Insulin-like growth factor-I gene polymorphisms and left ventricular function in Turkish obese women with insulin resistance

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Insulin-like growth factor I (IGF-I) is a peptide which is involved in the regulation of glucose hemostasis, stimulates bone growth, cell differentiation, and metabolism. Obesity may cause structural and functional changes in the heart even in the absence of hypertension and organic heart disease. The IGF-I may play an important role in the development of cardiovascular diseases.[1] Due to the interference of IGF-binding proteins, measuring circulating IGF-I levels may be misleading in reflecting the IGF-I bioactivity in diseased conditions. The polymorphism in the promoter region of the IGF-I gene may directly influence the expression of IGF-I. This polymorphism can be used to determine subjects with a genetic predisposition toward chronic low exposure of IGF-I in the cardiovascular system. Studying this polymorphism in relation to obesity and subclinical cardiac involvement may better reflect the effects of long term IGF-I exposure on cardiovascular system.

We have recently demonstrated that obesity is associated with low IGF-I levels, and the most frequent IGF-I gene polymorphism allele is longer than 194 base pair (bp) in both obese insulin resistant patients and controls, and the cause of lower IGF-I levels in obese patients may not be the IGF-I gene polymorphism and it may be insulin resistance (IR).[2] Tissue Doppler imaging has enhanced our capacity to identify early abnormalities in systolic and diastolic ventricular function. The objective of the study was to investigate the association between the IGF-I promoter polymorphism, and the occurrence of left ventricular subclinical involvement on echocardiogram of obese women with IR.

The study was carried out prospectively at the Cardiology, Endocrinology, and Medicine Outpatient Clinics of Pamukkale University, Denizli, Turkey from March to July 2009. The study was approved by the institution's Medical Ethics Review Committee. The study was conducted according to the principles of Helsinki Declaration. Patients were included in the study if they were obese women with IR. A total of 64 patients were included in the present study (34 obese women with IR, mean age 45.6 ± 11.9 and 30 age-matched healthy women). Individuals with smoking history, ethanol consumption (more than 60 g, 0.5 liters wine/d), diabetes mellitus, acromegaly, growth hormone deficiency, cardiac and/or ischemic cerebrovascular disease, thyroid dysfunction, hepatic insufficiency, impaired renal function, malnutrition, and other major pathologies were excluded. None of the study subjects were taking any medications, which might influence glucose, insulin metabolism, or secretion. Anthropometric measurements were obtained, such as body mass index (BMI), waist circumference, and waist-to-hip ratio (WHR)). A BMI >30 kg/m^2 was regarded as obesity. Routine biochemical values, insulin levels, thyroid function tests, IGF-1, GH (growth hormone), and IGFBP-3 (IGF binding protein) levels were examined. Polymorphisms in the promotor region of the IGF-1 gene consist of a highly polymorphic microsatellite composed of variable cytosine adenosine (CA) repeats. The number of CA repeats ranges between 10 and 24, with the most common allele containing 19 CA repeats (192 bp allele). Genomic DNA from the study subjects and controls were isolated. Interested genomic areas were studied using specific primers by polymerase chain reaction methods. Separation of amplified fragments was carried out by agarose gel electrophoresis. Amplified fragments were identified by using the ultraviolet gel documentation system. Genotyping of the 192 bp IGF-I promoter polymorphism resulted in 3 possible genotypes: shorter than192 bp allele, 192-194 bp allele, and longer than 194 bp. Standard and pulsed Doppler tissue echocardiograms were obtained in all participants using a Vivid 7 ultrasound machine (GE Vingmed, Milwaukee, Wisconsin, USA) with a 2.5-MHz phased array probe.

Values are expressed as mean ± standard deviation. Data were analyzed using Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, USA). Comparisons between obese women with IR and controls were performed by Mann-Whitney U test. Multiple genotype groups were analyzed by Kruskal-Wallis test. A $p<0.05$ was considered significant.

The 2 groups had similar mean ages however, the BMI, waist circumferences, fasting plasma glucose, fasting insulin, homeostatic model assessment (HOMA)-IR were markedly increased in obese women with IR as expected ($p=0.000$). The IGF and GH were markedly decreased in obese women with IR ($p=0.005$). Sixty six percent of obese women, and 93.5% of controls had longer than 194 bp IGF-I promoter polymorphism. Heart rate, left ventricle (LV) end-diastolic diameter, LV end-systolic diameter, interventricular septum, LV ejection fraction, the peak annular velocity during atrial contraction from the lateral wall, the peak annular

velocities during early diastole from the septum and the anterior wall, the ratios of mitral early (E) diastolic velocity to the peak annular velocities during early diastole from the lateral wall, the septum, the inferior wall, the anterior wall were similar in both groups. Posterior wall (PW) was thicker, mitral early (E) diastolic velocity was decreased, and mitral late (A) diastolic velocity was increased, their ratio (MVE/MVA) was lower, the peak annular velocities during early diastole from the lateral, and inferior walls were decreased, the peak annular velocities during atrial contraction from the septum, the inferior, and the anterior walls were increased, the ratios of annular velocities from the 4 walls were lower in obese women with IR. The left ventricular characteristics of obese women with IR when classified according to the IGF-I promoter polymorphism genotype are listed in **Table 1**. The shorter than 192 bp group consisted of 5 women; the 192-194 bp group, 4 women; and the >194 bp group, 25 women. These small sample sized groups obviously reduced the statistical power of the present study. However, heart rate, cavity dimensions, wall thicknesses, ejection fraction, E and late diastolic (A) velocities, mitral annulus early (Em) and late diastolic (Am) tissue Doppler velocities were found to be similar in all genotype groups (**Table 1**). The novel and important finding of the present study is that there is no association between IGF-I polymorphisms and echocardiographic measures of the left ventricle in obese women with IR. In addition, obese women with IR exhibited normal LV systolic function but an increased posterior wall thickness, decreased mitral early diastolic velocity, decreased peak mitral annular velocities during early diastole from the lateral and inferior walls. Our findings suggest that obese women with IR may have impaired LV relaxation and increased wall thickness, which are consistent with a previous study.[3]

An association between the IGF-I promoter polymorphism and left ventricular hypertrophy (LVH) on echocardiogram has been reported. Non-carriers of a 192 bp allele tend to develop LVH. This might be due to low levels of IGF-I resulting in faulty remodeling.[4] The present study investigated the association between the promoter region polymorphism of the IGF-I gene and subclinical cardiac involvement on echocardiogram in obese women with IR. Increased posterior wall thickness of the LV and impaired left ventricular relaxation in the obese subjects are not related to the IGF-I gene polymorphism in this study. Rather they may be related to low levels of IGF-I which might result from IR.[2] Diastolic annular Doppler tissue imaging patterns can be used as a marker of abnormal relaxation. Abnormal left ventricular relaxation can be identified by Doppler analysis of mitral inflow velocities, with a characteristic pattern of decreased peak early inflow velocity and prolonged early velocity deceleration. The early diastolic mitral Doppler tissue imaging velocity has also been found to be significantly lower in patients with abnormal relaxation compared to controls. Impaired LV relaxation observed in the obese subjects can either be due to obesity itself, or LV hypertrophy associated with it, or both.

Recent studies have showed an association between a 192 bp polymorphism of the IGF-I gene, and the risk of developing cardiovascular diseases. Measuring circulating IGF-I levels may be misleading in reflecting the IGF-I bioactivity. A polymorphism in the promoter region of the IGF-I gene is associated with serum IGF-I levels,[5] which may directly influence the expression of IGF-I, and can be used to determine subjects with a genetic predisposition toward chronic low exposure of IGF-I in cardiovascular system. Low serum levels of IGF-I have been shown to be associated with an increased risk of heart failure in elderly participants in the Framingham heart study, and Rotterdam study, and an increased risk for type 2 diabetes and myocardial infarction. Although these observations are not confirmatory, low levels of IGF-I levels may play a role in the pathogenesis of these diseases. In addition, we have recently reported that obesity is associated with low IGF-I levels which are not related to the IGF-I gene polymorphism.[2] The IGF-I counteracts apoptosis of cardiomyocytes and improves myocardial function. Since the majority of both groups in the present study had longer than 194 bp genotype, we think that the mechanism of the increased wall thickness could be consequent upon relative IGF-I deficiency leading to faulty remodeling, an increased intravascular volume, and possible disruption of leptin signaling in obesity.[4] Furthermore, we speculate that low IGF-I levels could play a role in the pathogenesis of impaired LV relaxation in the subjects. However, this can not be supported by IGF-I polymorphisms in the study, at least in Turkish subjects.

In conclusion, there is no association between IGF-I polymorphisms and echocardiographic measures of the left ventricle in obese Turkish women with IR. Obese women with IR have normal LV systolic function, but increased posterior wall thickness of the LV. Furthermore, obese women have impaired LV relaxation. Limitations of this study include the absence of measurements of invasive cardiac filling pressures, and small sample sized groups with reduced statistical power. However, the present measurements are comprehensive and performed on a careful selection of subjects; ensuring co-morbidities that frequently accompany obesity

Table 1 - The left ventricular echocardiographic parameters of the obese women with insulin resistance when classified according to insulin-like growth factor-I promoter polymorphism genotype.

Parameters	Group 1 <192 bp allele n=5	Group 2 192-194 bp allele n=4	Group 3 >194 bp allele n=25	*P*-value
Heart rate (beats/min)	76.60 ± 15.19	69.00 ± 10.73	77.36 ± 11.33	NS
Left ventricle (LD) end-diastolic diameter (mm)	48.00 ± 6.44	42.50 ± 3.69	44.36 ± 4.43	NS
LV end-systolic diameter (mm)	31.60 ± 4.39	28.75 ± 3.77	28.08 ± 3.44	NS
Interventricular septum (mm)	9.60 ± 0.55	10.50 ± 0.58	9.56 ± 1.80	NS
Posterior wall (mm)	8.20 ± 0.45	10.75 ± 0.50	9.28 ± 1.92	NS
LV ejection fraction (%, Simpson's)	61.80 ± 4.44	63.75 ± 4.50	64.32 ± 4.77	NS
Peak early mitral inflow velocity wave (MVE), m/sec	0.84 ± 0.26	0.71 ± 0.19	0.85 ± 0.20	NS
Peak late mitral inflow velocity wave (MVA), m/sec	0.67 ± 0.09	0.59 ± 0.15	0.71 ± 0.23	NS
MVE to MVA ratio	1.26 ± 0.44	1.30 ± 0.66	1.35 ± 0.60	NS
Tissue doppler echocardiographic variables				
Lat Em (cm/sec)	0.34 ± 0.47	0.11 ± 0.03	0.14 ± 0.04	NS
Lat Am (cm/sec)	0.13 ± 0.05	0.09 ± 0.01	0.10 ± 0.03	NS
Lat Em to Am ratio	1.30 ± 0.56	1.19 ± 0.52	1.42 ± 0.83	NS
Sep Em (cm/sec)	0.10 ± 0.03	0.26 ± 0.35	0.11 ± 0.03	NS
Sep Am (cm/sec)	0.27 ± 0.35	0.27 ± 0.35	0.10 ± 0.03	NS
Sep Em to Am ratio	1.05 ± 0.50	0.86 ± 0.10	1.26 ± 0.70	NS
Inf Em (cm/sec)	0.13 ± 0.04	0.12 ± 0.00	0.13 ± 0.03	NS
Inf Am (cm/sec)	0.10 ± 0.03	0.09 ± 0.00	0.15 ± 0.20	NS
Inf Em to Am ratio	1.35 ± 0.62	1.29 ± 0.08	1.28 ± 0.87	NS
Ant Em (cm/sec)	0.12 ± 0.03	0.09 ± 0.01	0.10 ± 0.03	NS
Ant Am (cm/sec)	0.12 ± 0.05	0.09 ± 0.03	0.09 ± 0.05	NS
Ant Em to Am ratio	1.17 ± 0.51	1.12 ± 0.28	1.28 ± 0.83	NS
MVE to Lat Em ratio	4.71 ± 2.62	6.83 ± 1.75	6.63 ± 2.08	NS
MVE to Sep Em ratio	8.19 ± 1.31	5.98 ± 3.25	8.43 ± 3.32	NS
MVE to Inf Em ratio	6.41 ± 1.32	6.04 ± 1.93	6.89 ± 2.03	NS
MVE to Ant Em ratio	7.10 ± 0.81	7.70 ± 2.50	8.92 ± 2.96	NS

Lat Em - the peak annular velocity during early diastole from the lateral wall, Lat Am - the peak annular velocity during atrial contraction from the lateral wall, Sep Em - from the septum, Sep Am - from the septum, Inf Em - from the inferior wall, Ant Em - from the anterior wall, Inf Am - from the inferior wall, Ant Am - from the anterior wall. A $p<0.05$ was considered significant. Data are expressed as mean±SD

do not affect our findings. Our study did not include morbidly obese patients. The present study is hypothesis-generating, and further research with a larger number of patients is warranted to confirm or dispute our findings. If an association between low IGF-I levels and IGF-I polymorphisms is found in further studies, some obese individuals with a specific IGF-I polymorphism may benefit from more aggressive therapeutic approach before manifesting cardiovascular disease.

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