Association between Angiotensin converting enzyme (ACE) polymorphisms and obesity in Palestinian individuals living at Gaza City.

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Abstract: In the present study Angiotensin converting enzyme gene was genotyped in 102 adult normal and obese individuals from both sexes living at Gaza city. Moreover, some biochemical parameters: Kidney functions (urea, creatinine, uric acid); liver functions (AST, ALT); lipid profile (cholesterol; triglycerides and high density lipoproteins, low density lipoproteins and very low density lipoproteins); glucose (fast blood sugar) were also studied to confirm that the obese individuals were free of other diseases. Blood samples were collected on vacutainer tubes supported with anticoagulant and each sample was divided to two equal parts, the first used for biochemical analysis while the second used for DNA isolation for gene typing. The PCR was performed with specific primer set for the gene understudy. The results showed the presence of two alleles I (insertion), D (deletion) which gives three genotypes of the gene II, ID and DD in the Palestinian individuals, the genotype DD was the more frequent genotype observed. It is concluded that no clear association was observed between the three ACE genotypes and the incidence of obesity in adult Palestinians living at Gaza city.
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1.Introduction:

The WHO has announced that obesity has reached epidemic proportions globally, in 2005 around 33% of the world's adult populations (1.3 billion people) were rated to be overweight, their body mass index (BMI≥25) or obese (BMI≥30) (Kelly *et al.,* 2008).

Overweight and obesity are generally defined as abnormal or an excess of body fat accumulation that presents a risk to health (Haslam and James, 2005). It is well known that the essential cause of obesity and overweight is an energy imbalance between calories consumed on the one hand and calories spend on the other hand (WHO, 2013). The WHO (2004) approved two parameters for measuring the degree of obesity: body mass index (BMI) and waist circumference. Accordingly obesity is classified as: overweight (Pre-obese); then three classes of obesity (I; II and III). Body Mass Index (BMI) is a statistical measure of body size based on an individual's weight and height values. It is calculated by dividing weight by height squared and apprise in units of kg/m^2 (Spruijt-Metz, 2011).

Overweight and obesity are main risk factors for a number of chronic diseases, inclusive diabetes, cardiovascular diseases and cancer (Mokdad *et al.,* 2003). Obesity also participates to shorter lifespan, depression and decreased quality of life (Roth *et al.,* 2004; Roos *et al.,* 2007). Overweight and obesity nowadays have dramatically on the rise in low- and middle-income countries. The essential cause of obesity and overweight is a lack of energy balance between calories consumed and that expended.

Increases in overweight and obesity are due to a global shift in diet across increased intake of energy-dense foods and a tendency towards decreased physical activity (Racette *et al.,* 2003).

Palestinians are not an exception country regarding obesity. In a study conducted by Abdul-Rahim *et al*. (2001) in the rural and urban Palestinian population, it is found that 49.1% of women and 30.6% of men to be obese in urban Palestinian, a positive association with urban residence in women was noticed. In another study performed on Palestinians at rural West Bank found that 37% obesity levels for women and 18% amongst men (Al-Rifai and Roudi-Fahimi, 2006). Moreover Abdeen *et al.* (2012) found that the prevalence of overweight in Palestinians were 35.5% in women and 40.3 % in men.

Angiotensin I converting enzyme (ACE) gene is one of the most intensely studied genes since it is one of the key role genes which plays an important role in the rennin-angiotensin system (RAS). The gene encoding ACE is located on the long arm of chromosome 17(17q23.3), it is 21 kilo bases (kb) long

and consists of 26 exons and 25 introns. This gene encodes both ACE isoforms, but has two different promoters resulting in different mRNAs (Hubert *et al.,* 1991). There is a genetic variation within the ACE gene, The insertion/ deletion (I/D) polymorphism in the gene refers to an Alu repetitive sequence 287 bp long, in intron 16 on chromosome 17, resulting in three different ACE genotypes: D/D and I/I homozygotes and I/D heterozygotes (Rigat *et al.,* 1992).

Although the polymorphism is located in an intron, it is theoretically will not affect the structure of the enzyme; however the polymorphism is strongly connected to the level of ACE in plasma, where I/I, I/D and D/D genotypes, have low, medium and high levels of enzyme secretion respectively (Rigat *et al.,* 1990; Tiret *et al.,* 1992). In addition, the expression of ACE in T-lymphocytes (Costerousse *et al.,* 1993) and in human cardiac tissue (Danser *et al.,* 1995) is also influenced by the ACE I/D polymorphism, suggesting that tissue ACE and circulating ACE are under similar genetic control (Montgomery *et al.,* 2002). Furthermore, increased conversion of angiotensin I to angiotensin II have been reported in carriers of the D/D genotype compared to I/I (Ueda *et al.,* 1995). It is noticed that the genotype frequencies for ACE are different in different ethnic groups, for example the frequency of the D/D genotype in Caucasian and African American populations varies from 25% to 30%, whereas in Asian populations less than 20% carry the D/D genotype (Mathew *et al.,* 2001; Sagnella *et al.,* 1999; Tamaki *et al.,* 2002).

ACE claimed by many authors to have a link to obesity and related complications like diabetes mellitus, hypertension, cardiovascular disorders and dyslipidemia (Hashimoto *et al.,* 2001;Riera-Fortuny *et al*., 2005; Bitigen *et al*., 2007; Muthumala *et al.,* 2007 and Tseng *et al.,* 2007)

The aim of the present study is identify the genetic polymorphisms for Angiotensin converting enzyme (ACE) in adult Palestinians live in Gaza city, the correlation of this gene polymorphisms with some biochemical parameters: Kidney functions (urea, creatinine, uric acid); liver functions (AST, ALT); lipid profile (cholesterol; triglycerides and high density lipoproteins, low density lipoproteins and very low density lipoproteins); glucose (fast blood sugar) and the incidence of obesity as well as the degree of obesity.

2.Materials and Methods:

2.1.Materials:

2.1.1.Subjects:

The target populations of the present work were two groups:

Group I (Tested group): from Gaza City, Gaza Governorate, Palestine. They were overweight or obese individuals was selected. They were free from any complications that might be related to obesity(hypertension, diabetes, heart disease). This group was consisted from 77 persons; 36 male and 41 female

Group II(Control group):A group of apparently healthy subjects having normal BMI and waist circumference comprised of 25 persons; 11 male and 14 female (table: 1).

Table 1: Characteristics of volunteers in the two groups participated in the study:

Group	Gender	Age(Mean ± SD)	BMI(Mean ± SD)
Healthy individuals	Male = 11	25.91±4.28	21.93±2.27
	Females=14	24.93±6.83	21.62±1.75
Overweight/obese individuals	Male = 36	34.56±9.96	37.61±4.93
	Females=41	34.95±9.92	41.82±9.82

2.1.2. Samples:

Totally 102 venous blood samples were withdrawn from antecubital vein (8 ml each) from normal and obese persons. The blood samples were collected in the morning after 14–16 hours fasting. Each blood sample was distributed equally into two vacutainer tubes; the first contains K_3-EDTA (used for DNA isolation) while the second tube contained clot activator and used for biochemical analysis. After sampling the tubes were inverted gently many times, transferred to the laboratory for the analysis. The tubes containing clot activator were used immediately for biochemical analysis, while the other tubes containing K_3-EDTA were stored at (-20 ± 2^{o}C) freezer until DNA extraction and genetic analysis.

2.1.3.Kits for biochemical analysis:

Kits for biochemical analysis were purchased from Globe Diagnostic, Milan, Italy and the procedures were done as manufacture instructions.

2.1.4. Kits for DNA Extraction:

QIAamp® DNA Blood Mini Kit, The extractions were done using manufacture instructions and by the aid of QIAcube apparatus (Qiagen Company, Germany).

2.1.5.Primers:

The primers used were:

5'-CTGGAGACCACTCCCATCCTTTCT- 3'

as forward primer and

5'-GATGTGGCCATCACATTCGTCAGAT- 3'

as reverse primer

2.2.Methods:

2.2.1.Biochemical analysis :

Each sample were subjected to full chemistry analysis including the following tests :

-Fast blood sugar,

-Kidney function tests (urea, creatinine, uric acid), ----Liver function tests (AST, ALT),

-Lipid profile (cholesterol, triglyceride, high density lipoprotein).

2.2.2. DNA analysis:

The concentration of the DNA was measured using Thermo Scientific NanoDrop™ 2000 Spectrophotometer instrument at 260 nm wavelength. The ACE I/D polymorphism was detected by PCR as described by Batzer *et al.,* (1996). Briefly, amplification was carried out in a final volume of 20 ml containing 40 ng genomic DNA, 1 mM of each primer, 200 mM dNTPs, 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris- HCl (pH 8.4) and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, USA). Amplification resulted in a combination of a 490-bp product and/or a 190-bp product depending on the presence or absence of the ACE I-allele fragment, respectively. PCR were done by using the T professional thermocycler (Biometra, Germany). The PCR program was: Primary denaturation: 94°C for 5 min. then: 35 cycles as: 94°C for 1 min.; 58-60°C for 1 min.; 72°C for 3 min, final extension: 72°C for 5 min., Storage at 4°C forever. Since the ACE gene alleles depend only on insertion of a 300 bp DNA fragment, so after the end of the PCR cycle, the PCR products were characterized directly on QIAxcel instrument (Qiagen company, Germany) for band size determination. Statistical analysis was carried out using two separate software programs: The SAS System for Windows, release 9.3 (SAS Institute, Inc., 2011); the Statistical Package for Social Sciences (SPSS) version 21 for Windows (IBM Corp., 2012).

3. Results:

3.1.Biochemical analysis:

The laboratory investigations for 11 male, 14 female with normal BMI (< 25) were carried out. The data of BMI, fasting blood sugar (FBS), urea, creatinine and uric acid are presented in table 2.

Table (2): Results of fasting blood surge and kidney functions test of the normal BMI investigated individual

Gender/no. of cases	11 Male		Normal range	14 Female		Normal range
	Range	Mean ± SD		Range	Mean ± SD	
BMI	18.53-24.98	21.93± 2.27	18.50-24.99	18.44-24.45	21.62±1.75	18.50-24.99
FBS (mg/dl)	71- 106	88.72±11.48	70-115	70- 94	80.57± 5.86	70- 115
Urea (mg/dl)	20- 39	30.00± 5.81	18- 53	21- 40	29.07± 5.95	18- 53
Creatinine (mg/dl)	0.69- 0.97	0.80± 0.097	0.60-1.30	0.60- 0.80	0.65± 0.053	0.50- 1.2
Uric acid (mg/dl)	3.2- 5.8	3.96± 0.81	3.6-8.2	3.6- 5.7	4.47± 0.66	2.3 - 6.1

All of the provided data were approached to the standard values. The data indicated non-significant differences comparing to the normal range of these enzymes.

Table (3) is showing the results of AST and ALT concentrations of the studied normal volunteers

Gender/no. of cases	11 Male			14 Female		
	Range	**Mean ± SD**	**Normal range**	**Range**	**Mean ± SD**	**Normal range**
AST (u/l)	14- 34	22.9±7.16	10- 50	17- 45	27.07±7.54	10- 35
ALT (u/l)	24- 46	29.09±6.72	10- 60	14- 27	23.57±3.99	8- 40

The lipid profile is included in table (4), all of the obtained results were found to be within the normal level with the exception of LDL, the mean of the examined female was 114.67 vs. < 100 for the standard range.

Table (4): Lipid profile results of the normal BMI investigated individuals

Gender/no. of cases	11 Male			14 Female		
	Range	Mean ± SD	Normal range	Range	Mean ± SD	Normal range
Cholesterol (mg/dl)	106- 244	157.36± 45.93	< 200	116- 257	183.57±41.90	< 200
Triglyceride (mg/dl)	86- 210	128.0± 37.82	< 150	66- 184	114.85±39.33	< 150
HDL (mg/dl)	40- 57	48.54± 5.24	≥ 60	39- 54	45.92±3.49	≥ 60
LDL (mg/dl)	30.6-175.8	83.21± 44.33	< 100	39.4- 186.2	114.67±42.99	< 100
VLDL (mg/dl)	17.2- 42.0	25.60± 7.56	5- 40	13.2- 36.8	22.97±7.86	5- 40

The mean of laboratory investigations of the studied obese individuals are tabulated in tables (5, 6 and7). As regards table (5) FBS approached to the normal level. The mean values were 82.5±13.52 and 87.36±12.33 for males and females respectively. Urea, creatinine and uric acid concentrations were also around the standard values. AST and ALT were in the ordinary level the mean was 32.36 and 33.66 for males and 29.78 and 29.80 for the overweight/obese examined females. The mean of the lipid profile in both sexes were exceeding the standard level in cholesterol, triglyceride and LDL (table: 7).

Table (5): The results of fasting blood surge and kidney functions tests of the obese investigated individual

Gender/no. of cases	36 Male		Normal range	41 Female		Normal range
	Range	Mean ± SD		Range	Mean ± SD	
BMI	26.33-46.44	37.61±4.93	≥ 25	26.28-61.84	41.82±9.82	≥ 25
FBS (mg/dl)	28- 107	82.5±13.52	70- 115	71- 110	87.36±12.33	70- 115
Urea (mg/dl)	23- 42	31.97±5.65	18- 53	21- 39	28.65±4.89	18- 53
Creatinine(mg/dl)	0.50- 1.10	0.82±0.14	0.60- 1.30	0.44-0.95	0.69±0.12	0.50 -1.2
Uric acid (mg/dl)	3.9- 6.8	5.40±0.78	3.6- 8.2	3.6- 6.4	4.81±0.76	2.3 - 6.1

Results of the analysis of liver enzymes AST and ALT in the obese individuals are presented at table 6.

Table (6): The results AST & ALT the liver function tests of the obese investigated individuals

Gender/no. of cases	36 Male			41 Female		
	Range	Mean ± SD	Normal range	Range	Mean ± SD	Normal range
AST (u/l)	14- 50	32.36±7.89	10- 50	14- 50	29.78±6.59	10- 35
ALT (u/l)	20-44	33.66±5.77	10- 60	19- 60	29.80±6.83	8- 40

Results of the analysis of lipid profiles in the obese individuals are presented at table 7.

Table (7): Lipid profile results of the obese investigated individuals.

Gender/no. of cases	36 Male			41 Female		
	Range	Mean ± SD	Normal range	Range	Mean ± SD	Normal range
Cholesterol (mg/dl)	141-307	213.02±02	< 200	161-327	216.92±36.40	< 200
Triglyceride (mg/dl)	86-281	177.80±53.58	< 150	87-262	165.26±44.03	< 150
HDL (mg/dl)	27-47	38.22±4.70	≥ 60	22-50	38.58±5.53	≥ 60
LDL (mg/dl)	69.6-225.6	139.24±36.67	< 100	95.0-242.8	145.28±32.92	< 100
VLDL (mg/dl)	17.2-65.2	35.56±10.71	5- 40	17.4-52.4	33.05±8.80	5- 40

3.2.ACE genotyping:

Results of alleles (I &D) frequencies and genotyping analysis for the different groups understudy are shown in table (8):

Table (8): frequencies and genotyping analysis of ACE for the different groups understudy

Group	Allele and genotypes frequencies				
	Allele frequency		Genotypes frequencies		
	I	D	II	ID	DD
Normal male	0.318	0.681	0.090	0.454	0.454
Normal female	0.076	0.923	0.000	0.153	0.846
Obese male	0.294	0.705	0.088	0.411	0.500
Obese female	0.217	0.782	0.051	0.333	0.615

Regarding the allele frequencies, the results showed that the allele I is less in frequency than the allele D in all the studied groups. The lowest allele I frequency was observed in normal female group (0.076) while the highest allele I frequency was observed at normal male group (0.318).

The lowest allele D frequency was observed in normal female group (0.681) while the highest allele D frequency was observed ACE gene (DD, II, ID), it is noticed a limited correlation between different genotypes and cholesterol, triglycerides and very low lipoprotein ($p<0.05$). It noticed that the genotype DD had the higher effect compared with the II genotype. From another side, no correlation was noticed for the rest of parameters included in the present study.

4.Discussion:

In the current study, we have focused on BMI and lipid profile parameters which including cholesterol; triglycerides; high density lipoprotein; low density lipoprotein and very low density lipoprotein because of their close connection to obesity. The other biochemistry tests including fast blood sugar; kidney functions tests (urea, creatinine and uric acid) and liver functions tests (AST & ALT) were done to make sure that all the participant individuals in the experiment were healthy.

Our results in BMI showed similar values such as the cut off points of Europe and USA in the normal weight male and female groups which were 21.93; 21.62 respectively and the cut off points range (18.50 - 24.99). Moreover the results of BMI in the obese male and female groups were 37.61; 41.82 respectively fall in the same cut off points of Europe and USA overweight / obese (≥25.00) according to WHO (2004) and Bray (1998).Similar results were observed by Abdul-Rahim *et al.* (2001) on their study in the obese individuals live in rural and urban Palestinian West bank populations. Moreover, Abdeen *et al.* (2012) was the first to highlight the statistics and the risk of obesity in Palestinian individuals. In their paper they presented the results of the first national survey of its kind in Palestine which clearly shows that more than sixty percent of the Palestinian population between 18 and 64 years old are overweight (38.0%) or obese (24.4%). This highlights the emergence of non-communicable diseases and their risk factors as major contributors to the burden of ill health in the Middle East, particularly among urban populations.

Our results showed a significant increase in BMI; cholesterol and triglycerides parameters values in the obese males than normal males ($p<0.05$; $p<0.01$ and $p<0.01$ respectively). Similar results were observed by Thakur and Bisht (2010), in their study on Indian obese and non –obese sedentary college men. Moreover, Mishra *et al.* (2012) reported the same results on their study in north Indian males.

In contrast, a significant increase was noticed only in body mass index parameter in the obese females over normal females ($p<0.05$).Similar results were observed by Lima *et al.,* 2004 in their study on the effect of obesity in over weight adolescent females and adolescents. Moreover similar result was observed by Al-Malki *et al.,* 2003 on their study on the obesity in Saudi females, they found increase in BMI in obese females. Abdul-Rahim *et al.,* (2003) in their study about the obesity in rural and urban Palestinian West bank populations, concluded that leisure-time physical activity is not a common concept in Palestinian context, especially for rural women. Many of Palestinian women are house wives; do not have time for exercise or moving as well as some habits which can lead to obesity. From these habits the exchange of visits between houses wives, hospitality and the consuming high amount of food calories during the visits exchange.

Angiotensin converting enzyme (ACE) play a role in catalyzes the formation of angiotensin I to angiotensin II. A polymorphism has been identified in intron 16 in which a 287 base-pair alu sequence was found to be present (insertion or I) or absent (deletion or D) in the population. ACE and the components of the renin-angiotensin system are expressed in adipose tissue. ACE is involved in adipocyte growth and function and the ACE-processed angiotensin II inhibits adipocyte differentiation and therefore, the I/D polymorphism within ACE may be associated with overweight/obesity.

The ACE gene has two alleles: I and D; the D allele was more frequent than the I allele, which reflects on the genotype DD which was more frequent than the other genotypes (II, ID). No correlation between the allele and genotype distribution and the sex were observed in our study. Similar results were observed by El-Hazmi and Warsy (2003) in their findings of high frequency of DD genotype and D allele in Saudi overweight and obese individuals living in Riyadh region. Again similar finding were reported by Settin *et al.,* (2009) in their study with non-complicated overweight or obese Saudi individuals living at Qassim Region.

In our study we did not find a correlation between the ACE genotypes and the BMI; blood pressure; fasting blood sugar, kidney and liver functions tests, while a positive correlation was observed between the ACE genotypes and the cholesterol, triglycerides and VLDL. The correlation was more prominent with the D allele than the I allele.

Conflicting reports regards the correlation of ACE genotypes and weight, our results agrees with the study of Bell *et al.* (2007) who stated that

functionally relevant sequence variation in ACE, whether it is defined at the level of SNPs, haplotypes, or clades, is not associated with obesity.

Kramer *et al.* (2005) on their study the association of ACE gene polymorphism and obesity in three black populations identified the presence of the I allele or the absence of D allele as well as an Alu element in intron 16 (I/D polymorphism). The authors performed haplotype analysis using data collected from participants of a community survey of hypertension among blacks living in Ibadan (Nigeria); Spanish Town (Jamaica) and Chicago (USA). In each study population transmission distortion of ACE gene polymorphisms and haplotypes from heterozygous parents to affected offspring was examined. Polymorphisms were divided into three groups based on their position on the ACE gene, to estimate haplotypes. In general their study suggested that ACE gene polymorphisms may influence the development of weight gain with a sex difference in males and females.

In the study of Wacker *et al*. (2008) they involved genotyping of two groups with different body mass indexes (BMI <or=25 and BMI >or=30) that were composed primarily of 421 middle-age Caucasian individuals. They noted that, the male groups differed significantly in allele frequency at the ACE locus with the I allele more frequent in the BMI >or=30 group ($p<0.05$). While the female BMI >or=30 group also had a higher I allele frequency than the BMI <or=25 group, the difference was not significant.

Kim (2009) reported that there is a trend towards association of ACE I/D polymorphism with hypertension but not with obesity after examined whether the angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism is associated with obesity, cardiovascular risk factors and 12-week exercise-mediated changes in Korean women. Total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels were higher ($P < 0.05$) in the DD genotype than in II or ID genotypes. D allele frequency in ACE I/D gene had a higher ($P = 0.063$) trend in the hypertensive group than the normotensive group. The DD genotype had a trend to develop (odds ratio 4.032, $P = 0.086$) more hypertension than the II genotype. The II and ID genotypes showed a significant ($P < 0.05$) decrease in intima media thickness of the carotid artery after an exercise intervention, whereas the DD genotype showed an increase.

In a review research conducted by Mao and Huang (2013) regarding the association between angiotensin-converting enzyme insertion/ deletion gene polymorphism and the risk of overweight/obesity, they used all eligible studies were included in this meta-analysis by searching PubMed, Embase and Cochrane databases through April 2013 according to a predefined criteria. Fourteen case-control studies including 3371 cases and 4490 controls were recruited for the analysis of the association between ACE I/D gene polymorphism and overweight/obesity susceptibility. A significant association was noticed between DD genotype and overweight/obesity risk in overall populations and Africans ($p=0.014$ and 0.010, respectively). D allele was associated with the risk of overweight/ obesity in Africans ($p=0.026$). But, II genotype might not be a protective factor against overweight/obesity risk in overall populations, Africans, Caucasians and Asians. And they concluded that DD genotype is a risk factor for the overweight/obesity susceptibility in overall populations, particularly in Africans. D allele is a risk factor for the overweight/obesity susceptibility in Africans.

Das *et al.* (2013) studied the Synergistic effects of ACE (I/D) and Apo E (Hha I) gene polymorphisms on obesity, fat mass and blood glucose level among the adult Asian Indians: A population-based study from Calcutta, India. they observed that neither ACE (I/D) nor Apo E (Hha I) gene polymorphisms showed any significant association with body mass index, waist circumference, fat mass, fasting, and post meal blood glucose levels. Even synergistically (ACE + Apo E), these two polymorphisms showed no significant association with obesity, fat mass, and blood glucose level. ACE (I/D), Apo E (Hha I), as well as ACE + Apo E seem to have no significant association with obesity, fat mass, and blood glucose levels in this population. Our study results are completely agrees with our obtained results.

In conclusion, more studies with more genes and individuals are needed for a deeper understanding of the determinants of obesity in the Palestinian habitant in general and Gaza city in specific. However, it is clear that the high levels of obesity, high energy and fat consumption and low physical activity levels in this population demand prompt public health action if the rise in no communicable diseases is to be contained in the resource-constrained Palestinian context.

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