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Physio-Genetic Study on Lipoprotein Lipase Gene Polymorphisms in Non-Alcoholic Fatty Liver Patients.

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is common and may progress to cirrhosis and its complications. Lipoprotein lipase (LPL) is a member of the lipase gene family, has a fundamental role in transport and metabolism of plasma cholesterol. This study aims to study the Lipase Gene polymorphisms in non-alcoholic Fatty liver patients. This study has included 100 patients affected with obesity and fatty liver. They were selected from the Internal Medicine Hospital, Mansoura University, Mansoura Egypt, between the times of January 2012 to January 2014.The LPL gene polymorphic alleles were determined by PCR-RFLP that includes polymerase chain reaction for gene amplification followed by digestion with PVU II enzyme and analysis according to the size of digested amplified DNA. Fatty liver cases had a significantly higher frequency of the homozygous mutated LPL PVU II (+/+) genotype and also of the (+) allele particularly among fatty liver cases compared to controls. Cases with the (+/+) homozygous genotype showed significantly higher frequency of fatty liver, lower frequency of positive family history than those with the (+/-) and (-/-) genotypes. These cases have showed also higher levels of total cholesterol. This study showed a significant association between the LPL PVU II gene polymorphism and Fatty liver among Egypt ian cases particularly when complicated with the fatty liver.

Keywords: Lipoprotein Lipase , Gene Polymorphism , Non-alcoholic Fatty Liver.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is common and may progress to cirrhosis and its complications. The pathogenesis of steatosis and cellular injury is thought to be related mostly to insulin resistance and oxidative stress. Therefore, management entails identification and treatment of metabolic risk factors, improving insulin sensitivity, and increasing antioxidant defences in the liver. Weight loss and exercise improve insulin sensitivity.

Nonalcoholic fatty liver disease (NAFLD) occurs across all age groups and ethnicities and is recognised to occur in 14%–30% of the general population. Primary NAFLD is related to insulin resistance and thus frequently occurs as part of the metabolic changes that accompany obesity, diabetes, and hyperlipidaemia. However, it is important to exclude secondary causes of hepatic steatosis by clinical assessment *(Nomura, et al., 1988 ; Browning, et al., 2004*).

Subjects with NAFLD exhibit increased levels of oxidative stress and lipid peroxidation that may play a part in disease progression(*Sanyal, et al., 2001 ; Yesilova, et al., 2005*). Vitamin E is a potent antioxidant and has been evaluated among paediatric and adult patients with NAFLD .Two small pilot trials have shown reduction of ALT levels among adult and paediatric patients with Nonalcoholic steatohepatitis (NASH). Subsequently, two small randomised controlled trials have failed to show any benefit of vitamin E on ALT levels; one study randomised 16 adult subjects to vitamin E (800 IU/day) or no treatment over three months (*Kugelmas, et al., 2003*).

Nonalcoholic fatty liver disease (NAFLD) is progressively diagnosed worldwide and is considered to be the most common liver disorder in Western countries, estimated to affect at least one-quarter of the general population (*Rector, et al., 2008; Lazo and Clark, 2008*). NAFLD used to be almost exclusively a disease of adults but is now becoming a significant health issue also in obese children. The prevalence of childhood obesity has significantly increased over the past three decades (*Janssen, et al., 2005 and Park, et al., 2005*) and boosted the prevalence of NAFLD in adolescents (*Barshop, et al., 2008*).

NAFLD is also very common in type 1 diabetes and is strongly associated with increased prevalence ofCardiovascular Disease(CVD) independent of other confounding factors (*Targher , et al., 2010*) In addition to the liver-related causes, CVD represents the major survival risk of patients with NASH (*Ekstedt, et al., 2006*) However, the nature of the relationship NAFLD/CVD is still under debate. McKimmie and coauthors (*Mckimmie et al., 2008*) did not find independent association between hepatic steatosis and CVD in a subset of participants in Diabetes Heart Study. They suggested that hepatic steatosis is more a secondary phenomenon than adirect mediator of CVD. Even so, sufficient evidence exists that CVD risk assessment seems mandatory in NAFLD patients.

Abnormal lipoprotein concentration in plasma reflects disturbances in homeostasis of major lipid components of lipoproteins, triglycerides, cholesterol, and cholesterol esters. Excessive accumulation of triglycerides in the liver is the hall- mark of NAFLD. The potential sources of fat contributing to hepatic steatosis are dietary fatty acids through uptake of intestine-borne chylomicron remnants, increased lipolysis of peripheral fat store, and de novo synthesis. Tracer studies in obese humans with NASH demonstrated that 60% of triglycerides in the liver arose from free fatty acids, 25% from de novo lipogenesis, and 15% from the diet (*Donnelly, et al., 2005*).

Lipoprotein lipase (LPL) is a member of the lipase gene family, which includes pancreatic lipase, hepatic lipase, and endothelial lipase. It is a water soluble enzyme that hydrolyzes triglycerides in lipoproteins, such as those found in chylomicrons and very low-density lipoproteins (VLDL), into two free fatty acids and one monoacylglycerol molecule. It is also involved in promoting the cellular uptake of chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids (*Henderson, et al., 1994; Rinninger, et al., 1998 and Mead, et al., 2002*). LPL requires ApoC-II as a cofactor(*Kinnunen, et al., 1977 and Kim, et al., 2006*).

LPL is synthesized in a number of tissues and is regulated in a tissue-specific manner by nutrients and hormones, Life stage is also important to LPL gene expression and regulation. For example, LPL is expressed in the liver during fetal and early postnatal life, but gene expression is then suppressed by a putative

transcriptional regulatory mechanism (*Schoonjans, et al., 1993*). Thyroid hormone and glucocorticoids also play roles in the extinction of the hepatic expression of LPL (*Peinado, et al., 1992*). In the mammary gland, LPL activity is induced during late pregnancy and lactation (*Del Prado, et al., 1999*), and it appears that the partially dedifferentiated and delipidated adipocytes rather than the epithelial cell are the source of the lipase (*Jensen, et al., 1994*). Recent evidence indicates that prolactin works through prolactin receptors to reduce LPL activity in cultured human abdominal adiposetissue (*Ling, et al., 2003*). However, this effect has not been demonstrated in the mammary gland. In brown adipose tissue, cold exposure stimulates LPL activity by a combination of transcriptional and translational/posttranslational mechanisms that involve β-adrenergic stimulation (*Giralt, et al., 1990*). Both chronic and acute stress decrease LPL activity in white adipose tissue but increase the LPL activity through the effect of catecholamines in cardiac and skeletal muscle as well as in the adrenal glands (*Ricart-Jane, et al., 2005*).

MATERIALS AND METHODS

This study has included 100 subjects affected with obesity with fatty liver. They were selected from the Outpatient's Clinic, Department of Obesity and Diabetes, Specialized Internal Medicine Hospital, Mansoura University, Egypt, between the times of January 2012 to January 2014. Their age Mean±SD was 45.5±15.2 years ranging from 18-60 years. They were in the form of 40 (40%) males and 60 (60%) females. Of them, 40 (40%) were positive for parental consanguinity, 60 (60%) hadpositive family historydefinition of "metabolic syndrome" (Alberti and Zimmet, 1998; Grundy *et al*., 2005 and Alberti *et al*., 2005), while the rest of cases were not complicated and were termed "simple obesity". These cases were compared to100 (16 males and 84 females) normal healthy controls from thesamelocality of an age Mean±SD of 29.62±9.73 years. They were taken from blood donors after confirming that they are free from obesity, hypertension or other cardiovascular disorders in addition to a negative family history of similar conditions.

Measurements of lipids: After obtaining informed consent, blood samples were obtained from all cases and controls in the morning after fasting for 12 h. Immediately following clotting, serum was separated by centrifugation for 15 min at 3000 rpm.. The levels of TC (Total cholestrol) according to Allain et al.,(1974), TG (Triglyceride)according toFossati and Prencipel.,(1982), HDL-C (High Density Lipoprotein Cholesterol according to(Warnick and Wood (1995) and LDL-C (Low Density ,Lipoprotein Cholesterol in samples were determined by enzymatic methods with commercially available kits, (Friedewald et al.,1972).

DNA extraction, purification and amplification: Another venous blood samples (3 mL) were collected on EDTA (ethylenediamine tetra acetate) containing tubes, DNA was extracted promptly using DNA extraction and purification Kit (Gentra Systems, USA) according to manufacturer's instructions and then stored at -20°C till use(Alberti *et al*., 2005).

LPL gene amplification was carried out by PCR using the selected sequences for 5' and 3' primers: SB-75: 5'-ATG GCACCC ATG TGT AAG GTG-3' and SB-76: 5'GTG AAC TTC TGA TAA CAA TCT C-3' (Georges *et al*., 1996). Quality analysis of the PCR products (430 bp-long) was performed by electrophoresis with a 50 bp marker (Pharmacia Biotech, Uppsala, Sweden) on 1.5% agarose gel.

Restriction fragment polymorphism analysis (RFLP) of amplified DNA: Samples of PCR products (8 uL) were then incubated withPVU II restriction endonuclease (Boehringer) overnight at 37°C. The 430 bp-long product was digested to 320 and 110 bp-long products if there was aPVU II restriction site (+) and remain as it is if it is absent (-)(Georges *et al*., 1996). The digested DNA was electrophoresed on a 2% agarose gel stained with ethidium bromide (90 V/1 h), visualized under UV light and photographed. The length of each amplified DNA fragment was determined by comparing migration of a sample with that of standard DNA marker (Fig. 1, 2)

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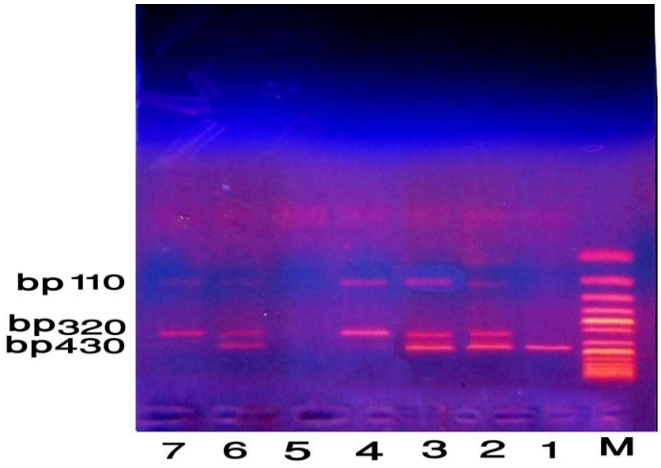
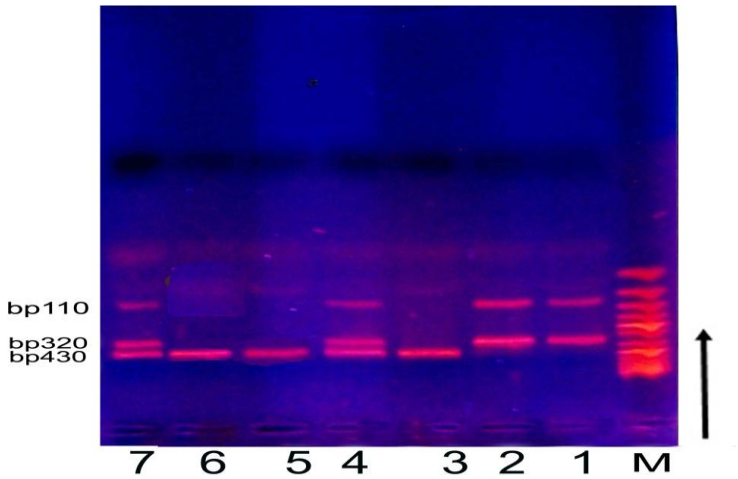


M= molecular marker
bp =base pair

Figure(1): **Digestion products of amplified segment of LPL gene (430 bp) using PVU II restriction enzyme showing lanes 4 and 7 digestion of both allele (+/+).Lane 1 no digestion of allele (-/-).Lane 2,3 and 6 digestion of alleles (+/-).**



M= molecular marker
bp =base pair

Figure(2): **Digestion products of amplified segment of LPL gene (430 bp) using PVU II restriction enzyme lanes 1 and 2 digestion of both allele (+/+).Lanes 3,5 and Lane 6 no digestion of allele(-/-).Lane 4 and 7 digestion of allele(+/-) observed.**

Statistical analysis: Statistical analysis of data was done using the software statistical package SPSS program version 17 (Chicago, USA). Student t-test was used to compare the numerical values related to lipid profile, body mass index and waist hip ratio, whereas Chi-square, Fisher exact and odds ratio with 95% confidence interval were used to compare frequencies of different genotypes and alleles among cases and controls

RESULTS

Table(1) shows lipid profile TC,TG,HDL-cand LDL-c of total cases was significantly higher than that of the controls.On the other hand, metabolic syndrome cases showed significantly higher TG with HDL-c levels than simple obesity casescomparison between cases with obesity and healthy controls regarding their

genotype and allelic distribution of LPL Gene PVU ll polymorphism is shown in Table(2) shows that obesity cases had a significantly higher frequency of the homozygous mutated (+/+)genotype of LPL Gene PVU ll polymorphism particularly among cases with metabolic syndromecompared to controls Cases of obesity had also a significantly higher frequencyof(+) allele LPL PVU ll gene polymorphism particularly also the metabolicsyndrome cases compared to controls Also from this Table(2) it is noted that simple obesity cases showed also a higher frequency of the homozygous mutated(+/+) genotype of LPL PVU ll gene polymorphism, this was statisticallynear significant.

On other hand, by comparing metabolic syndrome cases to simple obesity cases it noted that metabolic syndrome cases had a significantly higher frequency of the homozygous mutated(+/+) genotype of LPL Gene

PVU ll

Polymorphism (p<0.001,OR=9.86). Table(3) shows that cases with the (+/+) homozygous genotype showed significantly higher frequency of diabetes ,lower frequency of positive family history and lower values for waist hip ratio than those with the (+/-) and (-/-) genotypes. These cases have showed also higher levels of total cholesterol and LDL –c, yet not reaching statistical significance.

Table (1): Demographic data(general characteristics) of total cases of fatty liver compared to healthy controls

	Cases (n = 100)	Control (n = 83)	t	P
AGE (years)				
Age range	18 – 60	19 – 60		
Mean age ± SD	45.45 ± 15.29	45.55 ± 15.53	0.046	0.964
HB(g/dl)	11.31 ± 1.74	13.37 ± 1.93	7.586	< 0.001*
FBS(mg/dl)	131.83 ± 56.25	80.96 ± 9.31	8.898	< 0.001*
PBS(mg/dl)	223.23 ± 114.65	127.81 ± 23.39	8.122	< 0.001*
CHOL(mg/dl)	230.37 ± 48.58	82.89 ± 16.23	28.503	< 0.001*
LDL(mg/dl)	153.05 ± 34.31	83.98 ± 10.99	18.994	< 0.001*
HDL(mg/dl)	54.89 ± 11.44	60.96 ± 6.99	4.410	< 0.001*
TG(mg/dl)	131.43 ± 72.92	78.49 ± 14.87	7.084	< 0.001*
HbA1c%	7.00 ± 2.01	4.90 ± 0.38	10.270	< 0.001*
CPK(mg/dl)	156.75 ± 53.03	75.94 ± 13.41	14.684	< 0.001*
Consanguinity				
Positive	18 (18%)	0 (0%)	$\chi 2$= 19.780	< 0.001*
Negative	82 (82%)	83 (100%)		
Family history				
Positive	60 (60%)	0 (0%)	$\chi 2$= 85.714	< 0.001*
Negative	40 (40%)	83 (100%)		

* Significant P < 0.0

Table(2): Comparison between all cases with fatty liver and healthy controls regarding their genotype and allelic distribution of LpL gene polymorphisms.

	LPL	Cases (n = 100)	Control (n = 83)	P	OR (95% CI)
		N (%)	N (%)		
Genotypes	(-\-)	37 (37%)	43 (51.8%)	0.044*	0.55(0.30 – 0.99)
	(+\-)	36 (36%)	37 (44.6%)	0.238	0.70(0.39 – 1.27)
	(+\+)	27 (27%)	3 (3.6%)	<0.001*	9.86(2.87 – 33.89)
Alleles	(-)	110 (55%)	123 (74.1%)	<0.001*	0.43(0.27 – 0.67)
	(+)	90 (45%)	43 (25.9%)	<0.001*	1.63(1.24 – 2.15)

(%) = percentage of cases Significance using Fisher's Exact test:
* p= < 0.05 (Significant) ** p= < 0.001 (extremely Significant)

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Table (3): Comparison of the total cholesterol ,LDL,HDLand TG in the genotype of the LpL polymorphisms among cases and healthy controls

(Cholesterol) (mg/dl)			
Genotype	Cases (mean ± SD)	Control (mean ± SD)	P
(-\-)	238.95 ± 54.78	82.28 ± 16.37	< 0.001*
(+\-)	227.50 ± 50.21	82.22 ± 16.01	< 0.001*
(+\+)	222.44 ± 35.43	100.00 ± 10.00	< 0.001*

LDL-C(mg/dl)			
Genotype	Cases (mean ± SD)	Control (mean ± SD)	P
(-\-)	159.35 ± 41.30	84.14 ± 11.08	< 0.001*
(+\-)	149.39 ± 32.89	84.92 ± 10.70	< 0.001*
(+\+)	149.30 ± 23.96	70.00 ± 2.00	< 0.001*

HDL-C(mg/dl)			
Genotype	Cases (mean ± SD)	Control (mean ± SD)	P
(-\-)	54.41 ± 11.73	60.44 ± 6.94	< 0.008*
(+\-)	56.42 ± 12.39	61.65 ± 7.26	< 0.032*
(+\+)	53.52 ± 9.78	60.00 ± 5.00	0.273

TG(mg/dl)			
Genotype	Cases (mean ± SD)	Control (mean ± SD)	P
(-\-)	135.97 ± 81.59	78.14 ± 14.96	< 0.001*
(+\-)	127.72 ± 62.89	78.11 ± 15.20	< 0.001*
(+\+)	130.15 ± 75.23	88.33 ± 7.64	0.352

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome (*Marchesini, et al., 2003*) represents a spectrum of histopathologic abnormalities ranging from simple steatosis to the more aggressive non-alcoholic steatohepatitis (NASH), characterized by steatosis, parenchymal inflammation, hepatocellular ballooning and other evidence of hepatic injury (*Chalasani , et al.,2012*). Patients with NASH are at risk of developing progressive fibrosis; reported in up to 50% of cases over 6 years (*Musso , et al.,2011*). There is increasing recognition that NAFLD is a heterogeneous disease with multiple pathways of pathogenesis and patients with different phenotypes of NAFLD can present with diverse disease manifestations (*Younossi,et al., 2014*). Insulin resistance plays a dominant role in the pathogenesis of NAFLD (*Bugianesi, et al.,2005*). Patients with type 2 diabetes mellitus (DM) have an increased risk of developing NAFLD, NASH and hepatic fibrosis/cirrhosis (*Loomba, et al.,2012*). Furthermore, NAFLD patients with DM have three times the mortality compared to non-diabetic NAFLD patients (*Younossi, et al.,2004*). The importance of DM in NAFLD is reflected by its inclusion in the majority of the non-invasive composite predictive scores for NASH and advanced fibrosis (*Angulo, et al.,2007*). One such composite predictive score for predicting advanced fibrosis inNAFLD is the NAFLD fibrosis score (NFS), which has been validated and recommended for use in the American Society guidelines (*Chalasani., et al., 2012*).

Reiterating disease heterogeneity and that NAFLD may not conform to a “one size fits all approach”, McPherson and colleagues had reported a difference in the reliability of NFS in the context of normal and abnormal ALT levels (*McPherson, et al., 2013*).Other non-invasive fibrosis scores such as the BARD score and AST/ALT ratio have also been used to predict advanced fibrosis in NAFLD. This study sought to characterize the clinical spectrum of NAFLD in patients with and without DM. In addition, the study explored the utility of NFS and other established non-invasive fibrosis scores among these two groups.

Obesity is now recognized as the most prevalent metabolic disease worldwide, reaching epidemic proportions in both developed and non -developing countries and affecting not only adults but also children and adolescents. The prevalence of obesity has increased substantially over the past 30 years*(Christakis and Fowler, 2007).*

Obesity is a state of excess adipose tissue mass *(Spiegelman and Flier, 2001).*Prevalence is approximately 30% and is rapidly rising *(Misra and Khurana, 2008).*Obesity leads to increased morbidity more

than mortality and increases the prevalence of co-morbidities like hypertension, type 2 diabetes mellitus, dyslipidemia, endocrinal abnormalities and higher mortality from some cancers like esophagus, colon, rectum and breast*(Spiegelman and Flier, 2001).*

Lipoprotein lipase (LPL) is a key enzyme in lipoprotein metabolism. It was discovered in 1943 to play a central role in lipid metabolism by hydrolyzing triglyceride-rich particles in muscle, adipose tissue, and macrophages, thereby generating free fatty acids and glycerol for energy utilization and storage *(Goldberg, 1996).*LPL also plays a non catalytic bridging role as a ligand in lipoprotein-cell surface interactions and receptor-mediated uptake of lipoproteins with its ability to bind simultaneously to both lipoproteins and cell surface receptors *(Beisiegel and Weber, 1991).*The LPL gene is located on chromosome 8p22. It contains 10 exons and encodes a 448-amino acid mature protein *(Fisher and Humphries 1997).*

So far, more than 100 mutations have been identified in the LPL gene*(Murthy and Julien ,1996).*Dozens of rare mutations have been associated with markedly reduced enzyme activity *(Murthy and Julien , 1996),*whereas several relatively common variants have been associated with moderate changes in LPL catalytic function. The highly polymorphic LPL gene and its many single nucleotide polymorphisms in both the coding region and the non coding region have been studied for associations with lipids, lipoproteins, and atherosclerosis. The majority of these mutations are rare, although they can appear at a relatively high prevalence in specific subpopulations *(Wong and Schotz, 2002).*

In this work, it was explored the association of genetic polymorphisms of lipoprotein lipase PVU II site in the LPL gene, with clinical variables including gender, age of onset and biological variables as triglycerides, total cholesterol, HDL-C, and LDL-C, among Egyptian fatty liver cases.

Thus, a cohort sample including 100 cases presenting with obesity were studied regarding their genotype distribution of LPL gene polymorphisms. Their mean ± SD age was(45.4) ± (15.2)years ranging from (18-60)years. They were in the form of (40) males and(60)females. Also we compared cases according to consanguinity as being positive among 21 cases where negative cases were(79) Also we compared cases according to family history where positive cases(60)while negative cases (40). These cases were compared to (100) healthy unrelated subjects as controls.

The results obtained regarding the LPL polymorphisms showed that the frequency of homozygous mutated (+/+) genotype and mutant (+) allele of LPL gene polymorphism were significantly higher among cases-of obesity compared to controls (p=0.01).Thus (+/+) genotype and (+) allele may be considered as genetic risk factors for obesity

These results are in agreement with results of *(Shinji, et al 2012)*. Who reported that deregulation of LPL has been reported to contribute to many human diseases, such as atherosclerosis, chylomicronaemia, obesity, and type 2 diabetes. Recently, it has been reported that *LPL* gene deficiency, such as due to chromosome 8p22loss, *LPL* gene polymorphism, and epigenetic changes in its promoter region gene, increases cancer risk, especially in the prostate among Japan population.

These results are also in agreement with Wang, *et al., (2011)*, who studies LPL PVU II T>C in an LPL gene and measured the serum lipid levels in a case-control study of 124 obese children and 346 frequency-matched normal controls in preschool Chinese children. The variant genotypes of LPL PVUII CC (+/+) were associated with a significantly increased risk of childhood obesity among Chinese population.

In the present study it is proved that, non significant lower frequency of heterozygous mutant (+/-) genotype of LPL gene polymorphism was observed among cases of obesity compared to controls. So this genotype may be considered as low risk or protective genotype. This supports the autosomal recessive mode of the effect of this gene so that the homozygous mutant (+/+) is associated with the manifestation of obesity.

On the other hand, comparing cases of metabolic syndrome to that of healthy controls regarding the distribution of LPL geno types, it is noted that metabolic syndrome cases had a highly significant higher frequency of the homozygous mutated (+/+) genotype of LPL polymorphism (28:7% vs. 3,6%, p<.0001). while had no significant difference of the frequency of the heterozygous mutated (+/-),(-/-) genotype of LPL polymorphism compared to controls (31.5% vs. 44.5%, p =0.15 and 40.3% vs. 51.8%,p=0.22 respectively), and

by comparing the lipid levels between metabolic syndrome cases and healthy controls regarding LPL polymorphism, it was noted that the values of serum TC, HDL- C and LDL-C levels in metabolic syndrome cases were extremely significantly higher than those in healthy control individuals (P < 0.001 for each).

Moreover by comparing cases of simple obesity to that of healthy controls regarding the distribution of LPL genotypes, it is noted that simple obesity cases had a just (near) significantly higher frequency of the homozygo mutated (+/+) genotype of LPL polymorphism While they had no significant difference of the frequency of the heterozygous mutated (+/-), (-/-) genotype of LPL polymorphism compared to controls. Also by comparing the lipid levels between metabolic syndrome cases and simple obesity regarding LPL polymorphism it was noted that the values of serum HDL-Clevels in metabolic syndrome cases were significantly lower than those in simple obesity cases (p = 0.02 and P = 0.01, respectively), whereas serum TG levels in metabolic syndrome cases were extremely significantly higher than those in simple obesity cases (P = 0.001). There were no significant differences of serum TC and LDL-C levels between the two groups.

While by comparing the lipid levels between simple obesity cases and healthy controls regarding LPL polymorphism, it was noted that the values of TC, HDL-C and LDL-C levels in simple obesity cases were extremely significantly higher than those in healthy control individuals (P <0.001for each). Also, serum TG levels in simple obesity cases were significantly higher than those in health control cases.

These results might indicate that the mutant (+/+) genotype of the LPL gene is more associated with complicated obesity in the form of metabolic syndrome which denotes affection with obesity associated with hypertension, diabetes and dyslipidemias. Also these results are in agreement with results of *(Katedra etaL, 2011)*,who stated that, lipoprotein lipase (LPL) plays a central role in dyslipidemia and development of metabolic syndrome. The occurrence of polymorphisms of the LPL gene may result in the disturbance in the lipid metabolism among Polish population. The results of this work are also in agreement with Das (2009),who stated that, LPL may have strong genetic association with hypertensive individuals among Indian population. Hypertriglyceridemia and decreased adipose tissue LPL activity occur commonly in diabetic subjects ; therefore, it is relevant that the association we found was independent of triglyceride levels. Whether or not obese patients homozygous for the PVU II (+/+) genotype are at increased risk of developing non-insulin-dependent diabetes requires further exploration.

Conversely, these results are not consistent with the results of *Shen et al.,(2000),*who stated that the LPL PVU II is not significantly associated with type 2 diabetes mellitus in Chinese population, while agree with *Kisfali et al., (2010*), who stated that, the number of candidate genes in metabolic syndrome and coronary heart disease susceptibility increases very rapidly from the growing spectrum of the genes influencing lipid metabolism like the LPL among Hungarian population.

Comparing case-subgroups according to their family historyas regard their studied genotypes, it is noted that cases with positive family history of obesity has a significantly higher frequency of the heterozygous mutant (+/-) genotype. Whereas, cases with negative family history showed a significantly higher frequency of the wild type or normal (-/-) genotype. This supports the familial nature of obesity particularly when associated with the (+) mutant allele. Interestingly, however, the (+/+) genotype was not significantly different when comparing cases with positive to that with negative family history. This may be due to the fact that most of our cases were taken from the outpatient ambulatory cases when it is expected that most of the cases with the (-/+) genotype will have a severe form of complicated obesity that requires inpatient or intensive care unit (ICU) So, we recommend taking a wider scale sample of cases including inpatient and ICU cases to get a proper picture of the familial pattern of the disease.

Comparing case-subgroups according to their lipid profile, as regard their studied genotypes, significant differences in all lipid profile levels was noted among cases compared to controls. These results are in accordance with *Surendran et al .,(2012*), who stated that the severe forms of hypertriglyeeridaemia (HTG) are caused by mutations in genes that lead to the loss of function of lipoprotein lipase (LPL) and also in agreement with *Smart et al.,(2010),*who demonstrated that these common variants are associated with lipid levels in a healthy pediatric cohort, suggesting that even in these young children there may be potential in predicting their lifelong exposure to an adverse lipid profile. These results are also coincide with results of *Voruganti et al.,(2010)*,who stated that there is strong genetic influence on plasma fatty acid distribution and that genetic variation in LPL may play role in plasma fatty acid distribution among U.SA population. Conversely

these results are in disagreement with results of *Jemaa et al., (1995)*, who stated that, the PVUII and polymorphisms did not exhibit any significant association with the biochemical traits (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein) among French population.

Interestingly, although the (+/+) genotype had a higher value of cholesterol, HDL, LDL and TG among case than controls, it was statistically non significant. This may be due to the very rough and robust statistical estimation resulting from the fact that only 3 subjects of the controls had this genotype.

However by comparing cases with healthy controls according to their waist as regard their studied genotypes, there was a significant difference in distribution of LPL genotypes.

While comparing case-sub groups according, to their waist as regard their studied genotypes, significant differences in distribution of LPL genotypes among cases of obesity was noted. While comparing two sex groups of obesity subjects regarding their LPL genotypes there are no any significant differences between two sex groups (P =0.54). However comparing two consanguinity groups of obesity subjects regarding their LPL genotypes, there are no significant differences between two sex groups (P =0.45).

While these results are in agreement with results of *Sertic et al .,(2009),*in this study , LPL genetic polymer variants could represent predictive genetic risk markers for obesity -related metabolic disorders in young healthy subjects Mediterranean type of diet is also an important protective factor against abdominal obesity.

These results are also in agreement with results of *Liu et al.,(2005),*who stated that, LPL PVU II polymorphisms are determinants plasma of LPL concentration among Chinese population.

While these results are in agreement with results of Zhu et al.,(2003),who stated that, the conclusions could be primarily drawn that the variants of LPL-PVUII locus were important determinants of variation in serum cholesterol response to dietary change in hyperlipidemia population among Chinese population. While these results are in agreement PVUII (+/+) genotype were significantly more likely to have diabetes.

CONCLUSION

This study showed a significant association between the LPL PVU II gene polymorphism and Fatty liver when complicated with metabolic syndrome among Egyptian cases. The genotype (+/+) was mostly associated with the risk of complicated obesity. Although, Lipid profile was significantly higher among fatty liver cases compared to controls irrespective of the LPL genotype variants, it was non-significantly different between cases subgroups related to different LPL genotypes.

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