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STUDY OF ASSOCIATION BETWEEN IGF-1 GENE POLYMORPHISM AND OBESE ADULTS IN BABYLON GOVERNORATE

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ABSTRACT

The aims of the present study were to determine the association between IGF-1gene polymorphism with obese individuals. This case– control analysis was performed on 50 subjects ,obese (n=30) and control (n=20) groups for age ranged (20-40 year).The results showed that genotype distribution of AA and AB in IGF-1 gene polymorphism were 83.3% and 16.7% respectively in the patients of obese group, while it was 90.0% and 10.0% respectively in the control group .Meantime the allele frequency of A and B in IGF-1 gene polymorphism were 91.65% and 8.35% respectively in obese group, whereas it was 95.0% and 5.0% respectively in the control group. Several single nucleotide polymorphisms (SNPs), was obtained between the two resolved haplotypes and between the IGF1 gene, exon3 Primer3 plus reference of the study. The results demonstrate that there is no association between AA and AB polymorphisms with in obese (OR=0.87,CI[0.71-1.17]) as in comparison with control groups.

1. Introduction

Insulin-like growth factor 1 (IGF-1) is a polypeptide product of the IGF-1 gene which is mapped to 12q23.2 human genomic region and that shares near fifty percent sequence homology with insulin and is produced primarily by the liver following stimulation by growth hormone[1,2] .

IGF-1 regulates development and growth of many tissues, particularly during the prenatal period. IGF-1 in circulation is typically bound to IGF binding proteins (IGFBPs) that regulate the amount of free IGF-1 bioavailable to bind to the IGF-1 receptor (IGF-1R) and elicit growth or survival signaling[1]. The amount of bioavailable IGF-1 increases with obesity, possibly via hyperglycemia-induced suppression of IGFBP synthesis and/or hyperinsulinemia-induced promotion of hepatic growth hormone receptor expression and IGF-1 synthesis[1,3].

In adulthood, the decreased levels of IGF-1 have important health and life expectancy consequences. The regulation of IGF-1 circulating levels includes genetic and metabolic factors (e.g., growth hormone (GH), insulin) nutritional status, age, sex, weight gain, body fat distribution, and risk of obesity related diseases including cancer [2].

Obesity is a multifaceted metabolic disorder that is described by an increase in white adipose tissue mass and clinically it is defined as a BMI of ⩾30 kg/m2 [4,5, 6, 7].

Obesity is associated with an increased IGF-I response to GH, and increased GH-binding protein levels [6], so that an increase in expression of GH receptor may explain lack of suppression of total IGF-I levels. Several studies identified a large number of single nucleotide polymorphisms (SNPs) as determinants of body mass index (BMI, kg/m2), waist circumference, and body fat mass, genotype data of 41 SNPs in 18 candidate genes (ABCC8, ALPI, FABP1, FABP2, FTO, HSD11B1, IGF1, INSIG2, LEPR, MC4R, MTTP, NPC1, PPARG, PTGES2, SREBF1, TBC1D1, TCF7L2, TMEM18) distributed among 11 chromosomes were categorized into three groups (homozygote major allele, heterozygote, and homozygote minor allele). Single SNP effects on BMI and waist circumference were tested and observed that insignificant effect on waist circumference was observed for IGF1 rs1520220 (ß = 0.81, SE = 0.40, p= 0.042) ,meantime SNP features and associations of SNP alleles with obesity-related traits in 2,122 European middle-aged men and women (random population sample) was observed did no corrections with IGF1 rs1520220 (p= 0.436,chromosome =12, Position = 101320652, Minor/major allele= C/G)[7].

Circulating total IGF-I concentrations in simple obesity are reported as low, normal or high [8]. IGF-I has been cross-sectionally inversely associated with body mass index (BMI) or body weight, with some studies reporting that those with lowest or highest BMI have lower IGF-I [9,10].

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Meantime specific evidence from cross- sectional studies shows that IGF-I concentrations are suggesting that the decline in IGF-I may be implicated in age-related gains in fat and losses in lean mass while some studies have shown that ethnicity may also be associated with IGF-1 and IGFBP-3 levels in children and adults[11,12].

2. Material and Methods

The study was performed in Babylon university / College of Science / Laboratory bio-technology with department of nutrition at the Murjan teaching hospital / in babil province/Iraq for the period from June 2016 to January 2017 (The approval of the institutional research ethics committee and signed written consent of every patient included in the study was obtained. Ethical committee Iraq: Ethics Committee (University of Babylon/ College of Science), Reference number of approval: 215) where the excluded patients with from any chronic disease , but only suffering from obesity and all obese and control were from the same ethnic group (Arabic) for age ranged (20-40 year).

Where sampling was done by asking different questions about age, sex, individual's life style, diet, routine work and family background. Blood samples were collected from 30 obese and 20 control adult individuals from different areas of province of Babylon, and all the obese adults had the BMI above 30 kg/m2 and control adults had the BMI less than 25 kg/m2. DNA extraction was done by kit (FAVORGEN, then applied agarose gel electrophoresis to identified DNA, as show in Figure 1 A after that the polymerase chain reaction (PCR) applied on designing primer IGF-1.gene using computer based software Programs, Primer 3 plus by online reference through address http://www.ncbi.com, where type of primers used in our work to amplify exon 3 of IGF-1. gene of Homo sapiens is a designed one, as Sequences:

- F:5'GAGGGAGTGCAGGAAACAAG-3';
- R:5'CAGGACCATTTTTGCAAGGT-3' ,

Figure 1A - The electrophoresis pattern of DNA extracted fromblood. Lane 1 - lane 12 refers to extracted DNA from sample 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 , Size237 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 used, the first (49.9 – 62) °C was succeed in picking up the proper annealing temperature and each PCR amplicon before its being subsequently applied for SSCP its purity should be confirmed ,as show in Figure 1 B.

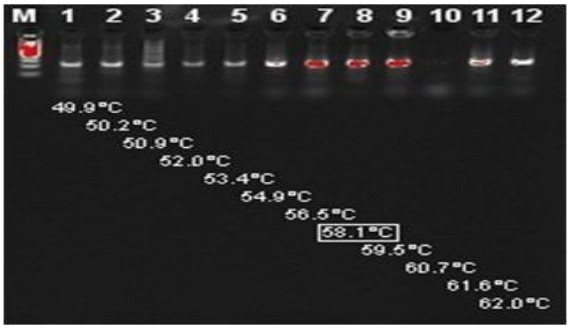

Figure 1 B - Agarose gel electrophoresis of *IGF1* gene, exon 3 gradient PCR fragments. Gradient PCR (49.9°C – 62°C) applied on *IGF1* gene, exon 3 to identify the optimum primer annealing temperature. M; refers to DNA size marker lane 1 - lane 12 refers to the variable gradient annealing temperature applied. Electrophoresis conditions: agarose concentration 1.5%, power applied: 75V(7 v/cm), time to run: 45 min. Staining method; precast ethidium bromide.

The sharp and apparent bands were found after performing the electrophoresis Figure 2, so only 50 from 70 PCR amplicons were included in the subsequent SSCP and the rest of the less quality PCR amplicons were excluded. The pattern of each SNPs that 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 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 12-G,49-C,83-G,184-C,193-T,111-C,133-G,148-T,217-G,229-G.

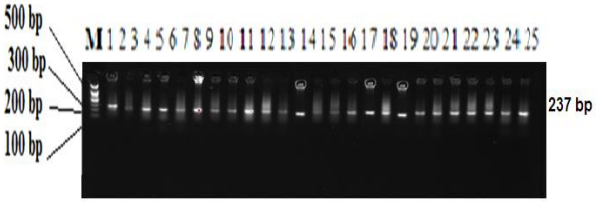

Figure 2 - Agarose gel electrophoresis of *IGF1* gene, exon3PCR fragments. M; refers to DNA size marker lane 1 into lane 25 refers to *IGF1* gene, exon 3 PCR fragments patterns. This amplification product was one band 237 bp for the sample. Electrophoresis conditions: agarose concentration 1.5%, power applied: 135V (7V / cm), time to run: 45 min. Staining method; precast ethidium bromide.

3. Results

The results showed that the present of two different haplotypes, as revealed in Figure 3. The first AA genotype, in which ssDNA constitutes only one bands, while the second AB genotype constitutes ssDNA two bands, however, it might be difficult to determine the pattern of all resolved SSCP bands using only the gel visualization. Accordingly, these two haplotypes must be confirmed using sequencing. Sequencing results confirmed the haplotypes observed in our work. Several single nucleotide polymorphisms (SNPs), was obtained between the two resolved haplotypes and between the haplotypes and the IGF1 gene, exon 3 Primer 3 plus reference as revealed in Figure 4.

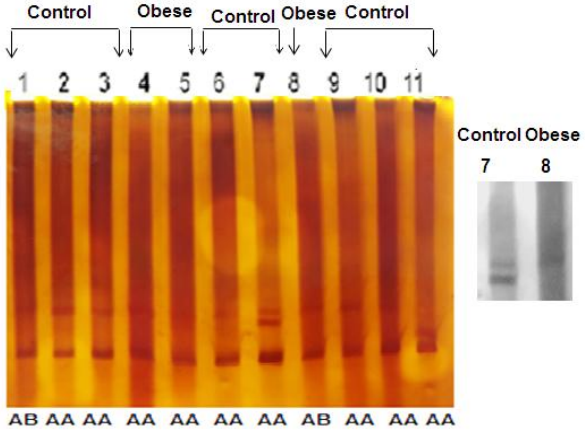

Figure 3 - PCR-SSCP Patterns that electrophoresed on non-denaturing polyacrylamide gel electrophoresis for *IGF1* gene, exon 3 PCR fragments. Selected lanes 1 into lane 11 (control and obese groups) refer to *IGF1* gene, exon 3 PCR-SSCP fragments two observed genotypes (genotype A and B) . Lanes 7, 8 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.

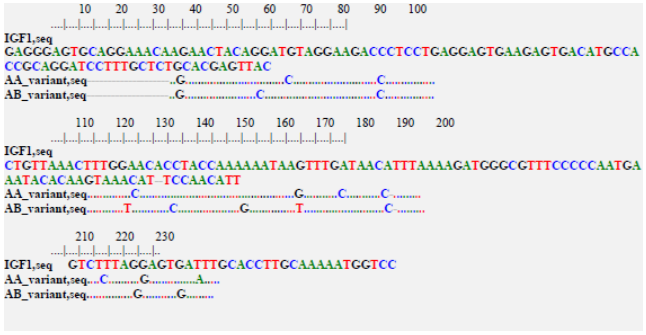

Figure 4 - Sequences alignment results for *Homo sapiensIGF1* gene fragment, two SSCP variants with their reference sequence (Bank accession number : NG_011713.1) using DNA STAR, Editseq software. By applying ClustalW alignment in the same software, several point mutations (SNPs) is discovered between the *IGF1*reference sequence and the genotypes AA and AB.

Several SNPs were demonstrated in IGF1, exon 3. Where this SNPs was found in two groups of the study The data in Table 1. refers to 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. The results revealed that there is was no significant association between AA and AB polymorphisms for obese and control group, as show in Table 2.

Genotype	Obese Group n (%)	Control Group n (%)	Allele frequency	
AA	25 (83.3%)	18 (90.0%)	obese	Control
AB	5 (16.7%)	2 (10.0%)	A (91.65%)	A (95%)
Total	30	20	B (8.35%)	B (5%)

Table 1 - The genotype of *IGF1* gen polymorphism with Allele frequency between the two group (obese vs. control).

IGF1 Polymorphism (genotype)	Obese Group n (%)	Control Group n (%)	OR (95% CI)
AA	25 (83.3%)	18 (90.0%)	0.87 (0.71-1.17)
AB	5 (16.7%)	2 (10.0%)	
Total number	30	20	

Table 2 - The Genotype distribution and odd ratio of *IGF1*gene polymorphism between the (obese vs. control).

4. Discussion

Obesity can affect the secretion of growth hormone, which would subsequently affect the secretion of IGF-1 by the liver but, IGF-1 levels in obesity have been reported to be variables [13]. The regulation of IGF-1 circulating levels effected by many factors involved genetic and metabolic factors ,nutritional status, age, sex, weight gain, body fat distribution, and risk of obesity related diseases including cancer [2].

Several studies identified a large number of single nucleotide polymorphisms (SNPs) as determinants of body mass index (BMI, kg/m2), waist circumference, and body fat mass, genotype data of 41 SNPs in 18 candidate genes including IGF1 rs1520220 , whereas single SNP effects on BMI and waist circumference were tested and observed that unimportant significant effect on BMI,waist circumference and body fat mass was observed for IGF1 rs1520220 [7].

The relationship between IGF-1 and obesity has different in previous investigations. In a sample of 351 subjects from the Baltimore Longitudinal Aging Study, IGF-1 was not significantly related to BMI or waist: hip ratio after controlling for age [3]. Similar results were seen in a Swedish cohort study [14]. Alternatively, Copeland et al. [15] found a negative association between IGF-1 and BMI in a sample of 62 men, but no association between IGF-1 and BMI in a sample of 45 women, whereas Nystrom et al. [16] found an association between BMI and IGF-1

in women and not in men. Only one small study of 27 obese men (BMI _25) has used X-ray computed tomography (X-ray CT) scanning to quantify adipose tissue distribution in relation to IGF-1 [17].

Hereditary variants in IGF axis genes and differential 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],thus Robert et al. (2002) reported that the lack of association between obesity and the IGF-1 axis suggests that the IGF-1 axis is not a likely mediator between of visceral adipose tissue and disease[23].

Inside our study, IGF-I concentrations were linearly inversely related to BMI, while these results contrast to some degree with those observed in Caucasian women, where results from cross- sectional studies indicate a nonlinear, correlation between adiposity and growth factor concentrations with the minimum IGF-I concentrations observed in women with BMI<20 as well as in women with BMI>30[24,9],thus these results provide evidence that IGF pathway polymorphisms have useful effects on growth and central obesity and indicate that genotype-phenotype relationships are ethnic specific [25].

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