Association of MspIpolymorphism and 3500Q mutation with Alteration of LDL and Cholesterol in Egyptian Obese patients

Waheba A. Zarouk[1], Hayat M.Sharada[2], Mohga S.Abdalla[2], Inas Mazen[3] and Ahmed I.Abdelneam[1]

[1]Department Of Molecular Genetics And Enzymology National Research Center, Egypt
[2]Chemistry Department Faculty Of Science Helwanuniversity, Egypt
[3]Department Of Clinical Genetics National Research Center, Egypt
ahmedabdelneam@yahoo.co.uk

Abstract: Obesity is associated with qualitative and quantitative abnormalities in lipid levels in plasma. Apolipoprotien B plays a central role in lipoprotein metabolism and in maintaining the normal homeostasis of serum cholesterol levels. To date, no data are available on relationship between MspI and 3500Q Apo B polymorphism and lipid levels in Egyptian population. Here we studied the effect of MspI polymorphisms and 3500Q mutation on lipid profile disturbance . Blood samples from 60 obese patients; 30 of them adult obese; 30 childhood obese in additional to 60 healthy volunteers; 30 adult and 30 childhood, were analyzed for total cholesterol and triglycerides. In parallel, genotyping by means of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was performed and we confirmed our result by sequencing. We found that MspI has no significant role in change LDL and Cholesterol levels, however, 3500Q mutation play important role in change LDL and cholesterol in obese patients in Egyptian population.
[Waheba A. Zarouk, Hayat M.Sharada, Mohga S. Abdalla, Inas Mazen and Ahmed I.Abdelneam **Association of MspIpolymorphism and 3500Q mutation with Alteration of LDL and Cholesterol in Egyptian Obese patients.** Nature and Science 2012;10(2):55-62]. (ISSN: 1545-0740). http://www.sciencepub.net. 10

Key Words: apo B, 3500Q mutation , MspI ,LDL and cholesterol

1. Introduction

Obesity is a medical condition in which excess body fat, due to imbalance between consuming and burning calories, accumulates. This may have an adverse effect on health and leading to reduced life expectancy (**Rankinen *et al.,* 2006**). Obesity is a known risk factor for many diseases, particularly heart disease, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis. It has been is a complex, multi-factorial chronic disease often has more than one cause such as genetic, environmental and psychological factors. It has been reported that polymorphisms in genes controlling appetite and metabolism are associated with the susceptibility of obesity when sufficient food energy present. More than 41 of these sites have been linked to the development of obesity when a favorable environment is present (**Poirier *et al.,* 2006**). The prevalence of obesity is rising to epidemic proportions at an alarming rate in both developed and less developed countries (**Mokdad *et al.,* 1999**).

The insoluble lipids of human body are transported through blood as lipoprotein complexes with one or more specific proteins, called apolipoproteins. Among lipoproteins, low density lipoprotein (LDL) is 75% lipid (cholesterol and cholesteryl esters) and 25% protein. The high level of LDL is a risk factor for cardiovascular disease (**Herbert *et al.,* 1983**). Apolipoprotein B (Apo B) is the primary apolipoprotein of LDL which is responsible for carrying cholesterol to tissues. While it is unclear exactly what functional role Apo B plays in LDL, it is the primary apolipoprotein component and is absolutely required for its formation. What is clear is that the Apo B on the LDL particle acts as a ligand for LDL receptors in various cells throughout the body. Through a mechanism that is not fully understood, high levels of ApoB can lead to plaques that cause heart disease (atherosclerosis) (**Brunzell *et al.,* 1984**).

There is considerable evidence that levels of Apo B are a better indicator of heart disease risk than total cholesterol or LDL. However, primarily for practical reasons, cholesterol, and more specifically, LDL-cholesterol, remains the primary lipid target and risk factor for atherosclerosis . Moreover, it has been reported that several Apo B restriction fragment length polymorphisms (XbaI, EcoRI, MspI) to be associated with variation in lipid levels and obesity(**Thangarajah *et al.,* 1999**). To date, no data are available on relationship between MspI and 3500Q Apo B polymorphism and lipid levels in Egyptian population. It the present study we examined the lipid levels and its association with the MspI and 3500Q polymorphisms in Apo B gene.

2. Material and Methods

Subjects

A total of 60 patients obese (30 adult and 30 childhood), their age ranged between 10 to 50 years,

were collected in Egypt. Hyber-normal controls (n=60) were recruited in Egypt. These individuals had no diagnosis of obesity, and no family history of obesity. Their ages and sex matched with the patients group. All subjects gave their informed consent and the protocol was approved by the Genetic Clinic at the National Research Center of Egypt.

Pedigree analysis; family history analysis, habit of food eating, history of drug intake such as corticosteroid, thorough clinical examination including cardiovascular and abdominal examination were performed for all patient. Dimorphic features if any with special emphasis on eye, skeletal malformations, to exclude syndromic obesity like Pradderwilli or Bardetbiedl syndromes. IQ evaluation, hearing test were also performed. Anthropometric measurements including height, weight, head circumference, waist circumference, triceps and subscapular skin folds were also conducted. Weight and height were measured on the subjects barefooted and lightly clothed. Body mass index (BMI, kg/m^2) was calculated and obesity was defined as BMI >30 kg/m^2.

Lipid measurements and other laboratory investigations

Blood samples were obtained after an overnight fast. Serum levels of total cholesterol, triglycerides and HDL were measured by standardized enzymatic procedures using Olympus AU 400 using Olympus kit. LDL was calculated by Freidewald equation ***(*****Friedewald** ***et al*****.,** ***1972).***

Estimation of cortisol level in 24 hours collection of urine and serum .Serum leptin and insulin assay to exclude monogenic obesity. Estimation of FT3, FT4, TSH, growth hormone, FSH, LH to exclude endocrinal causes.

DNA analysis and genotyping

Genomic DNA was prepared from white blood cells by phenol/chloroform extraction technique as described by **Sambrook** ***et al.,*** **1989 .**A region in exon 26 of apo B gene was amplified by PCR using primers flanking the nucleotides flanking the nucleotides 10.628 to 11.092.For this proposal we used the sense oligonucleotide (5"CCA ACA CTT ACT TGA ATT CCA AGA GCA CCC 3') described by (**Pullinger** ***et al.,*** **1995**), that creates an Msp I endonuclease cleavage site, allowing the identification of apo B 3500Q mutation and antisense oligonucleotide (5"GGA AGC TTA GGT GTC CTT CTA AGG ATC CTG 3") described by (**Hansen** ***et al.,*** **1991**). This PCR product is larger and allow identification ofMsp I polymorphism and 3500Q mutation at apo B gene.

Detection of 3500 Q mutation and MspI polymorphism was carried out by digestion of PCR-amplified product with MspI. Enzymatic digestion were carried out at 37C for 4hours, in total volume 15 ul , using 5 U of restriction endonuclease . PCR and restriction product were identified on 3 and 1.5 % agarose gel electrophoresis for 3500 Q mutation and MspI polymorphism(**Cavalli** ***et al.,*** **2001**) .where homozygous for mutation and M+/M+ MspI expected bands will be 362bp and 103bp .heterozygous for mutation and M+/M+ MspI expected bands will be 362 bp , 33,103and 29. Absence of mutation and M+/M+ MspI expected bands will be 333,103 and 29. homozygous for mutation and M+/M- MspI expected bands will be 464, 362 and 103.heterozygous for mutation and M+/M- MspI expected bands will be 465,436,362,333,103 and 29bp. Absence of mutation and M+/M- MspI expected bands will be 436,333,103 and 29bp. homozygous for mutation and M-/M- MspI expected bands will be 465bp. heterozygous for mutation and M-/M- MspI expected bands will be 465,436 and 29bp. Absence of mutation and M-/M- MspI expected bands will be 436 and 29bp (**Cavalli** ***et al.,*** **2001**).

DNA Sequencing:

Samples were run on 1.5% agarose gels and the bands corresponding to the predicted size were cut and purification was carried out using the gel extraction kit following the manufacturer protocol (QIA quick columns, Qiagen). Purified samples were subjected to cycle sequencing using Big Dye Terminator v3.1 Kit and injected to ABI 3100 Genetic Analyzer (Applied Biosystems, Germany).

Statistical analysis

Data was presented by means ± SD and percentages .The compiled data were computerized and analyzed by SPSS PC+, version 12. The following tests of significance were used : Analysis of variance (ANOVA) test between more than two means , t-test between means we used analyze mean difference , t-test between percentage to analyze percent difference and chi –square. A level of significance with $p \leq 0.001$ was considered highly significant and $p > 0.05$ was considered insignificant.

3. Results

Descriptive characteristics of all studied subjects are presented in Table 1. Childhood obese was 15 male and 15 females and their ages ranged between 3 – 15 years. A higher total cholesterol level was observed and higher LDL in compared to control group ($P<0.001$). However there was no significance difference in HDL and triglycerides. Similarly, adult obese were 15 male and 15 females and their ages

ranged between 35-50 years. In the present study, it has been shown that a significance different in total cholesterol level (P<0.003) and LDL (P<0.003). However, there was no significance difference in HDL and triglycerides between adult obese group and adult control group.

MspI polymorphism genotype and frequency

The homozygous (M+/M+) was observed to be higher in all control (31.66%) compared to the all obese patients (8.34%) (P<0.001). While the genotypes heterozygous (M+/M-) was more common in all obese patients (61.66%) compared to all control (43.44%). This was also shown in M-/M- where native (M-/M-) was more common in all obese patients (30%) than all control (25%). It was observed that allele M + was more prevalent in control (46.66%) compared to all obese patients (39.2%). However, M- allele was less frequent in control (53.44%) compared to all obese patients (60.8%).

3500Q mutation genotype and frequency

The homozygous form was not found in controls (0%) but was only found in obese (48.3%) (P≤0.001). Similarly heterozygous was not found in controls (0%) and was observed in obese (33.33%) (P≤0.001). However normal form found more common in controls (100%) compared to obese (18.33%) (P≤0.001). 3500Q mutation allele frequency in all obese compared to controls. It was observed that the 3500q + allele more common in obese (65%) compared to controls (0%) (P≤0.001). However, 3500Q - allele more common in controls (100%) compared to obese patients (35%) (P≤0.001).

Genetic forms of 3500Q mutation and MspI polymorphism associated with lipid profile disturbance

We found that patients with homozygous 3500Q mutation form have higher cholesterol and LDL levels compared to heterozygous and native formsof 3500Q mutation (Table 2). However, it was no significant difference in all lipid profile (LDL, HDL, triglycerides and cholesterol) between Msp I M+/M-, Msp I M-/M- and Msp I M+/M+ Table (3).

T
able 1: Anthropometric and metabolic variables in the studied obese population

Variable	Total population (n=60)	Adult (n=30)	Children (n=30
Age (years)	42±12	**50**±5	**10**±6
Mean BMI (Kg/m2)	45±5.6	**48**±5.6	**35**±3.5
Total cholesterol (mg/dl)	198.80±48.3	214.15±45.0	185.57±47.47
LDL(mg/dl)	126.99±40.61	136.02±36.13	118.52±42.65
HDL (mg/dl)	45.45±9.3	48.30±10.8	43.90±8.78
Triglyceride (mg/dl)	119.92±54.82	125.10±63.3	115.90±44.99

Data are expressed as means ± S.D.

Table (2) Plasma lipids for all obese in different 3500 Q mutation genotypes :

Parameter	+/+ n=29	-/- n=11	+/- n=20	P value (ANOVA)
Total cholesterol (mg/dl)	245.6± 40.8**	144±7.2*	199.8±50	0.001**
LDL(mg/dl)	164±38**	76±11.6*	132±41	0.001**
HDL (mg/dl)	51±9	46±8	46±8	0.328
Triglyceride (mg/dl))	149±58	109±57	123±50	0.850

**p highly significant

Table (3) Plasma lipids for all obese in different Msp I genotypes

Parameter	+/+ n=10	-/- n=12	+/- n=18	P value (ANOVA)
Total cholesterol (mg/dl)	204±51	149±28	225±50	0.023
LDL(mg/dl)	131±42	87±26	155±38	0.013
HDL (mg/dl)	48±6	37.3±5	46±14	0.119
Triglyceride (mg/dl))	125±54	122±40	133±57	0.915

Discussion

In the present study we found that 3500Q mutation in apolipoprotien B may play an important role in change lipids levels, in spit that MspI polymorphism has no significant role with the same parameters in Egyptian obese population. It has been observed that total cholesterol and LDL was higher in all obese compared with control. However, it was no significance different in obese patients compared to control in HDL and triglycerides. This in accordance with **Howard *et al.,* 2003** work who reported that all of the components of the dyslipidemia, including higher triglycerides, decreased HDL levels, and increased LDL particles, have been shown clearly in obese patients**.**

There were significant differences in total cholesterol, triglyceride, LDL-cholesterol, and VLDL-cholesterol between obese patients and control **(Timirci *et al.,* 2010)**. Similarlythe prevalence of the dyslipidemia increased significantly as the BMI increased (P<0.005) **(Ghergerehchi, 2009)**. Furthermore, obesity has been associated with hyperlipidemia, a high LDL cholesterol and a low level of HDL cholesterol **(Lai *et al.,* 2001).** Also Overweight and obesity were associated with hyperlipidemia **(Lauer *et al.,* 1997**).

Altered levels of LDL-cholesterol were observed mainly in overweight or obese males. HDL cholesterol was borderline in the overweight and obese groups **(Lima *et al.,* 2004)**. In a study of Fifty two obese individuals that the mean total obese patients cholesterol was 203.38±30.20 mg/dl, HDL was 49.35±8.9mg/dl, LDL was 127.9±24.32mg/dl and triglycerides was 138±66 mg/dl while in control subjects Lipid profiles were in their normal limits .this shed light on the frequency of dyslipidemia are increased in obese patients **(Horri and Vakili, 2006)**.

In the present study it was observed that total cholesterol level was (185.57±47.47 vs 113.60±32.73mg/dl) and higher LDL (118.52±42.65 vs 52.27±30.76mg/dl) in childhood obese group compared to control group (P 0.001). While there was no significance difference in HDL (P 0.112) and triglycerides (P 0.906).

Simsek *et al.,* 2010 indicated that total cholesterol, LDL-C, HDL-C, and TG in the obese children were significantly different from values in the control subjects (all p<0.001). In a study of 546 obese children aged 7–12 observed that Obese children are at risk of dyslipidemia and relate also Children with the highest BMI and lowest physical fitness have the lowest HDL-C**(Korsten-Reck *et al.,* 2008)**. High risks of hyperlipidemia, hypertension, insulin resistance and abnormal glucose tolerance are expected from children who are obese**(McBride, 2010)**.

In the Muscatine Study, 75% of school-aged children who had total cholesterol concentrations higher than the 90th percentile at baseline had total cholesterol concentrations of >200 mg/dl in their early twenties (**Daniels *et al.,* 2008**). In the Bogalusa Heart Study, approximately 70% of the children with elevated cholesterol levels continued to have increased cholesterol levels in young adulthood **(Webber *et al.,* 1991).**

In the present study it was observed that total cholesterol (214.15±45.02) and LDL (136.02±36.13) higher in adult obese compared to control (175.45±30.71 and 100.90±33.18) (P 0.003). However there was no significance difference in HDL (P 0.121) and triglycerides (P 0.809) between adult obese group and adult control group.

in a study of 230 (94 male and 136 female) overweight and obese adolescents **Tershakovec *et al.,* 2002** reported, 117 (50.9%) who were severely obese, 41 (17.8%) were moderately obese, and 72 (31.3%) were overweight. LDL-C in the severely obese group was significantly higher than in the normal group (P< 0.005). While others noticed that Hypercholesterolemia is associated with increased relative weight in women **(Mokdad *et al.,* 2003)**. Adults obese are strongly associated with dyslipidemia, type 2 diabetes, hypertension, osteoarthritis, gout, and cardiovascular disease **(Spady *et al.,* 1983)**.

LDL concentrations in plasma depend on the balance between synthesis and catabolism. Usually, ~70% of LDL is removed from the blood by LDL receptors located on the surface of most cells, primarily hepatocytes (**Sacks *et al.,* 1996**). Apolipoprotein (apo) B-100 is the major structural protein of LDL and acts as a specific ligand in the cellular binding and uptake of LDL by LDL receptors (**Goldstein *et al.,*** 2001).

The relevance of this catabolic pathway is illustrated by genetic disorders affecting the receptor or ligand. In familial hypercholesterolemia (FH), LDL catabolism is impaired because of mutations in the LDL-receptor gene **(Innerarity *et al.,* 1990)**, and in familial defective apolipoprotein B-100 (FDB) hypercholesterolemia is attributable to defects in the structure of apoB-100 caused by mutations in the gene encoding the apolipoprotein **(Innerarity *et al.,* 1987)**. FDB was first observed in individuals with moderate hypercholesterolemia **(Weisgraber *et al.,* 1988)**. However, FDB patients may have markedly increased cholesterol concentrations similar to those in FH patients, and clinical findings may also be similar (**Defesche *et al.,* 1993).**

Gene mutation detection has identified several hundred different mutations in the LDL-receptor gene that disrupt receptor function. In contrast, FDB has been associated with surprisingly few mutations (**Pullinger *et al.,* 1999**), of which only three have been linked with reduced LDL ligand function. Most common is R3500Q (**Shen *et al.,* 2010**).

In the present study it was observed that there is significantly higher level of cholesterol and LDL in R 3500 Q homozygous compared to heterozygous and normal control, where P value by ANOVA test ($P\leq 0.001$) which indicate effect of R3500Q mutation was significant in changing lipid values.

LDL in heterozygotes with Arg 3500 Gln bound defectively with the LDL receptor in competitive binding assays. The Arg 3500 Gln substitution was statistically more prevalent in patients with hypercholesterolemia ($P = 0.0003$). Total cholesterol and LDL-cholesterol were significantly higher ($P<0.0004$) in 34 carriers with apoB 3500 Gln than in the controls. The surprising result that only (Arg 3500 Gln) mutation of apo B in the receptor-binding domain was associated with a defective LDL binding and hypercholesterolemia **(Ludwig *et al.,* 1997)**.

R3500Q was strongly associated with DL-C levels (*P* 0.001) and accounted for an approximately 55-mg/dL increase in LDL-C levels ***(*Shen *et al.,* 2010*)***. The apoB gene for R3500Q mutation in 130 hypercholesterolemia patients, among whom 30 patients met the criteria of familial hypercholesterolemia (FH). They reported the presence of R3500Q mutation of the apoB gene which result in the decreased binding of LDL to LDL receptor **(Fard-Esfahani *et al.,* 2005)**. Defective binding of LDL to the LDL receptor is a major cause of hypercholesterolemia because alterations of the lipid composition of the LDL core aside, the three-dimensional structure of the receptor-bindingdomain of apo B-100 is subject to modification resulting fromgenetically determined changes in the primary structure of apoB-100**(Fard-Esfahani *et al.,* 2005)**.

In Chinese population hypercholesterolemia with the 3500Q mutation was observed in Chinese population where the cholesterol concentrations of the R3500Q carriers were moderatelyincreased (6.12–7.08 mmol/l), similar to those reported(6.43–7.29 mmol/l) for Chinese R3500Q carriers **(Teng *et al.,* 2000)**. Plasma cholesterol, LDL-cholesterol, and apoB were significantly higher in R3500Q heterozygotes than in healthy controls ($P<0.001$) **(Raungaard *et al.,* 2000)**. Using molecular biology assays to diagnose affected individuals with borderline cholesterol levels **(Robles-Osorio *et al.,* 2003)**.

In the present study it was observed that no significantly difference was detected in all lipid parameters (LDL , HDL , TG and cholesterol) compared to MspI polymorphism form (M+/M+, M+/M- and M-/M-). Similarly other study indicated that no significant difference in the lipid parameters were determined for the apoBMsp I polymorphisms study **(Hu *et al.,* 2009)**.

Moreover there was no significant difference in any lipid profiles between different alleles of the apoBMspI restriction site in 148 Chinese patients with coronary heart disease and 153 healthy subjects**(Pan *et al.,* 1995)**. Furthermore, in a study of 108 white Norwegian at risk of atherosclerosis, the frequency of the M- allele was significantly higher in cases with high TC, high LDLC and high apoB than in controls with normal TC, LDL-C and apoB **(Delghandi *et al.,* 1999)** .

apo B *Msp*I allele (*M*-) was associated with a higher basal cholesterol concentration **(Rantala *et al.,* 2000)**. There was no correlation between *Msp*I RFLP genotypes and levels of any variable.serum lipid **(Talmud *et al.,* 1987)** . There was no significant difference in BMI, serum protein and lipid parameters in determining the polymorphism of the *APOB*MspI locus ($p>0$ 0.05) **(Hong *et al.,* 2001)**. Also Japanese population showed the same trend **(Zaman *et al.,* 1997).**

Moreover there was no significant difference in cholesterol or LDL in individuals lacking M+ allele (M- / M-) with individuals possessing one or more M+ (M- /M- or M+/ M+) **(Xu *et al.,* 1989)**.

Conclusion:

3500Q mutation in apolipoprotien B may play an important role in change lipids levels, in spit that MspI polymorphism has no significant role with the same parameters in Egyptian obese population. It has been observed that total cholesterol and LDL was higher in all obese compared with control. However, it was no significance different in obese patients compared to control in HDL and triglycerides.

Corresponding author
Ahmed I. Abdelneam
Department of Molecular Genetics and Enzymology
National Research Center, Egypt
ahmedabdelneam@yahoo.co.uk

References

1. Brunzell JD, Sniderman AD, Albers JJ, Kwiterovich PO Jr. Apoproteins B and A-I and coronary artery disease in humans. Arteriosclerosis. 1984; 4(2): 79-83.
2. Cavalli SA, Hirata MH, Hirata RD. Rapid detection of 3500Q and 3531 mutations and MspI polymorphism in exon 26 at the

apolipoprotein B gene. J Clin Lab Anal., 2001; 15(1):35-9.
3. Daniels SR, Greer FR, the Committee on Nutrition. Lipid screening and cardiovascular health in childhood. Pediatrics. 2008; 122(1): 198-208.
4. Defesche JC, Pricker KL, Hayden MR, van der Ende BE, Kastelein JJ. Familial defective apolipoprotein B-100 is clinically indistinguishable from familial hypercholesterolemia. Arch Intern Med., 1993; 153(20):2349-2356.
5. Delghandi MThangarajah R, Nilsen M, Grimsgaard S, Bønaa KH, Tonstad S, Jørgensen L. DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls. Acta Cardiol. 1999; 54(4):215-25.
6. Delghandi M, Thangarajah R, Nilsen M, Grimsgaard S, Bonaa KH, Tonstad S, Jorgensen L . DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls. ActaCardiol., 1999; 54(4):215–225.
7. Fard-Esfahani P, Mohammadi-Torbati P, Khatami S, Zeinali S, Taghikhani M and Allahyari M. Familial Defective Apoliporrotein B 100: Frequency Of R3500q Mutation Of Apoliporotein B Gene In Iranian Hypercholesterolemic Patients. Acta MedicaIranic. 2005; 43(3): 193-196.
8. Friedewald WT, Levy RI, and Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem., 1972; 18:499 – 502.
9. Ghergerehchi R. Dyslipidemia in Iranian overweight and obese children. TherClin Risk Manag., 2009 ; 5: 739–743.
10. Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler K, Vogelstein B, editors. The Metabolic and Molecular Bases of Inherited Disease.New York: McGraw-Hill. 2001; 8: 2863 – 2913.
11. Hansen PS, Rüdiger N, Tybjaerg-Hansen A, Faergeman O, Gregersen N. Detection of the apoB-3500 mutation (glutamine for arginine) by gene amplification and cleavage with MspI. J Lipid Res., 1991; 32(7):1229-33.
12. Herbert, PN, Assmann G, Gotto AM Jr, Fredrickson DS. Familial lipoprotein deficiency: abetalipoproteinemia, hypobetalipoproteinemia, and Tangier disease. In: Stanbury JB, Wyngaarden JB,
13. Fredrickson DS, Goldstein JL, Brown MS. eds. The Metabolic Basis of Inherited Disease. 5th ed. New York: McGraw-Hill Book Co., New York .1983;587-621.
14. Hong SH, Song J, Kim JQ. The haplotype analyses using multiple markers of the apolipoprotein B gene in patients with coronary artery disease. J Korean Med Sci., 2001; 16(6):719-724.
15. Horri M and Vakili R. Evaluation of Cardiovascular and Lipid Profile Abnormalities in Obese Children and Adolescents. IJMS., 2006; 31(2):87-90
16. Howard BV, Ruotolo G and Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am., 2003 ;32(4):855-867.
17. Hu P, Qin YH, Jing CX, Lei FY, Chen P, Li MF. Association of polymorphisms at restriction enzyme recognition sites of apolipoprotein B and E gene with dyslipidemia in children undergoing primary nephrotic syndrome. Mol Biol Rep., 2009; 36(5):1015-21.
18. Innerarity TL, Mahley RW, Weisgraber KH, Bersot TP, Krauss RM, Vega GL, Grundy SM, Friedl W, Davignon J, McCarthy BJ. Familial defective apolipoprotein B-100: a mutation of apolipoprotein B that causes hypercholesterolemia. J Lipid Res., 1990; 31(8):1337-1349.
19. Innerarity TL, Weisgraber KH, Arnold KS, Mahley RW, Krauss RM, Vega GL, Grundy SM . Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. Proc Natl Acad Sci U S A., 1987; 4(19):6919-23.
20. Korsten-Reck U, Kromeyer-Hauschild K, Korsten K, Baumstark MW, Dickhuth HH, Berg A. Frequency of secondary dyslipidemia in obese children. Vasc Health Risk Manag, 2008; 4(5):1089-94.
21. Lai SW, Nga KC, Lin HF, Chen HL. Association between obesity and hyperlipidemia among children. Yale J Biol Med., 2001; 74(4): 205-210.
22. Lauer RM, Clarke WR, Burns TL. Obesity in childhood: the Muscatine study. Acta Paed Sin., 1997; 38(6): 432-437.
23. Lima SC, Arrais RF, Almeida MG, Souza ZM, Pedrosa LF. Plasma lipid profile and lipid peroxidation in overweight or obese children and adolescents. J Pediatr (Rio J). 2004; 80(1):23-8.
24. Ludwig EH, Hopkins PN, Allen A, Wu LL, Williams RR, Anderson JL, Ward RH, Lalouel

JM, Innerarity TL. Association of genetic variations in apolipoprotein B with hypercholesterolemia, coronary artery disease, and receptor binding of low density lipoproteins. J Lipid Res., 1997;38(7):1361-1373.

25. McBride D. Childhood obesity. Practice Nurse. 2010; 39 (11):40.
26. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS (2003): Prevalence of obesity, diabetes, and obesity-related health risk factors, .JAMA. 2001; 289(1):76-9.
27. Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP . The spread of the obesity epidemic in the United States, 1991-1998. JAMA, 1999; 282(16):1519-22.
28. Pan JP, Chiang AN, Tai JJ, Wang SP, Chang MS Restriction fragment length polymorphisms of apolipoprotein B gene in Chinese population with coronary heart disease. Clin Chem., 1995; 41(3):424–429.
29. Poirier P, Giles TD, Bray GA, *et al.* "Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss". Arterioscler.Thromb.Vasc. Biol., 2006;26 (5): 968–76.
30. Pullinger CR, Gaffney D, Gutierrez MM, Malloy MJ, Schumaker VN, Packard CJ, Kane JP.The apolipoprotein B R3531C mutation. Characteristics of 24 subjects from 9 kindreds. J Lipid Res, 1999; 40(2):318-327.
31. Pullinger CR, Hennessy LK, Chatterton JE, Liu W, Love JA, Mendel CM, Frost PH, Malloy MJ, Schumaker VN, Kane JP.Familial ligand-defective apolipoprotein B. Identification of a new mutation that decreases LDL receptor binding affinity. J Clin Invest., 1995; 95(3):1225-34.
32. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Pérusse L, Bouchard C. The humanobesitygene map: the 2005 update. Obesity (Silver Spring). 2006; 14(4):529-644.
33. Rantala M, Rantala TT, Savolainen MJ, Friedlander Y, Kesäniemi YA.Apolipoprotein B gene polymorphisms and serum lipids: meta-analysis of the role of genetic variation in responsiveness to diet. Am J ClinNutr, 2000; 71(3):713-24.
34. Raungaard B, Heath F, Hansen PS, Brorholt-Petersen JU, Jensen HK, Faergeman O. Flowcytometric assessment of LDL ligand function for detection of heterozygous familial defective apolipoproteinB-100. Clin Chem., 2000; 46(2): 224-233.
35. Robles-Osorio L, Ordoñez M, Aguilar-Salinas C, Aurón-Gómez M, Tusié-Luna M, Gómez J. Familial Hypercholesterolemia Due to Ligand-Defective Apolipoprotein B100. Archives of Medical Research, 2003;34(1):70-75.
36. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary event safter myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators', N Engl J Med. 1996;335(14):1001-1009.
37. Sambrook J, Fritsch E F, and Maniantis T .Molecular cloning .A laboratory manual 2th edition, cold spring Harbor, Laboratory press, New York;1989.
38. Shen H ,Damcott C M, Rampersaud E, Pollin TI, Horenstein R B, McArdle PF, Peyser P A, Bielak LF, Post WS, Chang Y C, Ryan K A, Miller M, Rumberger J A, Sheedy II P F, Shelton J, O'Connell J R, Shuldiner A R, Mitchell B D. Familial Defective ApolipoproteinB-100 and Increased Low-Density Lipoprotein Cholesterol and Coronary Artery Calcification in the Old Order Amish. Arch Intern Med., 2010; 170(20):1850-1855.
39. Simsek E, Balta H, Balta Z, Dallar Y. Childhood Obesity-Related Cardiovascular Risk Factors and Carotid Intima-Media Thickness. Turk J Pediatr., 2010; 52(6): 602-611.
40. Spady DK, Bilheimer DW, Dietschy JM. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. Proc Natl Acad Sci U S A., 1993; 80(11)3499-3503.
41. Talmud PJ, Barni N, Kessling AM, Carlsson P, Darnfors C, Bjursell G, Galton D, Wynn V, Kirk H, Hayden M R, Humphries SE. Apolipoprotein B gene variants are involved in the determination of serum cholesterol levels: a study in normo- and hypelipidaemic individuals. Atherosclerosis, 1987; 67(1) :81-89.
42. Teng YN, Pan JP, Chou SC, Tai DY, Lee-Chen GJ. Familial defective apolipoprotein B-100: detection and haplotype analysis of the Arg (3500)-->Gln mutation in hyperlipidemic Chinese. Atherosclerosis, 2000;152(2):385-90.
43. Tershakovec AM, Jawad AF, Stouffer NO, Elkasabany A, Srinivasan SR, Berenson GS. Persistent hypercholesterolemia is associated with the development of obesity among girls: the Bogalusa Heart Study. Am J Clin Nutr., 2002; 76(4):730-735.
44. Timirci O, Darendeliler F, Bas F, Arzu EH, Umit Z, Isbir T . Comparison of lipid profiles in relation to APOB EcoRI polymorphism in obese

children with hyperlipidemia. *In Vivo*, 2010; 24(1):65-9.

45. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum and lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. Am J Epidemiol, 1991; 133(9): 884-899.
46. Weisgraber KH, Innerarity TL, Newhouse YM, Young SG, Arnold KS, Krauss RM, Vega GL, Grundy SM, Mahley RW . Familial defective apolipoprotein B-100: enhanced binding of monoclonal antibodyMB47 to abnormal low density lipoproteins. ProcNatlAcadSci U S A., 1988; 85(24):9758-62.
47. Xu C, Nanjee N, Tikkanen MJ, Huttunen JK, Pietinen P, Biitler R, Angelico F, Del Ben M, Mazzarella B, Antonio R, Miller NG, Humphries S, and Talmud P J. Apolipoprotein B amino acid 3611 substitution from arginine to glutamine creates the Ag (h/i) epitope:the polymorphism is not associated with differences in serum cholesterol and apolipoprotein B levels. Hum Genet. 1989; 82: 322-326.
48. Zaman MM, Ikemoto S, Yoshiike N, Date C, Yokoyama T, Tanaka H. Association of apolipoprotein genetic polymorphisms with plasma cholesterol in a Japan serial population. Arterioscler Thromb Vasc Biol. 1997; 17(12):3495-3504.

1/12/2012