

Interleukin-6 (174G/C) Gene Polymorphism and Serum Levels of IL-6, their Association with Risk of Obesity in Iraqi Childhood Populations

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Abstract

Background: Childhood obesity is one of the most serious public health challenges of the 21st century.

Aim: The present study was conducted to investigate the association of *CD14* genotype and serum levels of IL-6 with risk of ischemic stroke.

Method : A total of 60 patients with obesity were included in the study who were admitted to hospital from the period between March to August 2020, and other groups consist of 60 apparently healthy individuals. A five ml of blood samples were collected, 3 ml of each sample for polymerase chain reaction amplification and detection of IL-6 technique. The remaining (2 ml) for IL-6 serum levels by ELISA assay procedure.

Results: *IL-6* (-174G/C) genotype was significantly associated with increased risk of obesity ($P = 0.004$). However, IL-6 serum levels were higher in subjects with GG genotype compared with those with CG or CC genotype ($P < 0.001$).

Conclusion: A significant correlation between *IL-6* genotype and IL-6 levels were higher level in subjects with GG genotype compared with those with CG or CC genotype.

Keyword: *Childhood Obesity, BMI, IL-6.*

Introduction

Childhood obesity is one of the most serious public health challenges of the 21st century⁽¹⁾. It is widely known that obesity reduces child health-related quality of life and is associated with several health and social consequences. These include the risk of premature death, as well as developing diabetes, cancer, heart disease⁽²⁾. It also causes undesirable psychological consequences, such as anxiety, depression, sleep disorders and low self-esteem, which affects the social and educational relationships of children⁽³⁾. A pooled analysis of 2416 population-based measurement studies showed that from 1975 to 2016 there was a rising trend in children's and adolescent's body mass index (BMI). In 2016, 124 million children and adolescents, aged 5–19 years, were estimated to suffer from obesity worldwide

and 213 million were overweight⁽⁴⁾. The number of obese children and adolescents (aged five to 19 years) worldwide has risen tenfold in the past four decades. If current trends continue, more children and adolescents will be obese than moderately or severely underweight by 2022⁽⁵⁾. Researchers believe that the increased prevalence of obesity is the result of changes in the life-style of societies, such as the inactivity, unhealthy eating patterns, collapse of energy balance, short sleep duration, increased use of fast food and animal proteins, and increased use of technology⁽⁶⁾. Interleukin-6 (IL-6) is an immune-modulator pro-inflammatory cytokine, secreted by many types of cells, mainly T cells, macrophages, endothelial cells, smooth muscle cells, adipocytes, and hepatocytes⁽⁷⁾. IL-6 is critical in the inflammatory signaling pathway and is involved in the development of obesity and insulin resistance⁽⁸⁾.

Furthermore, IL-6 regulates/stimulates production of cell adhesion molecules, acute phase protein, and mediates the release of other cytokines that amplify the inflammatory response which influence the function of adipose tissue⁽⁹⁾. The human *IL-6 gene* is located on chromosome 7p21, and the -174G/C polymorphism consists of a single nucleotide change from G to C at position -174 in the promoter region⁽¹⁰⁾. The association of the *IL-6-174G/C* polymorphism with obesity risk has been evaluated in several genetic studies. Some studies have suggested that *IL-6-174G/C* increased the obesity risk⁽¹¹⁾, while some found no association between *IL-6-174G/C* and obesity⁽¹²⁾.

Materials and Method

The current study was carried on 60 patients (25 males, 35 females) age range between 4-18 years from March to August 2020. Other groups consist of 60 apparently healthy individuals (30 male and 30 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group. We excluded patients have obesity secondary to a genetic or medical condition, such as diabetics, polycystic ovarian syndrome, hypothyroidism, Cushing's syndrome, growth hormone deficiency, insulinoma, hypothalamic disorders (e.g., Froelich syndrome, Bardet-Biedl syndrome, Prader-Willi syndrome), or medication use (e.g., antipsychotics). A five ml of blood samples were collected by vein puncture using disposable syringes under aseptic technique 3 ml of each sample were transferred into with EDTA tube and immediately frozen at -20 C till further use to avoid repeated thawing and freezing for polymerase chain reaction amplification and detection of *IL-6* (RFLP-PCR) technique. The remaining (2ml) were transferred into sterile test tubes (Plain tube) and allow sample to clot for few minutes at room temperature then followed by separation of serum from the clot by centrifugation for 10 minutes at 2500 r.p.m. and stored at -20°C for ELISA assay procedure. This study was in agreement with ethics of Al-Imam Al Sadiq Teaching Hospital and verbal informed consent was obtained from all participants.

Results

Sixty Childhood obesity patients included in this study. The socioeconomic and lifestyle variables distribution of the study population are summarized in Table (1). There were no significant differences between daily duration of sleep and risk for obesity ($P = 0.138$).

The present study found a statistically significant between family income and obesity ($p = 0.007$). The frequency distribution of obesity further increased with the number of hours per day spent watching television (33.4% of childhood patients spent more than four hours per day in watching television), although the relation was not significant ($P = 0.175$). The frequency distribution of childhood patients and healthy controls according to physical activity indicated the prevalence of obesity was lower among childhood who practiced physical activity ($P = 0.003$). Distribution of *IL-6-174G>C* polymorphism was detected by RFLP-PCR technique, at this locus there are three genotypes, CG, GG and CC with band sizes 198/140/158 bp, 140/158 bp and 198 bp respectively, table (2), figure (1). The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups and agree with the reports of Ibrahim et al⁽¹³⁾. Both groups were comparable in Median serum concentration IL-6 table (3), figure (2). Median serum concentration of IL-6 in obesity patients was significantly higher than that of healthy control 127.32 (62.43) ng/L versus 89.44 (29.75) ng/L and P-value was ($P < 0.001$). Median serum concentration of IL-6 were statistically significantly higher in patients bearing the GG genotype ($p < 0.001$) compared with heterozygotes (CG) and those homozygous for the CC genotype, and this indicate serum level of IL-6 was highly significant associated to *IL-6 genotype* polymorphism in childhood patients group, table (4).

Table 1: Frequency Distribution of Patients with Obesity and Healthy Control according to some Socioeconomic and lifestyle variables.

Characteristic	Patients n = 60	Control n = 60	P
Sleep duration (h/day)			
< 8, n (%)	19(31.7%)	26(43.3%)	0.138
9-10, n (%)	17(28.3%)	20(33.3%)	
> 10, n (%)	24(40%)	14(23.4%)	
Average family income			
Low, n (%)	9(15%)	24(40%)	0.007
Medium, n (%)	21(35%)	18(30%)	
High, n (%)	30(50%)	18(30%)	
Television viewing (h/day)			
< 1, n (%)	14(23.3%)	24(40%)	0.175
1-2, n (%)	8(13.3%)	9(15%)	
2-4, n (%)	18(30%)	15(25%)	
> 4, n (%)	20(33.4%)	12(20%)	
Physical activity			
Yes	26(43.3%)	42(70%)	0.003
No	34(56.7%)	18(30%)	

Table (2): Distribution of IL-6 -174G/C Genotype and Alleles Frequency

Genotype	Controls (n=60)	Patients (n=60)	OR	95% Confidence interval	p	PF	EF
IL-6 -174G/C							
GG	15	26	4.189	1.66 -10.57	0.002	0.52
CG	16	22	3.23	1.31 - 8.43	0.01	0.45
CC	29	12	Reference	Reference	Reference	Reference
Overall P value	0.004						
Alleles Frequency							
G	46	74	2.59	1.54 -4.35	0.001		0.378
C	74	46	0.386	0.23- 0.65		0.378

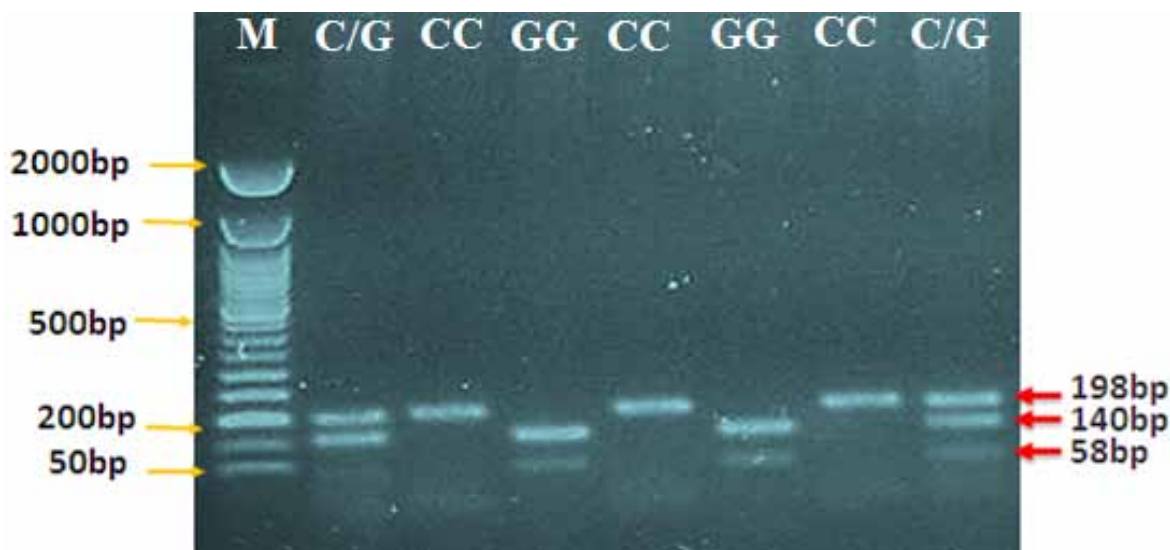


Figure (1): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of IL-6 gene-174G/C (rs1800795) gene polymorphism by using SfaNI restriction enzyme in 2.5% agarose gel. Where M: marker (2000-50bp). Lane (CC) wild type homozygote, the product undigested by restriction enzyme and still at 198bp bands. Lane (GG) mutant type homozygote that show digested by restriction enzyme into 140bp and 58bp bands. Lane (C/G) heterozygote, the product digested by restriction enzyme into 198bp, 140bp and 58bp bands.

Table (3): The comparison between the study groups regarding IL-6 level

IL-6(ng/L)	Patients n = 60	Control n = 60	P
Range	77.3 – 230	42.72 – 145.33	0.001 (S)
Median (IQR)	127.32 (62.43)	89.44 (29.75)	

Table (4): The Median Serum Interleukin-6 in patients with obesity and control subjects according to Interleukin-6 genotype

IL-6 POLY	Patients groups n = 60	Control groups n = 60
GG	149.86(60.7)	113(35.66)
CG	122.87(37.57)	90.33(13.79)
CC	93.4(10.41)	77.62(45.57)
P	< 0.001 HS †	< 0.001 HS †

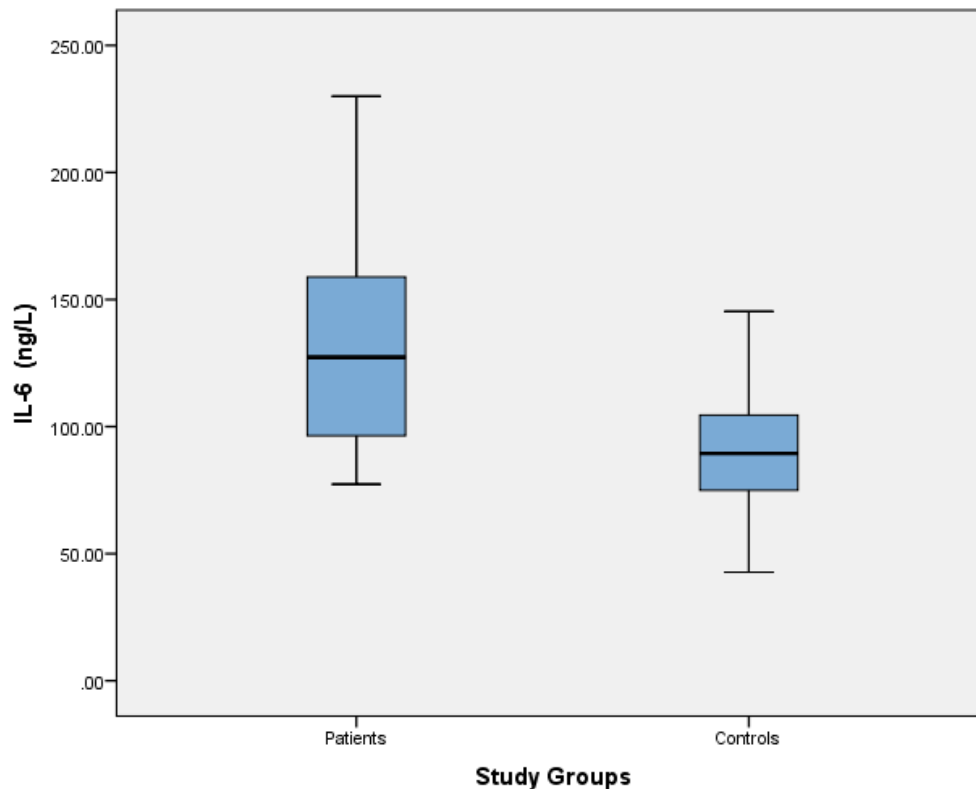


Figure (2): Distribution of patients with obesity and control subjects according to the level of Serum Interleukin-6

Discussion

Table (1) revealed that 24 (40%) of the patients with obesity with sleep duration more than ten hours and 14 (23.4%) of healthy control with sleep duration more than ten hours, and this result suggested increased obesity with increased sleep duration, but these results indicated no significant difference in the frequency distribution of patients and control subjects according to sleep duration ($P = 0.138$). The results of present study agree with results of El-Kabbaoui et al.,⁽¹⁴⁾ (2018), which indicated 78 (38.6%) of patients has had more than ten hours sleep duration and these result indicate no significant association between the daily duration of sleep and risk for overweight or obesity ($P = 0.75$). We found a statistically significant relation between family income and risk of obesity, where the obesity increasing with increasing family income 30 (50%) of patients have had high average family income. Several explanations have been proposed. The low prevalence of obesity in groups of low socioeconomic status in developing countries is related to food scarcity, patterns of high energy expenditure and the greater capacity of the elite to obtain adequate food supplies⁽¹⁵⁾. The correlations

may be due to the benefits of economic growth, notably better access to food and high energy expenditure by poorer social groups, difficulty in acquiring more expensive, less energy-dense foods and a trend towards less leisure time and fewer opportunities for exercise⁽¹⁴⁾. A similar finding was reported in a study of Moroccan adults, in which family income, used as a determinant of socioeconomic status, was strongly associated with obesity⁽¹⁶⁾.

Table (1) revealed that 20 (33.4%) of the patients with obesity was had more than four hours Television viewing, although watching television daily for ≥ 4 h was not significantly associated with obesity in our study when compared to healthy controls ($p = 0.175$). Watching television may reduce energy expenditure by replacing physical activity and also increase snacking, which is further encouraged by advertisements for energy-dense foods⁽¹⁷⁾. Our results agree with results of El-Kabbaoui et al.,⁽¹⁴⁾ which indicated non-significant correlation between watching television daily for ≥ 4 h with the risk of childhood for obesity ($p = 0.32$). The present results found inverse statistically significant relation between the prevalence of obesity and physical

activity ($P=0.003$), where the frequency distribution of childhood obesity patients according to physical activity was as following: 26 (43.3%) patients having physical activity and 34 (56.7%) don't having physical activity. Inadequate physical activity has been hypothesized to be an important contributing factor to the development of childhood obesity. The influence on energy expenditure can explain this relationship. Physiologically, sedentary posture lowers energy expenditure, and this in turn promotes weight gain⁽¹⁸⁾. A review of the influence of physical activity on adiposity among 5–18-year-olds showed that adiposity was reduced and aerobic capacity increased with more time spent in intense physical activity⁽¹⁹⁾. The present results in consistence with results of several studies. For instance, a study in Saudi Arabia showed that intense physical activity was inversely associated with adolescent obesity⁽²⁰⁾, and a strong negative association was reported between vigorous physical activity and total and central body fat in Spanish adolescents⁽²¹⁾.

Obesity is caused by several genetic, metabolic, social, and environmental factors. Among these internal and external factors, the contribution of genetic factors has been recognized widely, but the genes involved have not been fully elucidated⁽²²⁾. Genetic variants, especially functional polymorphisms in the promoter region of genes, may alter the function and expression of genes associated with energy intake and energy expenditure. Several indications of the linkage between single nucleotide polymorphisms (SNPs) and obesity phenotypes have been found⁽²³⁾. *Interleukin-6* polymorphisms have been investigated in many populations for associations with various chronic diseases. For example, single-nucleotide polymorphisms (SNPs) rs1800797(-597 G/A), rs1800796 (-572G/C) and rs1800795 (-174 G/C), located in the promoter region of IL6, have been shown to be associated with obesity and metabolic traits in different ethnic groups⁽²⁴⁾. Accordingly, *IL-6-174G>C* were selected and their genetic associations with obesity risk were evaluated using RFLP PCR analysis to identify if genetic variants in IL-6 might be a potentially genetic marker to predict the susceptibility of obesity. Overall, highly significant differences can be found in the distribution of the genotype and allele frequencies of *IL-6 (rs1800795)* between the obesity patients and control subjects ($P=0.004$). The present results consistence with results of Nelson et al.,⁽²⁵⁾, which indicated *IL-6-174G>C* polymorphism was highly associated with the risk of obesity.

The results of present study showed high frequency of (GG) genotype among patients when compared to healthy controls, these result identify (GG) genotype is associated with increased risk of obesity with statistically significant ($P=0.002$), and may be considered as the etiological factor for obesity. The results of present study consistence with result of Yang et al⁽²⁶⁾, which found the GG genotype was significantly higher in patients with obesity than in those without ($\chi^2=10.63$, $p=0.005$). The present study are relatively conflicting with previous reports which revealed that the *IL-6 rs1800795* SNP in the genotype GG play a protective role and present predominated in the control group (95.3%) with a statistically significant difference compared to that of obese children (28.2%)⁽¹³⁾. Moreover, the present result revealed frequency of allele C was more in healthy control compared to obesity patients. Also showed that (CC) genotype frequency in patients (20%) lower than in healthy controls (48.3%) and this results disagree with results Oana et al⁽²⁷⁾, which found that the G allele at *IL-6* as being more common in lean subjects and observed the C allele to be associated with indices of obesity.

Higher serum concentration of IL-6 may be due to its important role in pathophysiology of obesity. The adipose tissue is a major source of circulating IL-6, and the excessive secretion of IL-6 appears to be directly related to obesity⁽²⁸⁾. IL-6 also can influence adipocyte function, lipid metabolism and thus play a major role in the development of obesity⁽²⁴⁾. Other theory Saied IL-6 exerts strong effects on hormonal balance and may induce some endocrinological disturbances. It is suggested that IL-6 may affect the increase of free fatty acids level. IL-6 concentration is elevated in patients with lipid disorders and insulin resistance⁽²⁹⁾. The increased IL-6 levels in obese individuals may result in a state of insulin resistance and increased risk of cardiovascular complication⁽³⁰⁾. Our results agree with results of Ibrahim et al.,⁽¹³⁾, which found The mean serum level of IL-6 in obese children was significantly higher as compared to those of control children (7.7 ± 0.46 pg/mL and 5.46 ± 0.40 pg/mL respectively ($P=0.003$).

Table (4), showed significant association between serum IL-6 levels and *IL-6* Polymorphism in patients with obesity ($p < 0.001$), where we found higher median serum IL-6 levels in the GG genotype 149.86 (60.7) ng/L as compared with CG or CC genotype (122.87 (37.57)ng/L and 93.4 (10.41) ng/L respectively). The present study found higher promoter activity of the rs1800795 G-allele compared with the C-allele, where

CC homozygotes genotype had lower serum IL-6 levels when compared with G-allele carriers in both obesity patients and healthy controls. This suggests that rs1800795 G-allele carriers with higher levels of IL6 have a higher risk for obesity.

Conclusions

Our results confirm clinically relevant relationship of *IL-6* gene polymorphism with risk of obesity. Childhood Patients who undergone obesity showed a higher level of IL-6 than healthy control. In this study we found a significant correlation between *IL-6* genotype and IL-6 levels were higher level in subjects with GG genotype compared with those with CG or CC genotype.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Medical Microbiology and all experiments were carried out in accordance with approved guidelines.

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