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Apo lipoprotein E Polymorphism as risk factor for Lipid Profile disturbance among obese Egyptian females.

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ABSTRACT

Apo lipoprotein E (Apo E) plays a role in some diseases related to lipid metabolism. 122 obese adult females (BMI ≥ 30 Kg/m^2): 56 of them with visceral obesity (≥ 7cm by abdominal ultrasound) and 66 without visceral obesity and 36 age matched non-obese (BMI ≤ 25 kg/m^2) without visceral obesity were studied. Anthropometric assessment, visceral obesity and lipid profile evaluation were attempted. Genetic analysis of Apo E was performed. The homozygous (E3/E3) genotype was significantly the most prominent genotype among the control group (50.0%), and the heterozygous (E3/E4) genotype had significantly the highest frequency among the obese without visceral obesity (33.3%) . Comparison of the anthropometric parameters and lipid profile revealed that E4 allele had the highest significant values of total cholesterol and LDL. For the obese females without visceral obesity, Apo E3/E4 genotype had the highest significant values of total cholesterol and LDL. While for the obese females with visceral obesity, Apo E3/E3 genotype had the highest significant values of visceral fat. While E4/E4 genotype, had the highest significant values of total cholesterol and LDL, and Apo E3/E3 genotype had the lowest values. The homozygous (E3/E3) genotype was the most prominent type among this study sample, followed by E4/E4. Lipid profile (total cholesterol, LDL and HDL) has significant relation with E3/E4 in obese females without visceral obesity, and with E4/E4 among obese females with visceral obesity.

Keywords: Apo lipoprotein E, lipid profile, visceral obesity, adult females.

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INTRODUCTION

Obesity; an excess body fat; is the key to increased health problems and reduce life expectancy [1] . Abdominal obesity differ in structural composition, metabolic activity, and functional significance between Excess visceral (VAT) and subcutaneous (SAT) adipose tissues [2]. Do Nascimento et al., [3] stated that the Levels of visceral adipose tissue (VAT) may be related to differences in genetic make-up between different ethnic backgrounds and diseases.

Blood lipid profile are related to Genetic factors in Obesity [4]. One of the principal risk factors for the development of cardiovascular diseases is Dyslipidemia [5]. Identification of the genes responsible for increased risk of dyslipidemia has facilitated the detection of changes in the DNA sequence that can have a pathogenic effect [6] .

Apo lipoprotein E (Apo E) plays a role in determining gene risk for a number of diseases, particularly those related to lipid metabolism. It mediates the uptake of lipoprotein through ligand-receptor interaction arranged with the low-density lipoprotein (LDL) receptors [7] .

Apo E is polymorphic. It has Three major isoforms encoded by three alleles of chromosome 19 (E2, E3 and E4) [8] .The natural isoform, apo-E3 is the most common. Apo E2, in which arginine has been replaced by cysteine in position 158, and apo-E4, in which cysteine is exchanged for arginine in position 112 are two mutant forms [9] .

The aim of the study is to evaluate the relationship between Apo E genotype and plasma lipid levels in Egyptian patients with visceral obesity.

SUBJECTS AND METHODS

Subjects

Current study was a cross-sectional case control one, consisted of 158 females: 122 obese females (BMI ≥ 30 Kg/m^2) and 36 apparently normal healthy ones (BMI≤ 25 Kg/m^2) serving as control group; aged 30 - 62 years (mean: 49.88 $\pm$ 5.6). The obese females were classified; using cutoff point of 7 cm by abdominal Ultrasound; into 2 groups: obese with visceral obesity (56) and obese without visceral obesity (66). All subjects enrolled in the study were recruited from outpatient clinic of the "Visceral Obesity Management Unit" at "National Research Centre"; between October 2012 and December 2014**.** The participants were informed about the purpose of the study and their permission in the form of written consent was obtained. The protocol was approved by the "Ethical Committee" of the "National Research Centre". The agreement reference number is 10/119.

All obese and control females were subjected to detailed medical history, anthropometric assessment, ultrasound examination, and some laboratory investigations. ***Detailed medical history*** and information was obtained on demographic characteristics concerning history and duration of obesity and medications. Those with positive history of smoking, acute or chronic infections, coronary artery disease, congestive heart failure, chronic liver disease, diabetic nephropathy, rheumatic disease and cancer; were excluded from the study

Anthropometric assessment: Height and weight were measured following the recommendations of the International Biological Program [10]. Body weight was determined to the nearest 0.01 kg using a Seca Scale Balance, with the subject wearing minimal clothing and with no shoes. Body height was measured to the nearest 0.1 cm using a Holtain portable Anthropometer. Body mass index (BMI) was calculated as body weight divided by height squared (Kg/m^2).

Ultrasound (US) examination to each participant was done to evaluate visceral fat at the umbilicus (USVF) in cm. Intra-abdominal fat thickness measurement was obtained using the "Medison Sonoace X8" ultrasonography equipment. For the visceral fat, a 3.5 MHz transducer was transversely positioned 1 cm above the umbilical scar on the abdominal midline, without exerting any pressure over the abdomen. Visceral fat thickness attempted corresponding to the measurement in centimeters between the internal surface of the

abdominal rectus muscle and the posterior aortic wall in the abdominal midline, during expiration. In the current research, visceral fat thickness < 3 cm was considered normal, 3-< 7 Border line and ≥ 7 cm with visceral obesity.

Laboratory investigations

Sample collection

Five milliliters of venous blood samples were withdrawn after 12-14 hours overnight fasting into two sterile vaccutainer (Becton Dickinson, NJ, USA); one containing EDTA for assay Apo E gene variants and the other without additives to separate serum to assay lipid profile.

Biochemical analysis

Estimation of lipid profile:

Plasma levels of triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) were measured by standardized enzymatic procedures, using kits supplied by Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 automated clinical chemistry analyzer. Low density lipoprotein cholesterol (LDL-C) was calculated according to formula of ***Friedwald et al.[11]***:

LDL-C= Total cholesterol –Triglycerides/5+ HDL-C.

ApoE genotyping

DNA extraction

Genomic DNA was extracted from whole blood using DNA extraction kit (QIAamp DNA Blood Kit; Qiagen). The purity of extracted DNA was checked.

PCR amplification

A 318bp fragment from the ApoE gene was PCR amplified in a 50µl reaction containing 10µl (0.1-0.4ng) purified genomic DNA, 1x Qiagen PCR Buffer, 0.25µM each primer, 200µM each dTNP, 1xQ-Solution, and 1.5U QIAGEN Taq DNA Polymerase. Two primers were used in the amplification: Upstream primer E2mut (5' ACT GAC CCCGGT GGC GGA GGA GAC GCG TGC) and downstream primer E3 (t' TGT TCCACC AGG GGC CCC AGG GGC TCGCGG). Primer E2mut differs from the genomic sequence at one position (underlined) which creates an additional AflIII recognition site in the amplified fragment. Reaction mixtures were incubated at 94^{o}C for 3min, subjected to 40 cycles of amplification (94^{o}C, 10sec.; 65^{o}C, 30sec; 72^{o}C, 30sec.), and incubated **at 73oC for 7sec [12] .**

Restriction fragment length polymorphism analysis (RFLP)

The PCR product was digested with restriction enzymes AflIII (5.000 U/ml) and HaeII (20.000 U/ml), 10× buffer, and 0.2 µl BSA. The contents were incubated for 24 h at 37°C. The digests were resolved on ethidium bromide-stained agarose gel and the results were documented by photography and separation of the resulting DNA fragments on 4% agars gel.

Statistical analysis

The data analysis was carried out using the statistical package for social science (SPSS) software version 16 (Chicago, Illinois). All numeric variables were expressed as mean ± standard deviation (SD). Statistical analysis was performed; for the parametric data; using Student-t test; to compare between 2 groups; and one way analysis of variance (ANOVA) test followed by Post Hoc LSD multi-group comparison; for more than 2 groups. For non-parametric data, Chi-square test was used for comparison between any numbers of groups. For all tests a probability, $p < 0.05$ was considered significant.

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RESULTS

Baseline characteristics; age, BMI, visceral fat and biochemical characteristics; of the 3 groups; were shown in table1. There were insignificant differences between the 3 groups regarding age. Obese females with visceral obesity had the highest significant values in BMI, visceral fat and triglycerides; followed by the obese without visceral obesity, and the least values recorded in the control. However, there were insignificant differences in total cholesterol, LDL and HDL between the 3 groups.

Table 1: Comparison between controls and obese without and with visceral obesity regarding Lipid profile

Parameters	Controls (N=36)		Obese without Visceral obesity (N=66)		Obese with Visceral obesity (N=56)		P value
	Mean	± SD	Mean	± SD	Mean	± SD	
Age(year)	41.11	10.21	41.94	10.19	42.89	9.88	0.702
BMI (Kg/m2)	29.64	7.73	37.46	5.56	41.25	7.53	0.000**
Visceral fat (cm)	3.38	1.40	5.33	1.37	8.25	1.00	0.000**
Triglyceride (mg/dl))	106.00	34.91	108.46	37.31	134.37	56.38	0.005**
Total cholesterol (mg/dl)	208.93	37.10	209.85	39.05	211.71	39.52	0.929
LDL(mg/dl)	131.78	37.17	142.77	39.87	142.04	38.27	0.294
HDL (mg/dl)	50.52	9.58	47.85	9.23	46.86	9.79	0.130

** P<0.01= highly significant differences.

Genotyping results:

Comparing the allele frequencies of xbaI Apo E gene among the different groups (table 2), revealed that E3 allele (about 50%) was highly significant more frequent than E4 allele (about 40%) in all groups under study (total sample, females with visceral fat, without visceral fat and in control females). However, E3 allele was significantly more frequent among controls (52.8%) ,while there were significant higher prevalence of E4 allele among obese group especially those with visceral fat (41.1%).

Table (2): Allele frequency according to visceral obesity

Allele	Total	Controls (N=72)	Obese without Visceral obesity (N=132) N (%)	Obese with Visceral obesity (N=112) N (%)	P Value
E2	36 (11.4)	8 (11.1)	14 (10.6)	14 (12.5)	0.368
E3	156 (49.4)	38 (52.8)	66 (50.0)	52 (46.4)	0.023*
E4	124 (39.2)	26 (36.1)	52 (39.4)	46 (41.1)	0.011*
P value	0.000**	0.000**	0.000**	0.000**	

* P<0.05= significant differences.

** P<0.01= highly significant differences.

Comparing distribution of the genotypes of xbaI Apo E gene; it was found that the frequency of the homozygous (E3/E3) genotype was the most prominent type for total sample (39.2%), followed by the homozygous (E4/E4) genotype (22.8%) ; with highly significant differences (P<0.001). The homozygous (E3/E3) genotype was significantly the most prominent genotype among the control group (50.0% - p <0.01). The heterozygous (E3/E4) genotype had highly significant (p < 0.001) the highest frequency among the obese without visceral obesity (33.3%), followed by the obese with visceral obesity (14.3%). While the homozygous (E4/E4) genotype had insignificant the highest frequency (25.0%) among the obese with visceral obesity (Table 3) .

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Table (3): Distribution of ApoE genotype according to visceral obesity

ApoE genotype	Total (N=158) N (%)	Controls (*N*=36) N (%)	Obese without Visceral obesity (N=66) N (%)	Obese with Visceral obesity (N=56) N (%)	P Value
E3/E3	**62 (39.2)**	**18 (50.0)**	**22 (33.3)**	**22 (39.3)**	**0.773**
E3/E4	**32 (20.3)**	**2 (5.6)**	**22 (33.3)**	**8 (14.3)**	**0.000****
E2/E4	**28 (17.7)**	**8 (22.2)**	**8 (12.1)**	**12 (21.4)**	**0.565**
E4/E4	**36 (22.8)**	**8 (22.2)**	**14 (21.2)**	**14 (25.0)**	**0.368**
P value	**0.000****	**0.002****	**0.038***	**0.059**	

* P<0.05= significant differences. ** P<0.01= highly significant differences.

Comparing the anthropometric parameters and lipid profile between ***the different alleles*** of Apo E xbaI gene for total sample, revealed that E3 allele had the highest significant value of BMI, and E2 had the lowest value. While E4 allele had the highest significant values of total cholesterol and LDL, and E3 had the lowest value (table 4).

Table 4: Comparisons of the anthropometric parameters and lipid profile between the different alleles of Apo E xbaI gene for total sample

Variable	E2	E3	E4	P value (ANOVA)
	Mean ± SD	Mean ± SD	Mean ± SD	
BMI (Kg/m2)	**33.83 +7.09**	**37.63 +8.59**	**37.18 +7.43**	**0.037***
Visceral fat (cm)	**6.45 +2.11**	**5.91 +2.41**	**5.79 +2.09**	**0.297**
TG(mg/dl)	**120.67 +48.41**	**122.44+49.16**	**112.26+43.32**	**0.237**
Cholesterol(mg/dl)	**215.67+46.47**	**203.93+28.79**	**216.83+44.60**	**0.023***
LDL(mg/dl)	**144.60+46.66**	**130.90+29.76**	**145.51+43.36**	**0.007****
HDL(mg/dl)	**48.53+8.40**	**48.85+10.28**	**46.80+11.01**	**0.575**

* P<0.05= significant differences. ** P<0.01= highly significant differences.

For the obese females without visceral obesity (table 5), Apo E3/E4 genotype had the highest significant values of total cholesterol and LDL and Apo E4/E4 genotype had the lowest values. While for the obese females with visceral obesity (table 6), Apo E3/E3 genotype had the highest significant values of visceral fat. While E4/E4 genotype, had the highest significant values of total cholesterol and LDL, then E3/E4 genotype and Apo E3/E3 genotype had the lowest values. This means that lipid profile (total cholesterol , LDL and HDl) is related to E3/E4 genotype among the obese females without visceral obesity, and to E4/E4 genotype among the obese females with visceral obesity

Table (5): Comparisons of the anthropometric parameters and lipid profile between the genotype of Apo E xbaI gene for obese without visceral obesity

Variable	Apo E3/E3 (N=22)	Apo E3/E4 (N=22)	Apo E4/E4 (N=8)	Apo E2/E4 (N=14)	P value (ANOVA)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
BMI (Kg/m2)	**35.47+3.93**	**37.90+3.29**	**38.04+5.86**	**37.98+6.70**	**0.528**
Visceral fat (cm)	**5.87+0.87**	**4.53+0.95**	**5.04+1.60**	**5.58+1.36**	**0.077**
TG(mg/dl)	**99.17+35.67**	**93.67+39.90**	**123.73+38.90**	**100.00+30.80**	**0.099**
Cholesterol(mg/dl)	**189.64+21.55**	**252.33+70.86**	**230.17+31.28**	**213.00+32.17**	**0.000****
LDL(mg/dl)	**119.82+21.77**	**179.33+65.25**	**158.67+38.17**	**146.13+26.48**	**0.001****
HDL(mg/dl)	**50.33+9.70**	**44.91+9.48**	**46.67+2.73**	**47.00+11.79**	**0.506**

TG: triglycerides, HDL; high density lipoprotein , LDL: low density lipoprotein

** P<0.01= highly significant differences

DISCUSSION

Number of chronic diseases are related with Obesity. Cardiovascular risk, metabolic syndrome and type 2 diabetes are associated with rising body mass index and more closely intra-abdominal deposition of

visceral fat [2, 13] **.** Previous studies recorded disturbances in lipid profile have been associated with the pathogenesis of obesity especially with visceral fat **[14 -17]**. These findings come in agree with the results of present study where there were highly significant elevated serum triglyceride, visceral fat and BMI in females with visceral obesity and insignificant increased levels of cholesterol and LDL (hyperlipidemia).

Apo E gene polymorphisms were also found to be associated with Alzheimer's disease (AD), diabetes ,stroke , cardiovascular disease, Parkinson's disease and renal disease **[18].**

Apo E; is a protein in blood that carry lipids such as cholesterol and triglycerides. It helps to move lipids from body's cells to the liver, where the lipids are removed from the blood and excreted. It also affects the activity of enzymes that help to remove lipids from the body. Apo E has been identified as an important candidate gene for lipid abnormalities **[19].** Recently, Apo E has a role in regulating adipocyte **[20].**

The frequency of Apo E could be different according to environmental factors and lifestyle behaviors, obesity, gender and population related differences such as geographical differences **[21].** Three major isoform of apoE is related to their receptor affinity. Apo E3 allele in current study was the most common followed by E4, then E2 allele . These findings are in line with previous reports in Latwnia **[22],** in India and UK **[23].** While in Iranian population, Apo E3 was common allele, followed by E2, then E4 allele **[24].**

The present study showed higher prevalence of E4 allele in obese group: 41.1% of those with visceral obesity and 39.4% of those without visceral obesity .Also, Apo E4 allele had a significant higher total cholesterol and LDL compared with apo E2 and apo E3 alleles. These results are in agreement with those of recent studies of **Maxwell et al., [25]**; in African-American and European-American populations, and **Downer et al., [26]; in USA**. The apoE4 is critical risk factors for occlusive lipid disorders. However, the detailed mechanism E4 allele lipid a profile is partially understood **[18].** But With other factors such as inflammation, immunity and oxidative status might also influences on the lipid profiles **[27,28]**.

The current study found that the most common genotype is E3/E3 homozygous .while the heterozygous E3/E4 genotypes were more frequent in obese patients without visceral fat and had significantly higher total cholesterol and LDL cholesterol levels .However; the homozygous E4/E4 genotypic frequencies were significantly higher in the obese with visceral fat with higher significant total cholesterol and LDL cholesterol levels.

Researchers stated that ApoE affects the risk of developing heart disease, the E4 allele is associated with the increased prevalence of atherosclerosis and cardiovascular disease and this could be partially explained by E4 with higher LDL-c **[29,30].** Zhang et al. [31] reported that People who have the E3/E4 or E4/E4 genotype are at greatest risk of developing heart disease, and have high LDL cholesterol and triglyceride levels. But the E2/E4 or E3/E3 genotype, at normal risk for developing heart disease. Also, ApoE genotype can affect how changes respond to treatments or lifestyle. These can be considered as an indicator that E4 allele and E4/E4 genotype may increase the risk of heart disease especially among Egyptian females with visceral fat in the present study.

Further research, will be necessary to assess the variability in male with visceral fat in relation to genetic factors .Because previous studies demonstrated that the effects of ApoE genotype on lipid levels differ by sex. The reasons for those differences are not studied well; however, some authors stated that they might be related to the influence of sex hormones **[22].**

CONCLUSION

The homozygous (E3/E3) genotype was the most prominent type among this study sample, followed by E4/E4. Lipid profile (total cholesterol, LDL and HDL) has significant relation with E3/E4 in obese females without visceral obesity, and with E4/E4 among obese females with visceral obesity.

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