Detection of – rs53576 (C→T) polymorphism of OXTR gene in patients with DMT2 in Mosul city

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ABSTRACT

Background, Oxytocin it is a neuro-protein hormone, which is responsible for many process and behaviour in the body, study indicates that this hormone considered anti diabetic and anti-obesity, and play vital role in regulation of blood sugar, insulin secretion and metabolism of carbohydrate. The activation of Oxytocin hormone correlated with receptor of oxytocin in the brain.

This research aims to discover the association of OXTR (rs53576) gene polymorphism with risk factor of DMT2.

Methods, This study include 28 patients with DMT2 with 20 persons as control groups. The blood collected from each subject and divided in two types, one for serum that used to determine some biochemical test and another EDTA tube for DNA extraction the used for molecular test. And measured the R.B.S, Urea, Creatinine and Uric acid by Reflatrone technique, and the DNA extraction then the concentration and purity measured by Biodrop technique, and the determination polymorphism of OXTR gene (rs53576) done by ARMS-PCR technique.

Results, The result showed the distribution of allele and genotype for OXTR gene polymorphism in location rs53576 (C→T) different significantly between patients and control group, and also present all genotype for OXTR gene polymorphism in patients group, And the mutant allele frequency is high in patients comparing with healthy persons, On another side the every genotype and allele frequency for OXTR gene polymorphism different between patient and healthy people.

Conclusion: However, this study showed the mutant genotype TT and mutant allele T of OXTR gene in position rs53576 have significant difference between groups study.

Keywords: DMT2, OXTR gene polymorphism, Mutation, ARMS-PCR

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INTRODUCTION

The most common endocrine disease in the world is diabetes mellitus, which characterize with high level of sugar in blood, the increase level of sugar in blood result from depression in active of secretion of insulin, and correlation with defect in pathway of carbohydrate, lipids and proteins metabolisms, nowadays diabetes mellitus considered to be the main threat on population health (Henriksen et al., 2011; Whiting et al., 2011 and Anna et al., 2018).

The diabetes mellitus include two types, type1 and type 2. Type1 caused by the insulin secretion from the beta cell, however the body can’t benefit of it because the insulin is destroyed, therefore the imperative treatment is a daily insulin injection (Zhou et al., 2010). Type2 diabetes mellitus (DMT2), is either lack of production of insulin, however it’s not enough for body consumption, or the insulin receptor don’t work
effectively. Different studies have shown the stress and obesity can cause DMT2 and also considered as a risk factor for human mortality (Florian et al., 2010 and Andreou et al., 2018).

The oxytocin is considered a neuro-protein hormone, it is consisting of nine amino acids, synthesized in the hypothalamus and which responsible for many behaviours in the body, like control on the stress feeding metabolism disorders and fatness, also the oxytocin play a vital role for controlling the energy balance and loss or gain of the body weight (Quirin et al., 2011; Morton et al., 2018 and Milos et al., 2019).

Many studies give evidencethat oxytocin is considered as anti-diabetic and have oxytocin in DMT1, and it plays an important role in homeostasis of glucose as well as control carbohydrate metabolism in the body, and the oxytocin regulate the secretion of insulin and effect of the peripheral insulin (Florian et al., 2010 and Zhang et al., 2011).

The activation of Oxytocin correlated with Oxytocin Receptor (OXTR), which diffuse in the brain, Hypothalamus-pituitary-Adrenal axis (HPA), amygdala (Kogan et al., 2011 and Danielle et al., 2017). The position of OXTR gene in the q arms of chromosome 3, and this gene involve 4 exons and 3 introns, evidence study indicate the activation of OXTR is very important for many process (Olszewski et al., 2010 ; Zhang et al., 2011Feldman et al., 2012). The system of OXT-OXTR is very important for health of human, and in past few years the published information report the mutation in the gene of OXTR which is responsible for increase of pathogenesis of DMT2 and the polymorphism in OXTR gene have effect on the carbohydrate metabolism especially in cases with Diabetes Mellitus (Chen et al., 2011 ; Onaka et al., 2010 and Milos et al., 2019).

The aim of this study is to detect the correlation between the polymorphism of OXTR gene (rs53576) with risk factor of DMT2.

Materials and Methods

This study includes 28 patients with DMT2 in age groups (30-50) years with 20 persons as control groups with same age. The blood collected from each subject and divided into two types, one for serum that used to determine some biochemical test and another EDTA tube for DNA extraction the used for molecular test. We used the ARMS-PCR technique for molecular study, and Reflatron technique for Biochemical parameters.

In this research we measured the R.B.S , Urea, Creatinine and Uric acid by reflatrone technique, and the DNA extraction done by depend the manual that described by (Iranpour et al., 2010), then the concentration and purity measured by Biodrop technique, and the determination polymorphism of OXTR gene (rs53576) done by ARMS-PCR technique, that explained as below:

Table (1): Show the primers that used in PCR reaction for OXT R (rs53576)gene (Ramin et al., 2011).

<table>
<thead>
<tr>
<th>primer</th>
<th>Sequence</th>
<th>tm</th>
<th>Band size</th>
</tr>
</thead>
<tbody>
<tr>
<td>F- common</td>
<td>5'- TGTGATTTGTACCCAGAGG-3'</td>
<td>67.6</td>
<td>224 bp</td>
</tr>
<tr>
<td>R- wild allele</td>
<td>5'- CCTGTTCCTGTCCTGAGCTGACGTT-3'</td>
<td>65.4</td>
<td>224 bp</td>
</tr>
<tr>
<td>R- mutant allele</td>
<td>5'- CCTGTTCCTGTCCTGAGCTGTACGTTC-3'</td>
<td>62.9</td>
<td>224 bp</td>
</tr>
</tbody>
</table>

Table (2): show the program that used in ARMS-PCR reaction for OXTR (rs53576) gene(Thompson et al., 2011)

<table>
<thead>
<tr>
<th>No.</th>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Initial denaturation.</td>
<td>95.0</td>
<td>5 min.</td>
<td>1</td>
</tr>
<tr>
<td>2-</td>
<td>Denaturation.</td>
<td>95.0</td>
<td>45 Second</td>
<td>35</td>
</tr>
<tr>
<td>3-</td>
<td>Annealing.</td>
<td>60.0</td>
<td>1.0 minute</td>
<td></td>
</tr>
<tr>
<td>4-</td>
<td>Extension.</td>
<td>72.0</td>
<td>1.0 minute</td>
<td></td>
</tr>
<tr>
<td>5-</td>
<td>Final extension.</td>
<td>72.0</td>
<td>7.0minute</td>
<td>1</td>
</tr>
<tr>
<td>6-</td>
<td>Stop reaction</td>
<td>4.0</td>
<td>5.0minute</td>
<td>1</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
The result of this study, show the distribution of allele and genotype for Oxytocin Receptor gene polymorphism in location rs53576 (C→T) different significantly between patients and control group, and also in this study shows present of all genotype for OXTR gene polymorphism in patients group Fig (1).

![Image](224bp)

Fig. (1): Show the ARMS-PCR product for OXTR (C→T) gene polymorphism with band 224 bp in patients with T2DM.

For mutant homozygous genotype TT the Odd Ratio = 3.54, 95%CI =1663 to 75.5804 and P value = 0.4175. and for heterozygous genotype CT the Odd Ratio = 0.7500, 95%CI = 0.1635 to 3.4411 and P value = 0.7113, because the OR for TT genotype more than 1 its considered risk factor for DMT2 pathogenesis. And the mutant allele frequency in patients high compare with healthy persons 28.5-20 respectively (OR=1.6000, 95 % CI = 0.4632 to 5.5268 , P value = 0.4574), Table (3).

On another side the every genotype and allele frequency for OXTR gene polymorphism different between patient and healthy people, the wild homozygous genotype CC have same percentage in all groups study (57.5%-60%), and the heterozygous genotype CT its high in healthy persons compare with patients (40%-28.2%), but the mutant genotype TT have high distribution in patients but it didn’t appear in healthy group (14.3%-0%). Fig (2).
Fig (2): Distribution of genotype for OXTR (C → T) gene polymorphism in patients with DMT2.

In addition, the result show the frequency for alleles, the wild type allele C have high frequency compare to mutant allele in patients with DMT2 (71.5% - 28.5%) respectively fig (3).

Fig (3): Allele frequency for OXTR (C → T) gene polymorphism in patients with DMT2.

Other study don’t showed any significant different between patients and control croups about the distribution of allelic and genotypic for OXTR polymorphism at location rs2254298 A/G. (Kuessel et al., 2013 and Anna et al., 2018).

Nevertheless, the role of Oxytocin receptor gene polymorphism in DMT2 is unclear, a variety studies denote that 2 of the SNP of OXTR gene that correlated with certain disease (Ismail et al., 2013 ; Ramin et al., 2015 and Milos et al., 2019). It is believed that these SNP have important function in the human heterogeneity of stress reactivity, social interplay and autism disorder (Chen et al., 2011 ; Ismail et al., 2013 and Danielle et al., 2017) [20,15,12].

Some data indicate the C allele carriers increase their OXT relative to the T allele, few study have shown the OXTR gene SNP plays a vital role in DMT2 (Andreou et al., 2018 and Milos et al., 2019). Resent studies of whole genomic association demonstrated an correlation between OXTR gene and total blood sugar homeostasis, and thus an correlation with DMT2 in various ethnic groups (Kogan et al., 2011 ; Feldman et al., 2012 and Kuessel et al., 2013).
Table (4): Result of some biochemical parameters in study groups.

<table>
<thead>
<tr>
<th>Test</th>
<th>Patients ± SE</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.B.S (mg/dl)</td>
<td>222 ± 35</td>
<td>140 ± 23</td>
</tr>
<tr>
<td>B. Urea (mg/dl)</td>
<td>54 ± 4.2</td>
<td>39 ± 7.8</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>1.41 ± 0.2</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Serum Uric acid (mg/dl)</td>
<td>4.5 ± 0.81</td>
<td>4.2 ± 0.31</td>
</tr>
</tbody>
</table>

In table (2) the result showed increase in levels of R.B.S, B. Urea, S. Creatinine and Uric acid in patients with DMT2 compare with healthy people, and the reason for this increase due to the defect of metabolic pathway of this molecules in patients with DMT2 and depended on fatty acids as source of energy (Hamden et al., 2013 and Liu et al., 2010).

**Conclusion**

However, this study showed the mutant genotype TT and mutant allele T of OXTR gene in position rs53576 has significant difference between groups study.

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