
<td>5’-CATGGGGCTGAATGAACCCTCGC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlleleT T</td>
<td>5-GTCAGCAGGGCAGCAAAGCGGT-3</td>
<td>56</td>
<td>251 bp</td>
</tr>
<tr>
<td>AlleleA A</td>
<td>5-GCCGTCCCCCTCTGGGTGTTG-3</td>
<td>56</td>
<td>193 bp</td>
</tr>
</tbody>
</table>

Maxim PCR Pre-Mix Kit was used for amplification of Omentin-1 gene, original DNA (2 μL) from each tester and primers (0.25 μL) were additional to each master-mix tube (20 μL PCR Master Premix kit)

T-ARMS program and reagents use response of Omentin-1 gene rs2274907A>T SNP that gave results include: 5 μL of PCR product then 10 μL of dilution buffer and of nuclease free water 34 μL were added the incubated for 6 hr at 37°C. The products were analyzed on 2% agarose gel electrophoresis.

Agrose gel electrophoresis
After PCR product was estimated by agarose gels electrophoresis(according to Harisha method) (8). Rate movement or mobility of DNA in gel over the electric field depends its MW, concentration of the agarose, voltage applied and strength of the electrophoresis buffer. The location of DNA within the gel can be determined directly by staining with RedSafe nucleic acid staining solution, then gel was exposed to UV light and the photos was captured.

Statistical Analysis
Statistical analysis was carried out using SPSS version 20. Genotypes and alleles were uttered as frequency. (HWE) is a mathematical correlation relates genotypes to allele occurrences (9). HWE was measured using the chi-squared by likening the observed genotypes expected values(10).

The relationship between patients genetic patterns and control was examine using the Odd ratio (OR) with (CI).

3. RESULTS
Amplification of Omentin-1 gene exhibited an amplicon of 403bp. It contained the target SNP (rs2274907A>T), figure (1).

Two band (403 bp) and (193 bp) is the wild genotype (AA). This indicated the lack of polymorphism.

* Three (403 ), (193 bp) and (251 ) are heterozygous genotype (AT). indicated the occurrence of polymorphism.
*Two bands (403 bp) and (251 bp) are the homozygous genotype (TT). indicated the occurrence of polymorphism

Genotyping frequencies of rs2274907A>T of gene polymorphism were indicate to be consistent with Hardy-Weniberg equilibrium HWE (p > 0.05) in DFU and the controls group, as shown in table (2).

Table (2): Results of Hardy Weinberg Equilibrium for *Omentin-1* Gene (rs2274907A>T) SNP genotypes in Patients with DFU and the Controls Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.14</td>
<td>0.00</td>
</tr>
<tr>
<td>Patient</td>
<td>46.55</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The association of each genotype with DFU was further tested as depicted in table (3). Results of rs2274907A>T SNP of *Omentin-1* gene failed to indicate significant (p < 0.05) association with the occurrence of DFU under genotype distribution, inheritance models and minor allele frequency analyses.

Table (3): Association of rs2274907A>T genotypes with DFU

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control N=48</th>
<th>DFU N=58</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>32 66.66%</td>
<td>35 60.34%</td>
<td>Refe</td>
<td>Refe</td>
<td>Refe</td>
</tr>
<tr>
<td>AT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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The allele distribution and frequency of Omentin-1 gene (rs2274907A>T) SNP shown in Table (4). The allele frequencies of A and T of Omentin-1 gene (rs2274907A>T) SNP were found to be 62% and 38% in DFU patients respectively and 71.5% and 28.44% in the control group respectively. The minor allele frequencies (T) of Omentin-1 gene (rs2274907A>T) SNP in DFU patients and control groups were found to be 68.75% and 31.25. respectively. It was non-significantly change (P > 0.05) in DFU patients when compared with that of the controls group.

Table (4) Alleles Distribution and Frequency of Omentin-1 gene (rs2274907A>T) SNP in Patients with DFU and the Controls Groups

<table>
<thead>
<tr>
<th>Allele</th>
<th>Control</th>
<th>DFU</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No. 66</td>
<td>83</td>
<td>Refe</td>
<td>Refe</td>
<td>Refe</td>
</tr>
<tr>
<td></td>
<td>% 68.75</td>
<td>71.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>No. 30</td>
<td>33</td>
<td>OR=0.87</td>
<td>CI2=0.48-1.57</td>
<td>P=0.65</td>
</tr>
<tr>
<td></td>
<td>% 31.25</td>
<td>28.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NO 96</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The allele distribution and frequency of Omentin-1 gene (rs2274907A>T) SNP shown in Table (4). The allele frequencies of A and T of Omentin-1 gene (rs2274907A>T) SNP were found to be 62% and 38% in DFU patients respectively and 71.5% and 28.44% in the control group respectively. The minor allele frequencies (T) of Omentin-1 gene (rs2274907A>T) SNP in DFU patients and control groups were found to be 68.75% and 31.25. respectively. It was non-significantly change (P > 0.05) in DFU patients when compared with that of the controls group.

4. DISCUSSION

Several genetic polymorphisms of Omentin-1 gene have been identified. One of the most common polymorphism of the Omentin-1 gene is (rs2274907A>T) SNP located in (chr 1 position 160882036) located at exon 3 (https://www.ncbi.nlm.nih.gov/snp/rs2274907, GRCh38.p7) (11). The heterozygous genotype (AT) found to be significantly different and homozygous genotype (TT) of Omentin-1 gene (rs2274907A>T) SNP were found to be non-significantly different.

Omentin is uttered in visceral adipose tissue, it anti-inflammatory properties (12). It has linked to type 2 diabetes in some populations, signifying that omentin may be a candidate gene for type 2 diabetes susceptibility in humans (13).

In some studies, the connection between rs2274907 polymorphism in omentin-1 gene, diseases such as diabetes (14), rheumatoid arthritis (15), and coronary artery disease (16).

Omentin 1 gene, could be accompanied by T2D and DFU. Moreover, humans with this nucleotide variant are particularly vulnerable to this complication (17).

in this study, we investigated whether single nucleotide variance the omentin gene ITLN1 was related with the prevalence of DF.

The omentin gene is located in the 1q22-q23 chromosomal region. There occurs a polymorphic locus the position 3 exon, adenine is replaced with thymine (GAC→GTC, rs2274907), which involves the replacement of valine with asparagine in the position 109 of ITLN1 (Val109Asp) (18). (rs2274907) single nucleotide miss-sense
polymorphism, Val109Asp and additional sequence variations lead to a real disease-causing mutation. (Mrozikiewicz-Rakowsk, et. al., 2017) described that the rs2274907 different of Omentin gene is related with elevated occurrence of diabetic foot in patients with diabetes mellitus. The rs2274907 may influence the omentin function. we assessed the impact of genetic diversity by examining the effects of an ITLN1 allelic variant on the development of DF.

**Conclusion**

The rs2274907 A>T SNP of Omentin-I gene was studied previously in other population and showing the direct association of in the incidence of DFU.

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