Frequency of Distribution of Interleukin 6 Gene 174G/C Polymorphism in obese Egyptian Cohort

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Abstract: Elevated IL6 level is documented in obesity. 174G/C polymorphism in the promoter area of IL6 gene may alter its expression or its effect. Aim: Finding the frequency of IL-6 174G/C polymorphism, its sex distribution and its association with obesity and dyslipidemia. Subjects and methods: This study included 74 apparently healthy subjects (45 men and 29 women). BMI was calculated. Lipid profile was assessed by specific colorimetric assays. Serum IL6 level was measured by ELISA. IL6 genotyping was done by PCR-restriction fragment length polymorphism. Results: According to IL6 polymorphism, subjects were classified into 3 groups; CC, GC and GG. 71.42% of the GG group and 68.97% of the GC group were male. Similarly, 78.57% of the GG group and 72.41% of the GC group were obese. No significant difference as regarding sex or BMI was found in the CC group. No significant difference was found among the 3 groups as regarding IL6 level. According to BMI subjects were further classified into Obese (more than 25Kg/m^2) and Non obese (less than 25Kg/m^2) .IL6 and triglyceride levels were higher in the obese group while HDL cholesterol was higher in the non-obese group. Both GG and GC genotypes showed significant positive correlation of IL6 with BMI (P=0.024 & P=0.012 respectively). Moreover, the GC group showed a significant positive correlation between IL6 and LDL cholesterol (p=0.022). CC genotypes didn't show any correlation of IL6 with either BMI or any measure of the lipid profile. Conclusion: The G containing alleles, GG & GC carriers are prevalent in male Egyptians which make them more vulnerable to obesity and its deleterious outcome than CC carriers.

[Riham G.Mahfouz , Azza M. Abdu Allah, Seham A. Khodeer, Waleed F. Abd Elazeem, Mostafa Al Nagar and Walid A. Shehab-Eldin, Frequency of Distribution of Interleukin 6 Gene 174G/C Polymorphism in obese Egyptian Cohort. Journal of American Science 2011;7(8):788-794] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Interleukin 6, Polymorphism, Obesity, Dyslipidemia.

1. Introduction

There is no doubt that the prevalence of obesity is high and continually growing all over the world reaching to the level of an epidemic or a pandemic disease[1]. Facing the health hazards and consequences of obesity is a big challenge especially for the developing countries. To overcome this problem, understanding the pathogenesis of obesity is mandatory and is the main target of a lot of researches in the recent time.

Dealing with the adipose tissues is changed in the recent years from a big junk for fat to a highly biologically active organ. It releases huge amounts of hormones and inflammatory cytokines. Hence, The obese patient is in a state of continuous sub-clinical inflammation[2].

One of these mediators is interleukin 6 (IL6). It has been discovered for the first time as an acute phase reactant promoting inflammation[3]. On the other hand, it exerts anti-inflammatory action through stimulation of the hypothalamic-pituitary-adrenal axis[4]. In these acute conditions, the main source of IL6 is the immune cells[5]. Another important source of IL6 production is the adipose tissue where IL6 was the second molecule proved to be secreted by fat cells[6]. The pleotropic nature of IL6 gave it the very wide range of actions all over the body. It has endocrine functions such as growth hormone secretion, inhibition of thyroid-stimulating hormone secretion, and reduction of serum cholesterol. It also stimulates LDL receptor gene expression[7]. It is involved in the pathogenesis of rheumatoid arthritis and osteoporosis. Furthermore, it is secreted during stress, steroid withdrawal syndrome and in traumatic states associated with the inappropriate secretion of vasopressin. IL6 may contribute to illness during aging and chronic stress[8].

In the last decade, several studies showed the elevated IL6 in obese subjects. However, these studies failed to explain the relationship between this peculiar molecule and its source i.e. the adipose tissue[9-11]. Furthermore, IL6 deficient mice develop obesity and its weight is reduced after IL6 adminstration[12]. Up till now, no explanation links these conflicting data together.

The IL6 gene consists of 5 exons and 4 introns located on the short arm of chromosome 7[13]. A genetic polymorphism is discovered at the 174

nucleotides upstream of the major transcription initiation site of the IL6 gene. According to the presence of either guanine or cytosine at this position two different IL6 alleles exist. These two different alleles give rise to three possible IL6 genotypes: GG, GC, and CC[14].

Previous studies on G (-174) C polymorphism proved that the expression and effects of IL6 differ according to the genotype polymorphism. For example; IL6 concentrations were lower in patients with systemic onset juvenile chronic arthritis who had the CC genotype[14]. In addition; plasma IL6 concentrations were higher in patients with abdominal aortic aneurysm who had only the CC genotype[15]. Other studies have indicated that the G (−174) C IL6 genotype does not affect plasma IL6 effect[16].

This study aimed to evaluate the frequency of IL6 gene polymorphism according to BMI and sex of the subject and to find out if certain IL6 polymorphism is related to obesity or lipid profile in these subjects.

Subjects and Methods

Subjects:

The study group included 74 subjects; 45 men and 29 women aged 21-68 years. Written informed consent was obtained from all subjects during the enrollment. The subjects were volunteer relatives to patients admitted to the internal medicine department, Menoufya University hospital. Subjects with acute inflammation, pregnancy, hypertension, Ischemic heart disease or diabetes mellitus were excluded from the study.

All studied subjects were submitted to full history taking, clinical examination and laboratory investigations.

Sampling:

Under complete aseptic conditions, 5 ml of venous blood were collected after 12 hour fasting & divided into two tubes as follows: Tube A, 1ml of blood collected in citrate (to prevent clotting and DNA degradation) for DNA extraction and kept immediately at -20 C°. Tube B, 4 ml of collected blood, left to clot serum was separated and used for immediate assay of lipid profile .The rest of the serum was kept at -20 C° for assay of IL-6.

Laboratory Methods:

Total cholesterol, triglyceride, and HDL-C concentrations were determined by using an enzymatic colorimetric assay on Synchron Cx9. LDL-c concentration was calculated according to the Friedewald equation (Friedewald W.T. et al., 1972).Serum interleukin concentration was measured by Kit was supplied by AviBion Human IL-6 ELISA Kit (Orgenium Laboratories). Intra-Assay-Precision of this test was <9.4%, and sensitivity was <2 pg/ml.

DNA analysis:

PCR-RFLP method was used to determine the distribution of genotype frequencies of the IL-6 C174G of the interleukin-6 gene. The DNA was isolated and purified by genomic DNA purification kit (GeneJET™ Genomic DNA Purification Kit, Fermentas International Inc., Canada). The PCR was performed on 10 ug DNA in 20 uL sterile D.W (2uL vol contain 1 ug DNA be added to the master mix),10x dream buffer, 0.2 mL dNTP, 200 U Taq polymerase and 0.1 µM of each primer (Fermentas International Inc., Canada), Interleukin 6 (IL-6 C174G) forward and reverse primers (Fermentas International Inc., Canada) as follows: for. 5'-TGA CTT CAG CTT TAC TCT TTG T-3'and rev. 5'-CTG ATT GGAAAC CTT ATT AAG-3', for 35 cycles (30 s at 94 C°, 30 s at 60 C°, 30 s at 72 C°). Each PCR cycle consisted of denaturation for 60 seconds at 94°C, annealing for 95 seconds at 55°C, and extension for 60 seconds at 72°C; followed by a final extension at 72°C for nine minutes. PCR products were digested using the restriction enzyme ***Sfa***NI (Fermentas International Inc., Canada). The digested samples were separated by electrophoresis on 3% agarose gel stained with ethidium bromide and visualized on a UV trans-illuminator. The presence of a single 198 bp band corresponds to the CC genotype; bands at 140 and 58 bp correspond to the GG genotype; and the presence of three bands corresponds to the GC genotype (Fig. 1).

Statistical analysis:

Frequency of distribution analysis was performed with a Chi2 square test. The significance level was set at 0.05 or less. All data analysis was performed using SPSS 11.0 software.

3. Results

According to the polymorphism; subjects were divided into 3 groups; CC, GC and GG. According to BMI subjects were further divided into obese group (more than 25Kg/m^2) and non-obese group less than 25Kg/m^2.

Frequency of distribution of IL6 gene polymorphism:

According to the sex, both GC and GG polymorphism showed a higher male predominance with a high statistical significance of 0.041 and 0.023 respectively. On the other hand, no significant difference was found between male and female in the CC group (Fig. 2).

According to BMI, a statistically significant higher number of obese subjects were found in both the GC (69%) and the GG (71.43%) groups. On the other hand, no significant difference was found between obese and non-obese subjects in the CC group (Fig. 3).

Comparison between all types of polymorphism:

No significant difference was found between the three groups as regarding age, BMI, IL6 level or the lipid profile (Table 1).

Correlations between serum IL-6 levels and both basal measures and biochemical results:

Pearson correlation analysis was done in each group separately. In the CC group IL6 levels didn't show any correlation with BMI or any measure of the lipid profile. Both GC and GG showed correlation of IL6 with BMI (Figure 4). Furthermore, in the GC group, IL6 correlated also with LDL cholesterol (Figure 5). None of the three groups showed correlation of IL6 with total cholesterol, HDL cholesterol or triglycerides (Table 2).

Comparison between the obese and non obese groups:

Both the obese and non obese groups were similar as regarding age, sex, total cholesterol and LDL cholesterol. IL6 and triglycerides levels were significantly higher while HDL cholesterol was significantly lower in the obese group (Table 3).

Table 1: Comparison between all types of polymorphism of the whole population.

P1: the difference between CC & GC P2: the difference between CC & GG P3: the difference between GC& GG

Parameter	Polymorphism			P Value		
	CC (N=17)	GC (N=29)	GG (N=28)	P1	P2	P3
Age (years)	38.47 ±	49.00 ± 13.72	30.01 ± 5.82	0.366	0.978	0.257
Sex (M/F)	5/12	20/9	20/8			
BMI (Kg/ m^2)	28.38 ± 5.15	30.24 ± 5.87	30.01±5.8	0.709	0.781	0.923
IL6(pg/ml)	2.12 ± 0.83	2.1 ± 0.95	2.11 ± 0.91	0.367	0.520	0.756
Total Cholesterol(mg/dl)	215 ± 41	240 ± 25	226.1 ± 35.43	0.133	0.619	0.300
LDL-C(mg/dl)	130 ± 26.55	138 ± 26.28	145.71 ± 30.41	0.796	0.572	0.360
HDL-C(mg/dl)	58.76 ± 22.6	51.17 ± 24.73	45.79±17.85	0.976	0.057	0.078
Triglycerides(mg/dl)	160 ± 55	164 ± 58	176.5 ± 60.42	0.453	0.554	0.906

Table 2: Correlations between serum IL-6 levels and both basal measures and biochemical results.

Parameter	**Interleukin 6 level**					
	CC		**GC**		**GG**	
	R	**P value**	**R**	**P value**	**R**	**P value**
BMI (Kg/ m^2)	0.314	0.219	**0.460**	**0.012**	**0.424**	**0.024**
LDL-C(mg/dl)	0.214	0.410	**0.424**	**0.022**	0.253	0.194
Total Cholesterol(mg/dl)	- 0.102	0.698	0.026	0.892	0.017	0.931
HDL-C(mg/dl)	- 0.161	0.517	-0.303	0.110	0.033	0.868
Triglycerides(mg/dl)	- 0.077	0.770	0.214	0.265	0.174	0.375

Table (3): Comparison between obese and non obese patients in the whole group

Parameter	Non obese (No=20) Mean ± SD	Obese (No=54) Mean ± SD	P Value
Age (years)	46.7 ± 15	44.72 ± 13	0.251
Sex (M/F)	34/20	11/9	0.063
BMI (Kg/ m^2)	22.3 ± 1.17	32.47 ± 3.89	0.000
IL-6 (pg/ml)	1.16 ± 0.87	2.28 ± 0.86	0.041
Total Cholesterol	217 ± 27	233 ± 35	0.637
LDLc(mg/dl)	107 ± 15	151 ± 26	0.097
HDLc(mg/dl)	74.75 ± 22	42 ± 14	0.006
Triglycerides (mg/dl)	89 ± 52	197 ± 21	0.000

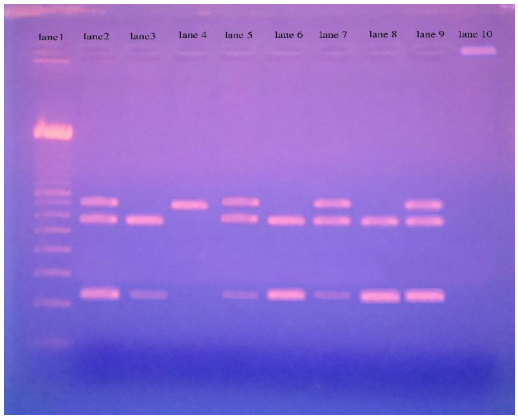

Figure (1): The three genotypes identified: lane 1 correspond to DNA (GeneRoler™ low range 25-700 bp) ladder; lane 2, 5 ,7 and 9 correspond to GC genotype (58,140 and 198 bp); lane 3,6 and 8 correspond to GG genotype (58 and 140 bp) and lane 4 correspond to CC genotype (198 bp).

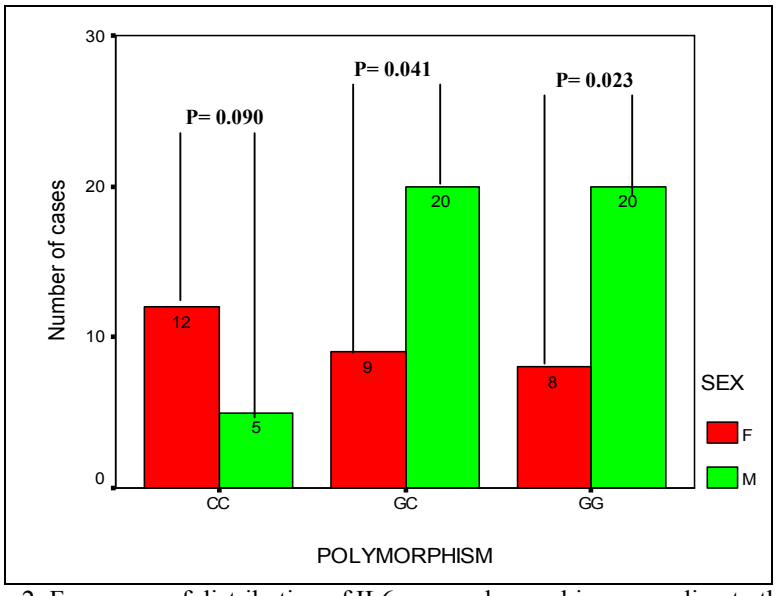

Figure 2: Frequency of distribution of IL6 gene polymorphism according to the sex

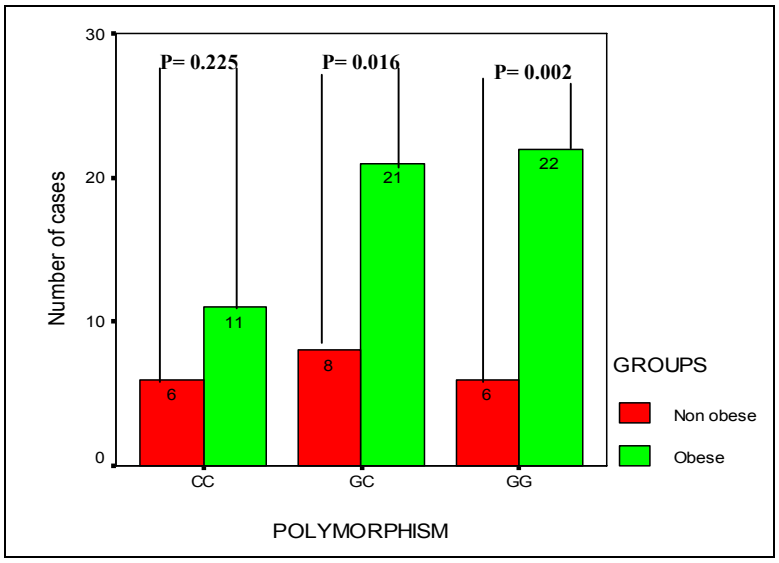

Figure 3: Frequency of distribution of IL6 gene polymorphism according to body weight

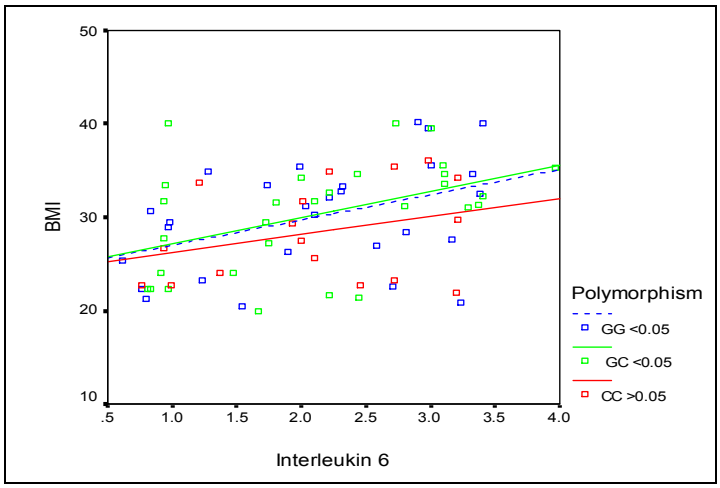

Figure 4: correlation between IL6 and Body Mass Index in different groups according to IL6 gene polymorphism.

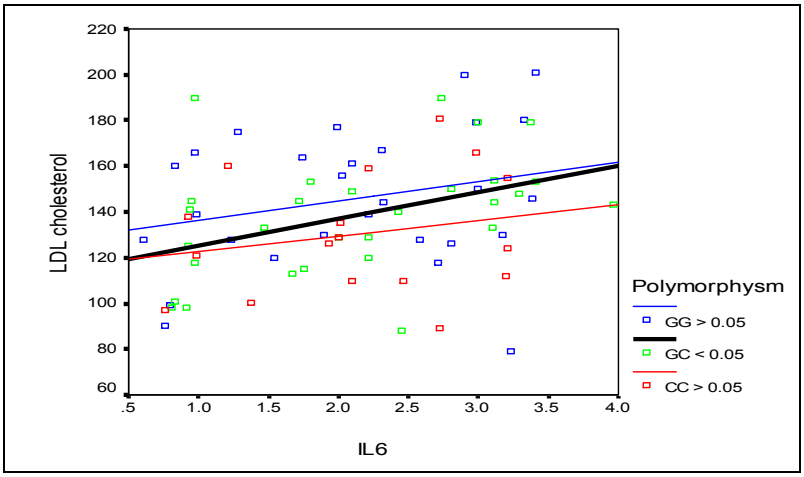

Figure 5: correlation between IL6 and LDL cholesterol in different groups according to IL6 gene polymorphism.

4. Discussion

The present work is a cross-sectional study on a group of apparently normal subjects. Its main purpose is to show the frequency of distribution of IL6 gene polymorphism according to the sex and its subsequent effect on obesity and lipid profile. In the GC and GG groups more than two thirds of the patients were male while there was no significant sex difference in the CC group. Interestingly, IL6 level correlate with BMI in the same two groups and not the CC group. Hence IL-6 (-174G>C) gene polymorphism; concerning GC and GG genotypes may be linked with male sex and obesity. This association was noted 3 years ago in elderly men by Strandberg et al., [17].

A striking finding, in this work, is the non significant difference in the distribution of the CC polymorphism between obese and non obese persons. On the other hand the GC and GG polymorphism showed a significantly higher distribution in obese subjects. 72.4% of the GC group and 78.57% of the GG group were obese.

With subgroup analysis, in the CC group, IL6 did not show any correlation with BMI or any measure of the lipid profile. On the other hand, in the GG group, IL6 showed a significant positive correlation with the BMI but not with the other measures of lipid profile. In the GC group, IL6 showed a significant positive correlation with both BMI and LDL cholesterol. This is in agreement with Fernandez-Real et al. who proved lipid abnormalities in subjects with the G allele [18]. Berthier et al., documented the correlation between IL6 and measures of obesity in patients with GC polymorphism[19]. The same results were confirmed in the EPIC-Potsdam study on 334 obese subjects[20]. However, Kubaszek et al., reached to a different result and linked the CC genotype with obesity and reduced energy expenditure [21].

The deleterious effect of the G allele was noticed in several studies. Bennermo et al., linked the GC polymorphism with inflammation and speculated its central role in the development of several diseases [22]. In the WOSCOPS study on Scottish patients, the G allele carriers had a higher risk for coronary artery disease than the C carriers[23]. Kocierz et al., in another recent study noticed that kidney transplant failure was associated with the GG and GC genotypes compared with the CC genotype [24]. Kayaalti et al., documented the advantageous effect of the C allele on the life span of Turkish people over the G allele [25]. Rheumatoid arthritis patients with the CC genotyping are resistant to treatment with Rituximab, an anti CD20 selective anti-inflammatory drug, confirming the absence of association between the C allele and inflammation [26].

Nevertheless, several studies failed to reach to a significant association between IL6 polymorphism and coronary artery disease [27] or diabetes mellitus [28].

Although there was no significant difference among all genotypes as regarding the IL6 level, there was association of the G allele only with obesity and elevated LDL cholesterol. This may refer to that the effect of gene polymorphism may be through its effect on other pathways rather than IL6 level itself. This is in agreement with Henningsson et al., who documented the absence of the association of IL6 genotyping and its plasma level [29]. Okada et al., proved the pivotal role of an IL6 locus in the regulation of serum CRP levels and inflammatory pathways [30]. Cardellini et al., showed that patients with GG genotype of the IL-6 gene have reduced insulin sensitivity which is a risk factor for a lot of deleterious vascular disorders [31].

Similar to the present result, different studies have documented IL6 elevation in obese subjects [32-34]. These studies, however, failed to reach to a consistent relation between IL6 and obesity. One decade ago, Roytblat and his co-workers tried to explain these opposing results. They suggested the complex etiology of obesity, diet and physical activity levels as factors affecting the relationship between IL6 and obesity. Moreover, this relationship may be additionally influenced by environmental, socioeconomic, and behavioral factors[32]. IL6 gene polymorphism may be a hidden factor at that time that may partially help to identify the heterogeneous results in the studies.

Conclusions:

IL6 is elevated in obese persons. G containing alleles are related to obesity and may be to its deleterious outcome while the CC genotype is not related to obesity. The effect of 174G/C polymorphism on obesity is not through its effect on serum IL6 level. Therefore 174G/C polymorphism in any future study on obese subjects should be considered. Dyslipidemia could be a mechanism promoting the deleterious effect of IL6. Other mechanisms, however, should be searched for.

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