Assessment the linking of Insulin-like growth factor 1 hormone and their gene polymorphism with excess adiposity

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ABSTRACT
This study aims to determine the correlation between the serum-IGF-1 concentrations hormone and their gene polymorphism with excess adiposity (obese adults) in Babylon province. The study has performed on 50 participants divided into obese (n=30) and control (n=20) groups with age range (20-40 years). No significant differences have been reported in the concentrations of IGF-1 hormone in obese group. However, the results showed that genotype distribution of AA and AB in IGF-1 gene polymorphism was represented by 83.3% and 16.7%, respectively. While in control group, it was 90.0% and 10.0%, respectively. Furthermore, in obese group, the allele frequency of A and B in IGF-1 gene polymorphism was represented by 91.6% and 8.35%, respectively, whereas the control group presented as 95.0% and 5.0%, respectively. Moreover, several single nucleotide polymorphisms (SNPs) were obtained between the two resolved haplotypes and between the IGF1 genes. The results demonstrate that there is no association between AA and AB polymorphisms in the obese group (OR=0.87, CI [0.71-1.17]) as compared with the control group.

INTRODUCTION
Insulin-like growth factor 1 (IGF-1) is a hormone consisting of 70 amino acids with a molecular weight of 7649 Da, similar in molecular structure to insulin with low affinity to the insulin receptor and their action to rapid metabolic effects and longer-term growth-promoting effects [1]. The IGF-1 gene is mapped to 12q23.2 humans’ genomic region. There is a sequence homology represented by nearly 50% between IGF-1 and insulin. The liver primarily produces IGF-1. Growth hormone stimulates its production [1][2]. The growth and development of many tissues are regulated by IGF-1 in particular during the prenatal period. IGF-1 in the circulation is typically bound to IGF binding proteins (IGFBPs). The free IGF-1 bioavailable level is regulated by IGFBPs to be bound to the IGF-1 receptor (IGF-1R). Also, this stimulates growth or survival signaling [1]. The bioavailable IGF-1 level increases with obesity. It is probably via hyperglycemia-induced suppression of IGFBP synthesis and/or hyperinsulinemia-induced promotion of hepatic growth hormone receptor expression and IGF-1 synthesis [1][3]. During the maturity period, the decrease in IGF-1 levels significantly affects life and health expectancy. There are metabolic and genetic factors regulating the levels of IGF-1 in the circulation including: growth hormone (GH), levels of insulin, nutritional state, age, gender, a gain of weight, distribution of body fat and risk of diseases related to obesity including cancer [2]. Excess adiposity or obesity refers to a multifaceted disorder of metabolism defined by the increase in the mass of white adipose tissue. Clinically, obesity is described as a BMI of ≥ 30 kg/m2 [4]-[7]. It is related to the response of IGF-1 to GH and the increased levels of GH-binding protein [6]. Consequently, when the GH receptor increases, this could indicate the lack of suppression of total levels of IGF-1. Various works defined

the single-nucleotide polymorphisms (SNPs) as factors of body mass index (BMI, kg/m2), the circumference of waist and mass of body fat. Genotype data of 41 SNPs in 18 candidate genes (ABCC8, ALPI, FABP1, FABP2, IGF1, INSIG2, LEPR, MC4R, MTPP, SREBF1, TBC1D1, TCF7L2, TMEM18) distributed among 11 chromosomes were classified into (3) groups (heterozygote, homozygote minor and major alleles). Effects of SNP on BMI and circumference of waist were examined and observed. Insignificant effect on circumference of waist was reported for gene IGF1 rs1520220 (β = 0.81, SE = 0.40, p = 0.042). SNP features and alleles associated with obesity-related traits in 2,122 European women and men of mid-age (randomly selected sample) observed did no corrections with IGF1 rs1520220 (p= 0.436, chromosome =12, Position=101320652, Minor/major allele= C/G) [7]. In simple obesity, the total concentrations of IGF-1 were described as low, normal or high [8]. A cross-sectional study demonstrated that IGF-1 is inversely associated with BMI or weight of the body. Another study reported that individuals with the lowest or highest BMI have lower level of IGF-1 [9,10]. Moreover, IGF-1 concentrations suggest that the decrease in the IGF-1 level may be associated with age-related gains of fat and losses in lean mass. However, other studies found that ethnicity may have a role in the levels of IGF-1 and IGFBP-3 in adults and children [11][12].

Patients and Methods
This study was conducted in Babylon University, College of Science, Laboratory of biotechnology with the department of nutrition at the Murjan Teaching Hospital in Babylon governorate, in cooperation with the Azadi Teaching Hospital in Kirkuk Governorate, Iraq from June 2017 to January 2019. The researcher obtained the approval of the
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institutional research ethics committee and signed written agreement of each patient enrolled in this study (Ethics Committee in University of Babylon, College of Science, the Reference number of approvals: 215). The study excluded patients with any chronic disease and kept those suffering only from obesity. All participants in both obese and control groups were from the same ethnic group (Arabic) with age range (20-40 years).

The sample was selected based on age, gender, individual’s lifestyle, diet, routine work and family background. Blood samples were collected from 30 obese and 20 control adult individuals from different areas of Babylon governorate. BMI in all of the obese group was above 30 kg/m², whereas it was less than 25 kg/m² in the control group. Levels of Insulin-like growth factor 1 hormone was measured by using specific kit (ELISA) supplied by Elascience - China company (Method Colorimetric).

The DNA extraction was done using (FAVORGEN) kit. Then, as illustrated in Figure 1, the agarose gel electrophoresis was applied to identify DNA. After that, the polymerase chain reaction (PCR) was applied to design primer IGF-1 gene using computer-based software, Primer 3 plus reference, retrieved from: http://www.ncbi.com. The type of primers used in this work to amplify exon 3 of the IGF-1 gene of Homo sapiens is a designed one, as sequences:

1. F: 5’GAGGGATGCAGAGAACAAAG-3’.
2. R: 5’CGGACCATTGTTCGAAAGT-3’.

Figure 1. The electrophoresis pattern of DNA extracted from blood.

Lane 1 - lane 12 refers to extracted DNA from the sample of study with electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (10 µL in each well).

Annealing Temp. 58.1 °C, Amplicon’s length Exon 3, Size 237 bp and a gradient PCR must be performed to identify the optimum temperature of annealing these primers to the template. Accordingly, gradient PCR experiments were employed. At first, the gradient PCR ranging between (49.9 – 62 °C) succeeded in picking up the proper annealing temperature. Then, as shown in Figure 2, the purity of each PCR amplicon, before being subsequently applied for SSCP, should be confirmed.

Figure 2. Agarose gel electrophoresis of the IGF1 gene, exon 3, gradient PCR fragments.

Gradient PCR (49.9°C – 62°C) is applied on the IGF1 gene, exon 3 to identify the optimum primer annealing temperature. M refers to DNA size marker lane 1; lane 12 refers to the variable gradient annealing temperature applied. Electrophoresis conditions: agarose concentration 1.5%, power applied: 75V (7 V/cm), time to run: 45 min. Staining method: precast ethidium bromide. The apparent and sharp bands were detected after applying the electrophoresis (Figure 3). Therefore, only 50 out of 70 PCR amplicons were included in the subsequent SSCP, and the rest with less quality PCR amplicons were excluded. The pattern of each SNPs was discovered by sequencing. The differences of nucleic acid patterns between the exon 3 of Homo sapiens IGF1 gene reference sequence and the two genotypes AA in nucleotide position were 21-C,49-C,83-G,151-G,163-C,184-C,206- C,217-G,233-A,195-C and AB in nucleotide position were 12-G,49-C,83– G,184-C,193-T,111-C,133-G,148-T,217-G,229-G.

Figure 3. Agarose gel electrophoresis of the IGF1 gene, exon3, PCR fragments.

M refers to DNA size marker lane 1 and lane 25 refers to the IGF1 gene, exon 3 PCR fragments patterns. This amplification product was one band 237 bp for the sample. Electrophoresis conditions: agarose concentration 1.5%, power applied: 135V (7 V/cm), time to run: 45 min. Staining method: precast ethidium bromide.

Results
As illustrated in Table 1 there are significant differences (P≤ 0.05) in mean of ages, which reached 30.10± 1.30 year in obese groups. The BMI was 33.25± 0.44 Kg/M2 in obese groups, as well as, the waist circumference was higher (P≤ 0.05) in obese groups which reached 102.96± 0.97 cm. The data demonstrated that the obese groups no significant differences in the concentrations of IGF-1 hormone comparison with control groups.

<table>
<thead>
<tr>
<th>Group Parameters</th>
<th>Control (Mean ± S.E) n=20</th>
<th>Obese (Mean ± S.E) n=30</th>
<th>P-value of groups</th>
</tr>
</thead>
</table>

Table 1- The differences among of age, BMI, WC and Serum concentrations of IGF-1 in obese and control groups
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| Age(years) | 22.05±0.63 | 30.10±1.30 | 0.001* |
| BMI(Kg/M²) | 21.41±0.33 | 33.25±0.44 | 0.02* |
| WC (cm)   | 85.20±1.71 | 102.96±0.97 | 0.04* |
| IGF-1 (ng/ml) | 2.51±0.30 | 3.24±0.32 | 0.12 |

t-test.
*P < 0.05
S.E: Standard error.
However, in Figure 4, the results showed the presence of two different haplotypes. The first was AA genotype where ssDNA constitutes only one band; while the second was AB genotype where ssDNA constitutes two bands. Nevertheless, determining the type of all resolved SSCP bands may be difficult through the use of only the visualization gel. Consequently, these two haplotypes should be proven by utilizing sequencing. Results of sequencing proved the haplotypes detected in this study. As demonstrated in Figure 5, Several single-nucleotide polymorphisms (SNPs) were found between the two resolved haplotypes and between the haplotypes and the IGF-1 gene, exon 3 and Primer 3 plus reference.

**Figure 4.** PCR-SSCP Patterns that electrophoresed on non-denaturing polyacrylamide gel electrophoresis for IGF1 gene, exon 3 and PCR fragments.

Selected lanes 1 to lane 11 (control and obese groups) refer to IGF1 gene, exon 3, PCR-SSCP and the two observed genotypes (genotype A and B). Lanes 7, 8 refer to the typical differences between genotype AA and AB respectively. Electrophoresis conditions: polyacrylamide gel concentration 8%, power applied: 200V (7.5V/cm) – 100mA, time to run: 90 - 120 min. staining method: silver staining.

**Figure 5.** Sequences alignment results for Homo sapiens IGF1 gene fragment, two SSCP variants with their reference sequence (Bank accession number: NG_011713.1) using DNA STAR, the Editseq software.

By applying ClustalW alignment in the same software, several point mutations (SNPs) are discovered between the IGF reference sequence and the genotypes AA and AB. Several SNPs were demonstrated in IGF1, exon 3. These SNPs were found in both groups of the study. Table 2 listed the frequencies of AA and AB of IGF1 polymorphism were 83.3% and 16.7% in the obese group, and 90.0% with 10.0% in the control group. Furthermore, as demonstrated in Table 3, the results revealed that there was no significant association between AA and AB polymorphisms for obese and control groups.

Table 2: The genotype of IGF1 gene polymorphism with Allele frequency between the two groups (obese vs control).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Obese Group N (%)</th>
<th>Control Group N (%)</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>25(83.3%)</td>
<td>18(90.0%)</td>
<td>Obese</td>
</tr>
<tr>
<td>AB</td>
<td>5(16.7%)</td>
<td>2(10.0%)</td>
<td>A (91.65%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>B (8.35%)</td>
</tr>
</tbody>
</table>

Table 3: The Genotype distribution and odd ratio of IGF1 gene polymorphism between both groups (obese vs control).

<table>
<thead>
<tr>
<th>IGF1 Polymorphism (genotype)</th>
<th>Obese Group N (%)</th>
<th>Control Group N (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
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</tr>
</tbody>
</table>

Discussion
The growth hormone can be affected by Excess adiposity or obesity. This, in turn, would affect the IGF-1 hormone secretion by the liver but, IGF-1 levels in obesity have been reported to be variables [13]. However, in obesity, the levels of IGF-1 are described to be variable [14]. The regulation of IGF-1 circulating levels is affected by many factors including genetic and metabolic factors, nutritional status, age, gender,
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a gain of weight, distribution of body fat and risk of diseases related to obesity including cancer [2]. Various works defined SNPs as factors of BMI (kg/m²) and circumference of waist. Genotype data of 41 SNPs in 18 candidate genes, including IGF1 rs1520220, were described. Additionally, the effects of SNP on BMI and circumference of waist were examined and observed. A non-significant effect on BMI, circumference of waist and mass of body fat was observed for IGF1 rs1520220 [7]. Previous works found a different association between IGF-1 and obesity. A longitudinal aging study conducted on 351 participants from Baltimore revealed that there was a non-significant association between IGF-1 and BMI or waist: the ratio of the hip after observing age [3]. Similarly, a study of the Swedish cohort showed the same results [14]. On the contrary, in the study of Copeland et al. [15], a negative relationship was found between IGF-1 and BMI in 62 patients of men group, while there was no relationship between them in 45 patients of women group. On the other hand, Nyström et al. [16] revealed that there was an association between BMI and IGF-1 in women but not in men. Only a study conducted on a small sample (consisting of 27 obese men) employed scanning of X-ray computed tomography (X-ray CT) to examine the distribution of adipose tissue concerning IGF-1 [17]. Hereditary variants in IGF axis genes and variance expression of such genes have been related to diabetes or its complications [14,18], cardiovascular diseases [14], cancers [18,19,20], open-angle glaucoma [21] and Alzheimer’s disease [22]. In the study of Robert et al. [2002], the absence of correlation between obesity and the axis of IGF-1 proposes that the latter is not a possible moderator between visceral adipose tissue and disease [23]. The current study revealed that the concentrations of IGF-1 were linearly, inversely associated with BMI. These results contradict to some extent shown in Caucasian women. Results obtained from other cross-sectional studies indicated a nonlinear correlation between adiposity and growth factor with the minimum concentrations of IGF-1 observed in women with BMI<20 and those with BMI>30 [24,9]. These results demonstrated that there are useful effects of IGF pathway polymorphisms on growth and central obesity. Also, they indicated that genotype-phenotype relationships are of ethnic specificity [25].

Conclusion
In this article, we evaluated there was no linking between Insulin-like growth factor 1 (IGF-1) hormone and IGF-1gene polymorphism with excess adiposity or obese adults in ethnicity of Babylon, Iraq.

Acknowledgements
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REFERENCES
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