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Research Article

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APOA1 polymorphisms in relation to lipid profiles in hypercholesterolaemiac Egyptian adolescent females

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ABSTRACT

The aim of this study was to investigate association between of two MspI polymorphisms in ApoA-I gene (G-75A and C83T) and lipid profiles in Egyptian adolescent females with hypercholesterolemia and explore their effect on variations of lipid parameters. A total number of 120 adolescent females with hypercholesterolemia and 120 age matched normolipidemic healthy controls were enrolled for the study. Genotyping of the APOA1 was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) combined with gel electrophoresis, and then confirmed by direct sequencing. The frequencies of GA genotype were significantly lower in patients than in controls. Serum total cholesterol, low-density lipoprotein cholesterol and triglycerides were significantly higher and the HDL-cholesterol levels were significantly lower in subjects with GG genotype than those with GA + AA genotype. Moreover, the heterozygous CT and the T allele were significantly lower in patients than in controls. Carriers of T allele showed lower levels of cholesterol and triglycerides levels and the higher level of protective HDL than subjects with CC genotype. Significant association between GG/CC haplotype and increased lipid parameters was observed in patient group. In conclusion, both the G–75A and C83T polymorphisms of APOA1 gene have adverse effects on HDL levels and were associated with elevated lipid parameters. The study suggests that G and T alleles may be susceptible alleles for hypercholesterolaemia in Egyptian adolescent females and have a significant effect on lipid metabolism.

Keywords: apolipoprotein A-I; gene polymorphism; hypercholesterolaemia; adolescent females; Egyptians

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INTRODUCTION

Apolipoprotein A-I (apoAI) is the predominant protein of high-density lipoprotein (HDL) and is a key component of the reverse cholesterol transport process (1). The gene encoding apoAI (APOA1) is part of the APOA1-C3-A4 gene cluster located on the long arm of chromosome 11 (2). Genetic studies have identified polymorphisms and mutations in APOA1 and other genes associated with variation in HDL-cholesterol plasma levels. Two common APOA1 polymorphisms, G–75A and C83T, have been associated with variations in apoAI and HDL-cholesterol serum levels. G–75A is a G-to-A transition located 75 bp upstream from the transcription start site, while C+83T is a C-to-T at +83 bp transition located in the first intron of the APOA1 gene (3–4)

MspI polymorphic site has been identified in the first intron of the apoA-I gene [5], in which two consecutive transitions at +83 bp (C to T) and +84 bp (G to A) sites occur together or independently. The objective of the present study was to investigate the effect of APOA1 gene polymorphisms G–75A and C83T on lipid profiles and evaluate the genetic susceptibility for hypercholesterolaemia among adolescent females.

EXPERIMENTAL SECTION

We studied 120 unrelated non smoker hypercholesterolaemic Egyptian females, aged 16–19 years) and 120 normolipidemic age- matched control. The average age was 14.56± 2.55 years in controls and 15.68± 2.55 years in patient group. Hypercholesterolaemia was considered as defined by the National Cholesterol Education Program. They were taken from the outpatients clinics of National Research Centre, Egypt. Subjects with personal history of smoking, gastrointestinal, thyroid, liver or renal disease, diabetes, or under treatment with lipid-lowering drugs, hormone replacement therapy or oral contraceptives at the time of blood sampling for lipoprotein analysis were excluded. The study was approved by the National Research Centre Ethics Committee.

Subjects were defined as hypercholesterolaemic if their total cholesterol level was more than 6.2 mmol/L or total cholesterol level was less than 6.2 mmol/L, but >5.2 mmol/L and total cholesterol to HDL-cholesterol ratio was more than 5.0 or were on treatment to lower LDL-cholesterol. Subjects with more severe hypertriglyceridaemia who have triglycerides more than 2.3 mmol/L were excluded as they were considered that may have other genetic causes and the close inverse relationship between triglycerides and HDL-cholesterol would mask any relationship between the ApoA-I gene polymorphisms and hypercholesterolemia as defined by the National Cholesterol Education Program (NCEP) (5).

DNA extraction and genotyping

Total genomic DNA was isolated from peripheral blood leukocytes by the commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA). Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was performed to assess the APOA1 gene polymorphisms. Based on the Gen Bank reference sequence, the PCR primer pair used as follows: forward: 5′-AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG-3′, and reverse: 5′-TTA GGG GAC ACC TAG CCC TCA GGA AGA GCA-3′. The amplified PCR products were digested with 10 units of the restriction endonuclease enzyme MspI overnight. The digested fragments were resolved on a 3 % agarose gel and stained with ethidium bromide for visualization under UV light. The presence of the MspI restriction site at -75 bp (G allele) and at +83 bp (C allele) in the 433 bp product resulted in four fragments of 45, 66, 113 and 209 bp. The absence of the restriction site at-75 bp (A allele) resulted in three fragments of 45, 179 and 209 bp. The absence of the restriction site at +83 bp (T allele) created a larger fragment of 254 bp instead of two fragments of 45 and 209 bp. For quality control, we performed double sampling PCR-RFLP in more than 10 % of the samples and found no differences, and then confirmed by direct sequencing.

Biochemical analysis

Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were measured using commercial kits following the manufacturer instructions. Serum low density lipoprotein cholesterol (LDL-C) was calculated using the Friedwald's formula.

Statistical analysis

Statistics Package for the Social Science (SPSS version 11.0, 2002, SPSS, Chicago, II) was used in the data analyses. Allele frequencies and genotype distributions were estimated by gene counting. Differences in allele frequencies and genotype distribution between the studied groups and deviation from Hardy-Weinberg equilibrium were estimated using the x2 test. Differences in lipid concentrations between groups were compared by Student's t-test. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Values of $p<0.05$ were considered statistically significant. The effect of polymorphisms or haplotypes on lipid levels was evaluated by one-way analysis of variance (ANOVA). Variables without normal distribution were log-transformed for analysis. Significance was assumed for p-0.05.

RESULTS AND DISCUSSION

Clinical characteristics and serum concentrations of lipids for unrelated Egyptian hypercholesterolaemia cases and controls are presented in Table 1. Serum concentrations of TC-C, LDL-C, and TG were significantly higher and HDL-C was significantly lower in patients as compared with controls. There were no significant differences in the mean BMI, systolic, diastolic blood pressure levels and age between patients and controls.

Genotype and relative allele frequencies of the G–75A and C83T polymorphisms in the patient and control groups are presented in Table 2. The GG genotype was found in 94 (78.33%) of the subjects in the patient group compared with 78 (65) of subjects in the control group. The GA genotype was found in 25 (41.66%) of the subjects in the study group compared with 38 (31.66%) in controls subjects (p=.03) and the AA genotype in was found in 1 (.83%) of the subjects in the patient group compared with4 (3.33%) in controls (p=.03). Moreover, the frequency of A allele was higher in the control group was found in 46 (19.16%) compared with the study group was found in 27(11.25%)(p=.02).The frequency of the G allele more frequent 213 (88.75%)in the study group than in the control group 194 (80.83%).

Table 1: Clinical characteristics and serum concentrations of serum lipids in patients with hypercholesterolemia and controls

	Controls	hypercholesterolemia	P
Age (years)	14.56± 2.55	15.68± 2.55	0.72
Systolic BP (mmHg)	107 ± 7.99	117 ± 10.77	0.06
Diastolic BP (mmHg)	84 ±10.88	89 ± 9.66	0.07
BMI (kg/m2)	23.3 ± 4.32	27.3 ± 3.21	0.06
Fasting glucose (mmol/L)	4.7±0.4	4.8±0.3	0.06
Cholesterol (mmol/L)	2.5±0.8	7.5±1.3	0.002
Triglyceride (mmol/L)	.91±.45	2.00±.74	0.001
HDL-cholesterol (mmol/L)	2.31 ±.29	.95±.35	0.001
LDL-cholesterol (mmol/L)	1.7±0.7	4.7±1.3	0.001

Table 2: Genotypes and Alleles distribution of APOA1 (G–75A and C83T) gene polymorphisms in hypercholesterolaemiac and control subjects

	G–75A		C83T		Haplotype
	GG (n=94)	*GA+AA (n=26)*	*CC (n= 114)*	*CT+**TT** (n=6)*	*GG/CC (n=70)*
Cholesterol (mmol/L)	4.51±0.8*	2.5±1.3	5.8±0.9$^{£}$	2.7±1.2	5.5±0.8$^{€}$
Triglyceride (mmol/L)	2.09±.07*	.90±.05	1. 81±.45$^{£}$	.98±.04	3.98±.35$^{€}$
HDL-cholesterol (mmol/L)	1.31 ±.29*	3.95±.35	1.31 ±.29$^{£}$	5.05 ±.35	1.21 ±.29$^{€}$
LDL-cholesterol (mmol/L)	4.9±0.7*	2.7±1.3	5.7±0.7$^{£}$	2.7±1.3	4.7±0.7$^{€}$
SBP (mmHg)	107 ± 7.91	116 ± 9.08	112 ± 7.99	110 ± 8.66	117 ± 7.99
DBP (mmHg)	88±9.99	84±10.88	85 ± 9.88	87±10.22	80 ± 9.99

$^{*}p<0.05$ versus *GA+AA;* $^{£}p<0.05$ versus *CT+TT;* $^{€}p<0.05$ versus *others*

Table 3: Serum levels of lipid, BMI and blood pressure levels according to the APOA1 G–75A and C+83T genotypes in hypercholesterolaemiac cases

Genotype	Normolipidemic	Hypercholesterolaemic	OR (95%CI)	p-value
GG	78 (65)	94 (78.33)	1.00(Reference)	
GA	38 (31.66)	25 (41.66)	0.57(0.32-1.02)	0.03
AA	4 (3.33)	1 (.83)	0.24(0.03-2.21)	0.18
Allele				
G	194 (80.83)	213 (88.75)	1.00(Reference)	
A	46 (19.16)	27 (11.25)	0.34 (0.21-0.55)	0.02
C83T			0.28(0.11-0.74)	
CC	101 (84.16)	114(95)	1.00(Reference)	
CT	18(15.00)	6 (5)	0.28 (0.11-0.78)	0.008
TT	1(.83)	0	0	0.49
Allele				
C	220 (91.66)	234 (97.50)	1.00(Reference)	
T	20 (8.33)	6 (2.50)	0.28 (0.11-0.71)	. 003

The frequencies of GA genotype [(OR = 0.57, 95 % CI =0.32-1.02; P = 0.03)] and A allele [Odds ratio (OR) = 0.34, 95 % confidence interval (CI) % CI= 0.21-0.55; P = 0.02)] were significantly lower in patients than in controls. Serum total cholesterol, low-density lipoprotein cholesterol and triglycerides were significantly higher and the HDL-cholesterol levels were significantly lower in subjects with GG genotype than those with GA + AA genotype. The APOA1 -75 A allele was significantly associated with decreased serum concentrations of TC, TG, LDL-C and higher levels of HDL-C (Table 3). Patients with GG genotype had significantly lower HDL-C and higher lipid parameters than those with GA + AA genotypes. Patients with hypercholesterolemia had a higher frequency of homozygous CC (95%) compared with controls (84.16%). The heterozygous CT was significantly lower in patients (5%) [odds ratio[(OR) = 0.28, 95 % confidence interval (CI) = 0.11-0.78; P = .008] and the T allele was found in 6 (2.50) [odds ratio (OR) =0.28 , 95 % confidence interval (CI) = 0.11-0.71; P = .003] compared to 20 (8.33%) in controls (Table 2). In addition, results showed a significant association between GG/CC haplotype showed variations in serum lipids among patient subjects, showing higher levels of lipid parameters than others ($p<.05$). The APOA1 C83T allele was significantly associated with increasing serum concentrations of HDL-C and decreased abnormal lipid levels (Table 3). Serum total cholesterol triglyceride levels and LDL were significantly lower and HDL cholesterol was significantly higher in the subjects with the CT+TT genotype compared to those subjects with the CC genotype ($p<0.05$ for all).

Annual stroke incidence ranged from 27.5 to 63 per 100,000 population in Arab countries Benamer and Grosset (6) and the commonest subtype in all series was ischemic stroke. The most common risk factors were hypertension, diabetes mellitus, hyperlipidemia, and cardiac disease. In Egypt the mortality rate from cardiovascular disease and diabetes was 384 for women per 100,000 populations as reported by the World Health Organization indicated that (7).

The national survey on risk factors for chronic diseases in Egyptian adolescents aged 10–18 years reported that 10% with borderline and 6% with high levels more than >200 mg/dL of total cholesterol and 9.4% of adolescents have a low high-density lipoprotein cholesterol levels, while 7.5% and 10.3% had high and borderline levels, respectively (8).

Association between higher serum concentrations of TC, LDL-C and TG and the *ApoA1gene* polymorphism was found in our hypercholesterolaemiac patients. Differences were identified in the frequency distributions of the *ApoA-I G-75A* genotypes or alleles between hypercholesterolaemia patients and controls in several studies. In our study the A allele was found to be more frequent in control subjects compared to the patient with hypercholesterolaemia. 3In vitro and in vivo studies suggest that the A allele at-75 site increases apoA-I gene expression and hence leads to elevated plasma apoA-I and HDL-cholesterol concentrations (9, 10, 11, 12, 13). Conversely, the A allele has also been reported to be associated with decreased apoA-I gene expression in vitro [14]. The APOA1 gene polymorphisms were also extensively studied and reported to be associated with other diseases. the effect of the A allele on HDL and apo A1 levels and found a positive association for those and, thereby, a decreased cardiac risk Saha et al. (15) Pischon et al.(16) Yangchun et al. (17) and Kamboh et al. (18). It has been found that the IL-6 -174 CC genotype was associated with an increased risk for renal cancer (19). The APOA1 -75 G/A and +83 C/T genotypes were also associated with susceptibility to breast cancer and lymph node metastases occurrence, respectively (20). A pilot study found that APOA1 polymorphisms (−75 G/A and +83 C/T) might be susceptibility to myocardial infarction in a north Indian population (21). The APOA1 -75G/A promoter polymorphism was associated with cognitive performance in multiple sclerosis (22). It has been found that the *APOA1* polymorphisms (G-75A and C83T) could be as risk factors for hypertension and obesity in a Brazilian elderly cohort (23). Data previously reported from Caucasian subjects (n=534), where the frequency of the GG, GA, and AA genotypes were 65.7%, 32.0%, and 2.3%, respectively, with an allele frequency of 81.7, and 18.3%, for the G and A alleles, were in consistent with the distribution within the current control subjects. Whereas, no differences were observed for the C83T polymorphism, whose frequencies in Caucasians were 93.4%, 6.6% for the CC and CT genotypes and 96.7%, 3.3% for the C and T alleles(17). The linkage disequilibrium has been reported between APOA1 polymorphisms, the present found a significant association between GG/CC haplotype and variations in the serum lipids, as has been previously demonstrated in Caucasians (24). Although our results revealed that the individuals with the APOA1 -75 A allele were likely to have a lower risk of hypercholesterolaemia as a result of its effect on higher serum concentrations of HDL-C, the exact mechanism is still unclear. However, the results have been inconsistent and inconclusive, with few studies reporting either no association or negative association between APOA1 -75 A allele and plasma lipids (25-27). Our study was consistent with some other studies reported association between-75A and 83T alleles and serum HDL-cholesterol variations, individuals with *APOA1* mutations

had higher concentrations of HDL-cholesterol than those with the wild types (28-31). In summary, the study showed that the rare alleles of *APOA1* gene polymorphisms (G-75A and C83T) in Egyptian adolescent females with hypercholesterolaemia might be associated with the variation of lipid profiles.

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