ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Spectrum of Human Growth Hormone Receptor Gene Polymorphisms in Physiological Obese Subjects in Babylon Province, Iraq

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Abstract

Background: A peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals is called growth hormone. In human, growth hormone gene is encoded the protein growth hormone receptor that located on chromosome number 5. Obesity is a condition in which excess fat has accumulated in the body, such that it can have an adverse effect on health. Obesity is defined as a body mass index (BMI) of greater than 30 kg/m2. Aim: assessment of spectrum of human growth hormone receptor (HGHR) gene polymorphisms in obese subjects in Babylon province, Iraq. Subjects and Methods: this case-control study included two groups, the first 30 obese subjects (OS) and 30 non-obese subjects (NOS). We investigated GHR genetic polymorphism in both study groups by assessment of the genotyping, Fl/Fl, F1/D3, and D3/D3 of two alleles of GHR. We used allele specific PCR method to investigate the all genotypes and comparing the results with the levels of GH and leptin in serum of study groups. Results: the results of present study showed significant differences (p-value< 0.05) in levels of leptin (LEP) and human growth hormone (HGH) between OS and NOS groups. The genetic results suggested that F1/F1 genotypes have high prevalence in OS compare to NOS (odd ratio was 3.54, CI 95% 1.77-7.94). Conclusion: F1/F1 genotypes have higher levels of leptin and HGH and so it having more risk factor to induce obesity in Babylon population.

Keywords: *Human growth hormone, growth hormone receptor, obesity, leptin.*

Introduction

A peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans is called human growth hormone (HGH) [1]. HGH is thus important in human development, stimulates production of IGF-1, and controlling on the levels of glucose and free fatty acids [2]. In human, growth hormone gene is encoded the protein growth hormone receptor (GHR) that located on chromosome number 5. This gene encodes a protein that is a trans-membrane receptor for growth hormone [3].

Binding of growth hormone (GH) to the receptor (GHR) leads to reorientation of a pre-assembled receptor dimer dimerization, the receptor may however also exist as monomers on the cell surface [4] and the activation of an intra- and intercellular signal transduction pathway leading to growth [5]. A condition in which excess fat has accumulated in the body, such that it can have a complication effect on health is called obesity. Obesity is defined as a body mass index (BMI) of greater than 30 kg/m^2. GHR is differs from other types of polymorphisms, so the GHR bears a common micro-deletion leading to complete numbers of exon 3 (full-length GHR, Fl) or not complete number of nucleotides of exon 3(deleted GHR, D3) [6]. Genetic factors may also influence individual GH sensitivity [7]. The GHR consists of an extracellular domain of 246 amino acids, a single trans membrane domain and a cytoplasmic domain [8].

The encoding gene of GHR has 9 exons, but there are two isoforms of the GHR in humans generated by deletion of exon 3 of the gene resulting in 3 genotypes, (Fl/Fl, Fl/D3, and D3/D3) [9]. Population studies have shown that the heterozygote frequency is between 25 and 40%, while the GHR-D3/D3 genotype occurs in 7-15% of the population [10].

Obesity arises as a result of a complex interplay between genetic and environmental factors including diet, physical exercise and sleep behavior [11, 13]. Other various risk factors are important affecting factors on the pathophysiologic and developing process of obesity. The life style and psychological factors are also related to hormone changes in obesity [14, 15]. The aim of this study is to examine the association of obesity with known SNPs of HGHR in obese subjects in Babylon province, Iraq.

Materials and Methods

Study Design

This study performed on sixty subjects, thirty obese and the other thirty were non-obese. BMI is calculated based on the weight (kg) divided by the square of height (m) and the obesity was applied on all subjects as OS whose having BMI≥ 25, and NOS were BMI ≤ 25 [16]. The mean± SD of age of OS group was 32±7.6 and for NOS was 30±5.6 and the both groups have matching with age and sex.

All OS were divided into two subgroup depending on age (10-25) and (26-45), BMI (≤30), and (>30), gender (M and F), dwelling (rural and urban), and education status (yes and no).

Determination of Leptin and Human Growth Hormone Levels

Human LEP and HGH assays were based on standard competitive enzyme-linked immune-sorbent assay technology (ELISA). The kits were buy from Elabscience® and the assays performed depending on the manufactured instructions that provided with kits.

Genotyping Analysis

Genomic DNA was extracted from peripheral whole blood of all subjects (OS and NOS) who participating in this study by using the AccuPrep® genomic DNA mini kit (Bioneer, Korea) that providing an efficient method for purifying of total DNA from whole and frozen blood. Allele specific PCR was performed by used unique primers [17] for analysis of HGHR genotyping, as shown in Table 1.

Table 1: Primers used in genotyping analysis

Genotype	Primer(5'-3')	No. of bands , Amplicon size
F1/F1	TGTGCTGGTCTGTTGGTCTG	1, 935 bp
D3/D3	AGTCGTTCCTGGGACAGAGA	1, 592 bp
F1/D3	CCTGGATTAACACTTTGCAGA CTC	1, 250 bp

PCR was carried out in a total volume 25 µl of reaction mixture with Taqman polymerase and carried by the thermocycler (Exycycler 96, bioneer, Korea) and subjected to denaturation at 95 C^{o} for 3 min, followed by 40 cycles of 94 C^{o} for 30 sec, 58 C^{o} for 60 sec and the final extension phase at 72 C^{o} for 7 min. The final PCR product was electrophoresis by agarose gel (2%) and photo documentation the products.

Results

Clinical characteristics of OS group are shown in Table 2.

Table 2: Clinical characteristics of Obese Subject group

Clinical Variables	NO. Total=30	Percentage (%)
Age		
10-25	**12**	**40**
26-45	**18**	**60**
Gender		
Male	**17**	**57**
Female	**13**	**43**
BMI		
≥30	**19**	**63**
<30	**11**	**37**
Education status		
Yes	**15**	**50**
No	**15**	**50**
Dwelling		
Rural		
Urban	**21**	**70**
	9	**30**

The results of this study showed significant differences (p-value< 0.05) in levels of leptin (LEP) and HGH between OS and NOS groups, as show in Table 3:

Table 3: LEP and HGH levels in study groups

Groups	LEP (ng/ml) mean± SD	P-value	HGH(ng/ml) mean± SD	P-value
OS n=30	**16.9±1.4**	**0.0001**	**12.9±1.6**	**0.006**
NOS n=30	**11.7±1.2**		**10.6±2.2**	

The allele specific PCR amplification of HGHR gene showing one band for specific genotypes F1/F1, F1/D3, and D3/D3 were 935bp, 592bp, and 250bp, respectively, as showing in Figure-1 A, B, and C.

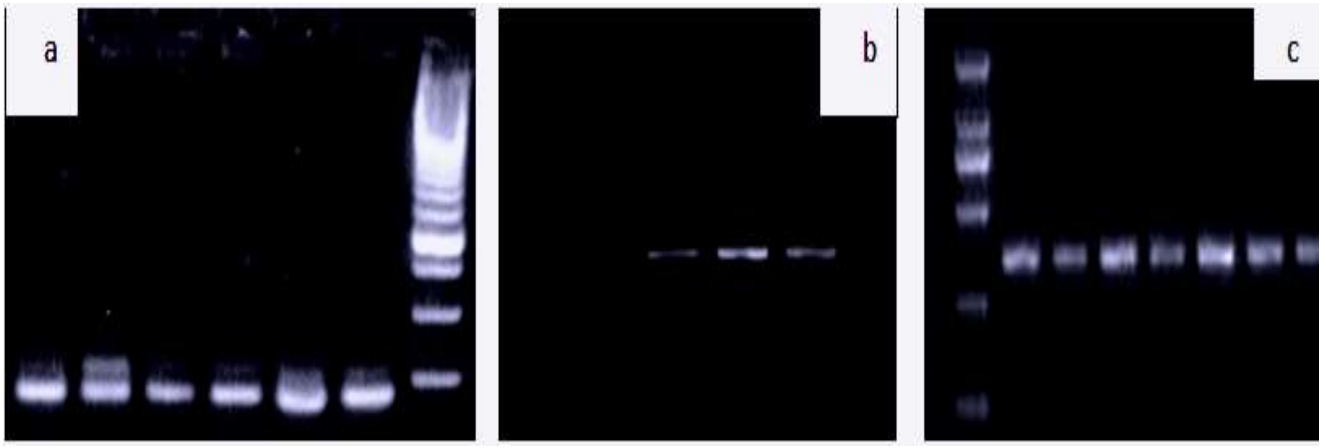

Figure 1: Electrophoretic pictures represents the genotypes, a: F1/F1, b: F1/D3, and c: D3/D3 of HGHR gene

The results showed that frequency of F1/F1allele of HGHR gene was (0.66) (0.34) in OS and NOS group respectively, and found significant difference between F1/D3 and D3/D3 alleles in OS and NOS (OR=3.54), CI $_{95\%}$ (1.77-7.94), as shows in Table 4.

Table-4: Comparison of three genotypes incidence in OS and NOS groups

Genotypes	OS	NOS	ODD RATIO	CI $_{95\%}$**
F1/F1	15 (50%)	11 (37%)	**3.543****	**1.77-7.94**
F1/D3	10 (33%)	10 (33%)	**0.415**	**0.28-0.52**
D3/D3	5 (17%)	9 (30%)	**0.775**	**0.43-4.87**
TOTAL	30(100%)	30(100%)	-	-

The results suggesting the positive correlation (r^2=0.346) between ages of OS and levels of HGH (ng/ml), as shows in Figure-2:

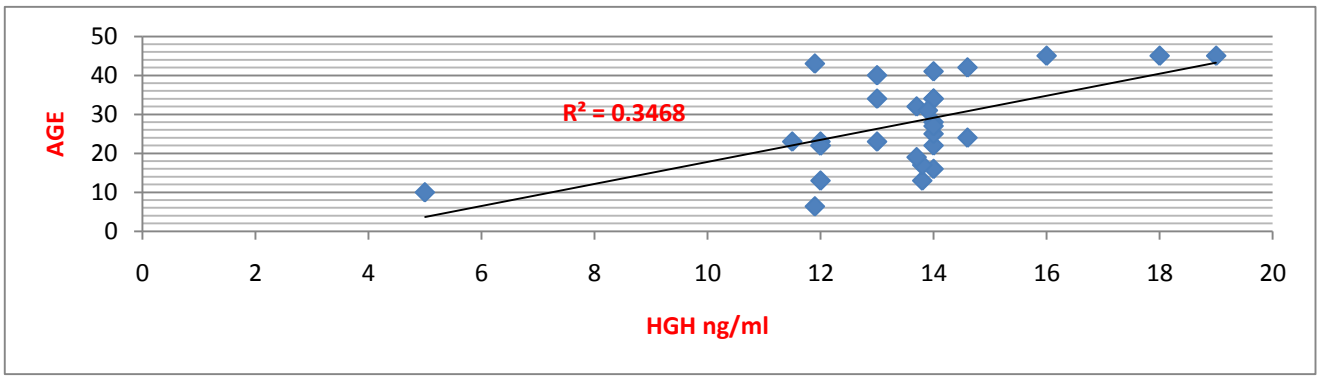

Figure 2: Correlation between ages of OS with HGH level

Discussion

Growth hormone (GH) promotes height growth by stimulation the bone and the cartilage cells proliferation, and influences carbohydrate and lipid metabolism through a direct stimulatory effect by GH, and an indirect effect mediated by insulin- like growth factor-1 [18, 20]. The effects of GH are mediated by the interaction between GH and the GH receptor (GHR) [21]. In response to GH signals, the GHR polypeptides form dimers and transport a cascade of signal transduction leading to activation of gene transcription [22].

In this study we examine the HGHR gene polymorphism in different genotypes with the levels of HGH and LEP to show the effects of it on increased risk of obesity in part of Babylon population. The results of this study suggested direct correlation between the levels of HGH and age, this indicate of independent factor to raise the risk of obesity and this results agreement with previous studies [23, 25]. In present study, the distribution of the genotype for HGHR in obese subjects is similar to previous studies reported in the patients with acromegaly because of the main role of GH in incidence of obesity and acromegaly [26, 27].

The results disagreement with other studies from Asian [28, 29], this indicate the effects of locally on differentiation of HGHR genotypes. The prevalence of F1/F1 genotypes was found in this study (50%) of OS compare to (37%) in NOS, this indicates to increase obesity risk by these genotypes and this same in other study [30]. Previous studies showing the effects of differentiation in genotypes on increased risk of other diseases such as cancers [31, 32].

The results showed that frequency of F1/F1allele of HGHR gene was (0.66) (0.34) in OS and NOS group respectively, and found significant difference between F1/D3 and D3/D3 alleles in OS and NOS (OR=3.54), CI $_{95\%}$ (1.77-7.94). Our findings describe an impact of the HGHR genotypes on the relationship between GH and LEP levels on obesity status. For this reason, a polymorphism in the HGHR was observed to influence physiological endogenous GH and LEP concentrations in OS. Apparently, lowering HGH and LEP concentrations are required for NOS those carrying a D3/D3 allele to produce a given low serum LEP concentration and not developing obesity. It has been reported that physiological obesity may be more closely related to LEP rather than HGH levels (33).

However, the theory of leptin as an anti-obesity hormone was called into question because obesity is typically associated with raise levels of LEP and not deficiency of these hormones (34). In conclusion, our findings demonstrate that the HGHR genotype F1/D3 have low relationship between HGH and LEP concentrations in OS. HGH concentration was higher in subjects carriers of the F1/F1 and low percentage of the D3/D3 compared to carriers of the F1/F1 genotypes. Thus, OS carrying a HGHR F1/F1 genotype have higher risk to obesity rather than other genotypes of HGHR gene.

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