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Non Alcoholic Fatty Liver Disease, Insulin Resistance, Dyslipidemia and Atherogenic Ratios in Epileptic Children and Adolescents on Long Term Antiepileptic Drug Therapy

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Abstract: This study explores the occurrence of Non-alcoholic Fatty Liver Disease (NAFLD), Insulin Resistance (IR), dyslipidemia and atherogenic ratios in epileptic children and adolescents receiving Valproic Acid (VPA), Carbamazepine (CBZ) or both (combination therapy) compared to healthy controls. Abdominal Computerized Tomography (CT), measurements of serum fasting insulin, glucose, serum lipids and liver enzymes were performed in VPA (n = 14), CBZ (n = 14) or both (n = 10) treated non-diabetic non-obese epileptic patients compared to healthy controls (n = 10). Abdominal CT demonstrated characteristics of fatty liver disease in 42.8% of VPA, in 21.4% of CBZ, in 60% of combination therapy treated patients and none of the healthy controls. All of them were overweight and 53.3% had IR. In conclusion VPA therapy was associated with increased risk of IR and NAFLD, while CBZ therapy was associated with dyslipidemia and combination therapy was associated with all these risks.

Key words: Epilepsy, insulin resistance, dyslipidemia, non-alcoholic fatty liver disease

INTRODUCTION

Epilepsy is not a disease, but a syndrome of different cerebral disorders of the Central Nervous System (CNS) which is characterized by paroxysmal, excessive and hypersynchronous discharges of large numbers of neurons (Nikalje *et al*., 2011). It is very often a disabling condition, rendered especially disturbing because of its unpredictability and its seriousness for being a common neurological disorder worldwide (Ahmad, 2011).

Multiple side effects of commonly used antiepileptic drugs which become obvious with high doses and in combination therapy directed researches toward the possible use of natural products in management strategies of epilepsy (Abdel-Wahab and Metwally, 2011).

Long term antiepileptic therapy in patients with epilepsy has long been associated with adverse metabolic changes. First generation AED such as VPA and CBZ has an increased potential for interactions and side effects due to enzyme induction and/or inhibition. (Nejad *et al*., 2009).

Several studies have demonstrated weight gain, hyperinsulinemia and IR in those receiving VPA (Verrotti *et al*., 2009, 2002), others demonstrated dyslipidemia and increased risk of atherosclerosis in those receiving CBZ therapy (Demircioolu *et al*., 2000; Eiris *et al*., 1995; Franzoni *et al*., 1992; Sonmez *et al*., 2006; Verrotti *et al*., 1997).

Non Alcoholic Fatty Liver Disease (NAFLD) which is a clinicopathological syndrome that ranges from simple steatosis to steatohepatitis, fibrosis or cirrhosis (Ramzan *et al*., 2009) is closely associated with visceral adiposity, dyslipidemia and insulin resistance and has been described as the hepatic component of the metabolic syndrome (Akbar and Kawther, 2006; Manco *et al*., 2008).

Ultrasound-diagnosed NAFLD was previously demonstrated in adults (Luef *et al*., 2004) and in obese adolescents (Verrotti *et al*., 2011) undergoing long-term treatment with VPA. Moreover, Gastaldelli *et al*. (2009) showed that the presence of fatty liver assessed by fatty liver index was significantly associated with increased Coronary Artery Disease (CAD) risk and reduced insulin sensitivity in non diabetic subjects. Recently, it is strongly suggested that NAFLD is likely to be a marker as well as an early mediator of atherosclerosis (Targher and Arcaro, 2007).

Therefore, we choose to study IR, dyslipidemia, atherogenic ratios and NAFLD in children and adolescents with epilepsy on long term antiepileptic therapy as indicators of metabolic derangements due to

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the impact of dietary programming and early metabolic changes on long term cardiovascular risk.

MATERIALS AND METHODS

The study was a cross-sectional case-control study that included 38 children and adolescents with epilepsy and epileptic syndromes classified according to the recommendations of the International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Engel and International League Against Epilepsy (ILAE, 2001). Diagnosis was made on the basis of clinical history, seizure semiology, EEG and brain imaging. Patients were treated with VPA (n = 14), CBZ (n = 14), or both (combination therapy) (n = 10) for at least 18 months. AEDs dose was unchanged during the last 3 months. Liver function tests done before AED therapy was started and at routine laboratory tests during the first 6-month period were normal in all patients. Moreover, each group was further divided equally using the BMI into overweight and lean subjects (WHO, 2006; De Onis *et al*., 2007). They were 21 males and 17 females with ages ranging between 2.5 and 14 years and a mean age of (8.40±3.42) years. They were recruited from those following up in the Pediatric Neurology Clinic, Children's Hospital, Ain Shams University in the period from October 2009 and February 2010. Ten children and adolescents, selected to match the patients group for age, sex, weight and socioeconomic aspects with no history suggesting epilepsy or other medical, neurological or psychiatric disorders served as controls. They were 5 males and 5 females with ages ranging between 3.5 and 13 years and a mean age of 7.70±3.19 years.

Subjects with any condition or medication that may affect the liver, body weight and lipid profile or glucose metabolism (eg., obesity, diabetes, metabolic, endocrine, liver diseases and family history of atherosclerosis) were excluded from the study.

Methods: The local ethical committee approved the study and consents were obtained from the parents of the included subjects. Detailed medical history and clinical assessment were done with special emphasis on the duration of the disease, type of seizures, its frequency and calculation of AED cumulative dose.

Measurements: Height and weight were measured using a wall-mounted stadiometer and a calibrated weight scale, respectively, wearing underwear only. Body-mass Index (BMI) was calculated by using the formula: BMI = weight (kg)/height (m^2). Blood pressure was obtained using a mercury sphygmomanometer and cuff sizes were selected so that the inflatable bladder width was at least 40% of the arm circumference. The cutoffs for overweight (WHO, 2006; De Onis *et al*., 2007) and elevated blood pressure (systolic or diastolic) (NHBPEP, 2004) were fixed.

Laboratory workup: Venous blood was collected from patients and controls after 12-14 h fasting. The samples were divided into two aliquots. One was taken on plain tubes and the serum sample was used for assay of liver function, fasting glucose, fasting insulin, lipid profile and AED drug levels. The other aliquot was taken on EDTA and plasma was stored at -20°C for subsequent measurement of Apolipoprotein A and B.

Serum glucose level, Total Cholesterol (TC) and Triglycerides (TG) were assayed using Synchron CX9 system auto analyzer applying enzymatic colorimetric method (Carl *et al*., 2006; Dietschy *et al*., 1976; McGowan *et al*., 1983).

High density lipoprotein-cholesterol (HDL-C) was assayed also on Synchron CX9 after precipitation of Low Density Lipoprotein-cholesterol (LDL-C) and Very Low Density Lipoprotein (VLDL) by dextran sulphate and magnesium in the separating reagent (Assmann *et al*., 1983). LDL-C was calculated according to Friedwald equation: LDL-C = TC-(HDL-C+TG/5) (Warnick *et al*., 1990).

Apolipoproten A and B were assayed by RID (Sindarid, Birmingham, United Kingdom). The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate monospecific antibody.

Insulin was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxSYM* for the quantitative determination of insulin in human serum (Abbott Ireland, Diagnostic Division-Lisnamuck, Longford Co. Longford, Ireland).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the equation:

$$\text{HOMA-IR} = \frac{\text{fasting glucose (mg dL}^{-1}) \times \text{fasting Insulin (IU mL}^{-1})}{405}$$

The cutoffs for high TGs (≥110 mg dL^{-1}), low HDL-C (≤40 mg dL^{-1}) and high fasting glucose (≥110 mg dL^{-1}) (Cook *et al*., 2003). Cutoffs for high TC (≥200 mg dL^{-1}) and high LDL-C (≥130 mg dL^{-1}) (National Cholesterol Education Program (NCEP, 1994). Abnormal ALT (>40 U L^{-1}) (Park *et al*., 2005) and insulin resistance (HOMA-IR≥3.16) (Keskin *et al*., 2005) were fixed.

CT scan of the abdomen: Assessment of the liver fat content was done by a non-contrast abdominal CT scan.

The children were sedated with chloral hydrate (50 mg kg^{-1}) before scan according to standard radiological practice. To reduce radiation exposure, a single cross sectional CT scan 10 mm thickness was taken at the level of the intervertebral disc between the body of the 12th thoracic and 1st lumbar vertebrae to include both lobes of the liver and the spleen. For all scans, the window level and the window width were kept constant and the machine was operated in the tissue optimization mode. A Region of Interest (ROI) was placed on 3 areas of the liver and 1 area of the spleen: at depths of 1.5 and 3.0 cm from the liver capsule on the right lobe of the liver, at a depth of 1.5 cm from the liver capsule on the left lobe and at a depth of 1.5 cm from the splenic capsule on the spleen. Splenic attenuation was measured from the later RIO. Care was taken not to include major portal, arterial and venous vessels. For each ROI, the attenuation measured in Hounsfield Units (HU) was recorded. The mean liver attenuation was calculated from the 3 liver ROIs and the ratio of mean liver attenuation to spleen attenuation (L:S) was ascertained. L:S is inversely related to hepatic fat content such that the lower the L:S, the greater the amount of fat in the liver and a ratio <1 denotes significant hepatic steatosis (Rockall *et al*., 2003).

Statistical analysis: Standard computer program SPSS for Windows, release 10.0 (SPSS Inc, USA) was used for data entry and analysis. Comparison of different variables in various groups was done using student t test and Mann Whitney test for normal and nonparametric variables respectively. Qualitative variables were compared using chi square test. Spearman's correlation test was used for correlating non-parametric variables. For all tests a probability $p<0.05$ was considered significant. Graphic presentation of the results was also done (Daniel, 1995).

RESULTS

Baseline clinical characteristics of patients and controls are presented in Table 1. A total of thirty-eight patients and ten controls fulfilling the inclusion criteria agreed to participate in the study.

Lipid profile, atherogenic ratios and apolipoproteins: The results of our study showed increased TC levels in 21.4% of VPA, 78.5% of CBZ and 80% of combination therapy groups. LDL-C levels were also increased in 21.4% of VPA, 78.5% of CBZ and 90% of combination therapy groups. TC and LDL-C levels were significantly higher in the CBZ and combination groups when compared to VPA and control groups, as shown in Table 2.

TG and HDL-C levels were within normal range for age and sex in all groups. However, HDL-C levels were significantly higher in the CBZ group when compared to VPA group ($p<0.05$). TG and Apo B100 levels were not significantly different between the studied groups, whereas, Apo-A1 levels were significantly higher in the CBZ group when compared to control group ($p<0.001$). Atherogenic ratios were significantly higher in combination therapy group when compared to controls ($p<0.05$).

Body mass index (BMI), blood pressure and the fasting methods for assessment of insulin resistance: BMI and blood pressure measurements were comparable between the studied groups.

IR was demonstrated in 16 (42%) of our patients, they were as follows 9 (64.3%), 7 (70%) and none in VPA, combination therapy and CBZ treated patients, respectively. They were 6 males and 10 females.

The HOMA-IR index was significantly higher and G/I ratio was significantly lower in the VPA, CBZ and combination therapy treated groups when compared to controls and in the VPA when compared to CBZ treated group as shown in Table 3.

Liver enzymes and liver/splenic attenuation: Liver enzymes were significantly higher in the VPA and combination therapy treated groups when compared to controls ($p<0.05$) but they were within the normal reference ranges for age and sex.

NAFLD was demonstrated in 15 (39.4 %) of our patients, they were as follows 6 (42.8%), 3 (21.4%) and 6 (60%) in VPA, CBZ and combination therapy treated patients, respectively. They were 7 males and 8 females, all of them were overweight and 8 (53.3%) had IR. Of note,

Table 1: Clinical and treatment data of the included patients

	Groups (Mean±SD)		
Variables	VPA (n = 14)	CBZ (n = 14)	Combination therapy (n = 10)
Age (years)	8.11±3.66	9.30±3.17	7.60±3.46
Duration of AED therapy (months)	42.00±26.73	41.57±28.38	47.40±28.90
VPA cumulative dose (g)	26.93±18.17		20.20±12.44
Serum level of VPA (μg mL^{-1})	70.59±5.70		72.34±7.53
CBZ cumulative dose (g)		15.40±10.42	12.60±6.43
Serum level of CBZ (μg mL^{-1})		7.29±0.95	13.19±19.13

Table 2: Comparison of the lipid profile, atherogenic ratios and apolipoproteins between the studied groups

Variables	VPA group (group I) Mean±SD median (IQR) (n = 14)	CBZ group (group II) Mean±SD median (IQR) (n=14)	Combination therapy group (group III) Mean±SD median (IQR) (n = 10)	Controls (group IV) Mean±SD median (IQR) (n = 10)	Group I vs. IV t/z*(p)	Group II vs. IV t/z*(p)	Group III vs. IV t/z*(p)	Group I vs. II t/z*(p)
TC (mg dL^{-1})	181.57±17.85	225.36±18.20	228.30±19.20	176.70±12.02	-0.70*	-4.00*	-3.80*	-3.80*
	179.00 (35)	232.00 (21)	230.00 (26)	177.50 (20)	(>0.05)	(<0.001)	(<0.001)	(<0.001)
TG (mg dL^{-1})	106.79±28.70	98.07±16.20	108.40±32.03	95.10±15.60	1.20	0.50	1.20	1.00
	106.50 (45)	99.50 (21)	102.50 (61)	97.50 (20)	(>0.05)	(>0.05)	(>0.05)	(>0.05)
LDL-C (mg dL^{-1})	105.57±24.00	141.14±24.90	151.30±14.47	98.00±15.40	-0.50*	-3.40*	-3.80*	-3.10*
	96.50 (40)	145.50 (39)	146.50 (25)	100.50 (27)	(>0.05)	(<0.001)	(<0.001)	(<0.05)
HDL-C (mg dL^{-1})	54.57±11.50	64.50±13.28	55.30±14.82	59.60±10.80	-1.10	1.00	-0.70	-2.10
	56.50 (21)	61.50 (16)	53.00 (27)	59.50 (13)	(>0.05)	(>0.05)	(>0.05)	(<0.05)
TC/HDL ratio	3.48±0.87	3.65±0.82	4.31±0.84	3.06±0.60	-1.30*	-1.80*	-3.10*	-0.60*
	3.20 (1)	3.70 (1)	4.50 (2)	3.00 (1)	(>0.05)	(>0.05)	(<0.05)	(>0.05)
LDL/HDL ratio	2.05±0.81	2.32±0.76	2.90±0.80	1.74±0.60	-1.00*	-2.00*	-3.00*	-0.80*
	1.90 (1. 5)	2.40 (1.1)	3.10 (1.7)	1.70 (0.7)	(>0.05)	(>0.05)	(<0.05)	(>0.05)
Apo A1 (mg dL^{-1})	206.86±78.40	254.50±57.90	228.10±98.30	180.00±63.90	0.89	2.98	1.30	-1.80
	190.50 (112)	248.50 (89)	210.50 (157)	182.50 (115)	(>0.05)	(<0.001)	(>0.05)	(>0.05)
Apo B100 (mg dL^{-1})	181.64±55.11	199.00±56.88	179.80±52.60	162.40±66.07	0.78	1.45	0.65	-0.80
	178.00 (81)	204.50 (77)	174.00 (50)	164.50 (112)	(>0.05)	(>0.05)	(>0.05)	(>0.05)

TC: Total cholesterol, TG: Triglycerides, LDL-C: Low density lipoproteins, HDL-C: High density lipoproteins; Atherogenic ratios = TC/HDL ratio and LDL/HDL ratio, Apo A1: Apolipoprotein A1 and Apo B100: Apolipoprotein B100; *Non-parametric data detected by Shapiro-Wilk test and presented as median (interquartile range). The test of significance used here is Mann-Whituey test $p<0.05$ is significant, $p<0.01$ is highly significant, $p<0.001$ is very highly significant and $p>0.05$ is non-significant

Table 3: Comparison of BMI, blood pressure, HOMA-IR, G/I ratio, liver enzymes and liver/splenic attenuation between the studied groups

Variables	VPA group Mean±SD median (IQR) (n = 14)	CBZ group Mean±SD median (IQR) (n=14)	Combination therapy group Mean±SD median (IQR) (n = 10)	Controls Mean±SD median (IQR) (n = 10)	Group I vs. IV t/z*(p)	Group II vs. IV t/z*(p)	Group III vs. IV t/z*(p)	Group I vs. II t/z*(p)
BMI	17.77±2.73	17.69±2.70	17.87±2.08	17.11±2.07	-0.41*	-0.35*	-0.91*	-0.18*
	17.50 (2.2)	17.15 (4.4)	17.50 (1)	17.20 (3.3)	(>0.05)	(>0.05)	(>0.05)	(>0.05)
Systolic BP	109.64±5.71	112.50±6.43	105.00±7.82	106.50±5.80	-1.30*	-1.60*	-0.40*	-1.20*
	110 (10)	112.50 (11)	105 (11)	105 (11)	(>0.05)	(>0.05)	(>0.05)	(>0.05)
Diastolic BP	71.79±4.20	72.14±4.67	63.50±20.28	70.50±6.85	-0.60*	-0.60*	-0.60*	-0.30*
	70 (5)	75 (6)	67.50 (14)	70.00 (11)	(>0.05)	(>0.05)	(>0.05)	(>0.05)
HOMA-IR	3.37±0.69	2.95±0.28	3.39±0.54	2.62±0.22	3.30	3.10	4.20	-2.60
	3.50 (1.2)	2.95 (0.3)	3.50 (0.8)	2.60 (0.4)	(<0.05)	(<0.05)	(<0.05)	(<0.05)
G/I ratio	5.81±0.60	6.64±0.73	5.85±0.70	7.88±0.70	-7.80	-4.10	-6.40	-3.30
	5.80 (1)	6.70 (1)	6.00 (1)	7.80 (1)	(<0.001)	(<0.001)	(<0.001)	(<0.05)
ALT (IU L^{-1})	20.14±7.02	16.57±5.30	18.00±2.83	15.40±2.20	2.40	0.70	2.30	1.50
	20.50 (8)	17.50 (8)	18.00 (9)	15.00 (7)	(<0.05)	(>0.05)	(<0.05)	(>0.05)
AST (IU L^{-1})	23.71±7.70	19.71±5.82	23.30±6.40	17.10±2.73	2.60	1.30	2.80	1.60
	23.50 (11)	20.50 (10)	22.50 (10)	17.00 (5)	(<0.05)	(>0.05)	(<0.05)	(>0.05)
L/S attenuation	1.02±0.08	1.04±0.09	0.97±0.88	1.25±0.06	-7.60	-6.20	-8.20	-0.70
	1.05 (0.16)	1.03 (0.13)	0.94 (0.17)	1.28 (0.10)	(<0.001)	(<0.001)	(<0.001)	(>0.05)

BMI: Body mass index, BP: Blood pressure, FBG: Fasting blood glucose, FI: Fasting insulin, HOMA-IR: Homeostasis model assessment of insulin resistance = multiplying fasting serum insulin (μIU mL^{-1}) and fasting plasma glucose (mg dL^{-1}) divided by 405, G/I ratio: Glucose/insulin ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and L/S attenuation: Liver/spleen attenuation

the serum levels of VPA and CBZ were within the normal ranges excluding a toxic drug effect.

The median values of L/S attenuation were significantly lower among all studied subgroups when compared to controls ($p<0.001$) as shown in Table 3 without significant difference between subgroups.

Subgroup group analysis: We compared overweight versus lean patient groups and found that 15 patients (78.9%) of the overweight group had NAFLD. TG levels were significantly increased in the overweight group when compared to lean group ($t = -2.10$, $p<0.05$).The rest of laboratory results were otherwise comparable between both groups.

Correlation studies: Our results showed significant negative correlation between L/S attenuation and VPA, CBZ cumulative doses and the duration of AED therapy

Table 4: Correlation between liver/spleen attenuation and BMI, lipid profile, HOMA and G/I ratio

	BMI (mg dL^{-1})		TC (mg dL^{-1})		TG (mg dL^{-1})		LDL-C (mg dL^{-1})		HDL-C (mg dL^{-1})		TC/HDL ratio		LDL/HDL ratio		Apo A1		Apo B100		HOMA-IR		G/I ratio	
Variables	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
L/S rattenuation	-0.11	>0.05	-0.10	>0.05	-0.29	>0.05	-0.10	>0.05	-0.04	>0.05	-0.08	>0.05	-0.05	>0.05	0.03	>0.05	-0.07	>0.05	-0.16	>0.05	0.04	>0.05

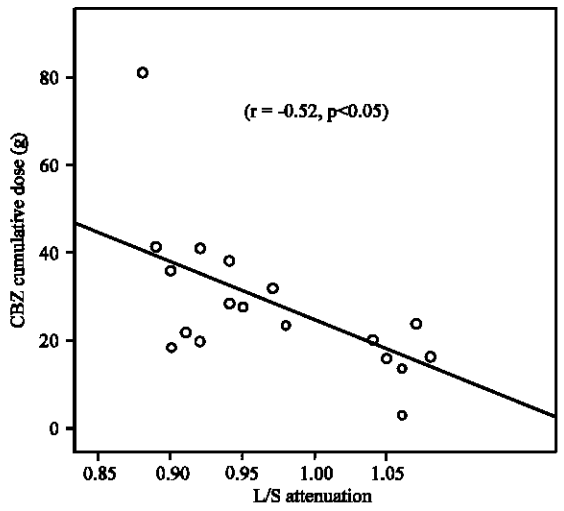

Fig. 1: Correlation between CBZ cumulative dose and liver/splenic attenuation

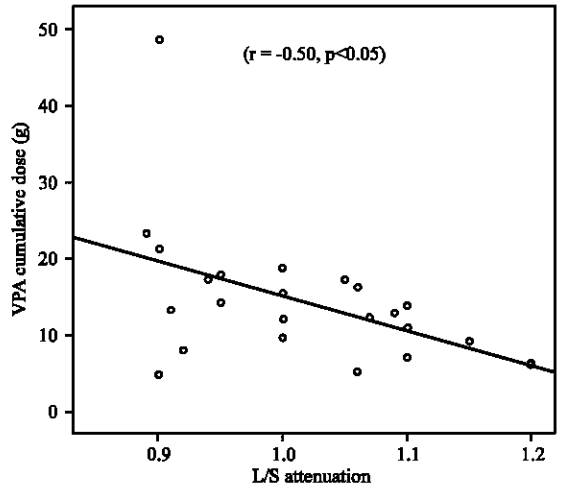

Fig. 2: Correlation between VPA cumulative dose and liver/splenic attenuation

(i.e., liver fat content increased) as shown in Fig. 1-3, respectively). L/S attenuation was correlated negatively with (BMI, all fractions of lipid profiles and HOMA-IR index) and positively correlated with (G/I ratio) but it didn't reach statistical significance as shown in Table 4.

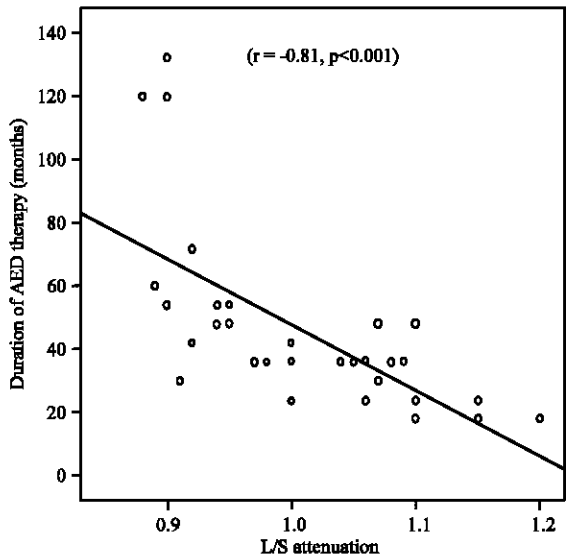

Fig. 3: Correlation between duration of AED therapy and liver/splenic attenuation

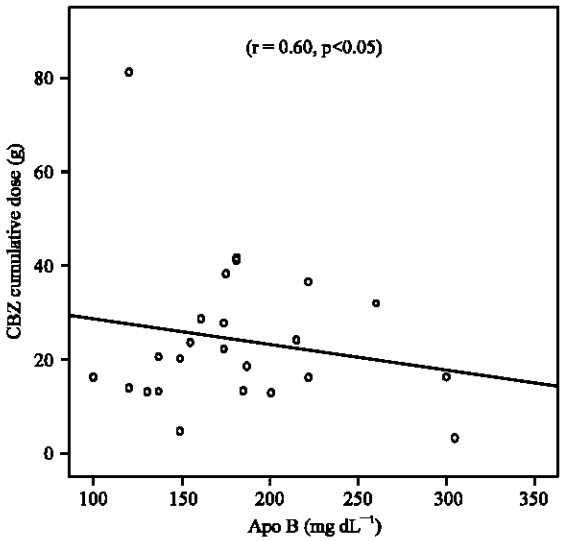

Fig. 4: Correlation between Apo B levels and CBZ cumulative dose

Moreover, there was significant positive correlation between Apo B levels and CBZ cumulative dose as shown in Fig. 4.

DISCUSSION

This is the first study evaluating the occurrence of dyslipidemia, atherogenic ratios, IR and CT-diagnosed NAFLD in non obese epileptic children and adolescents who are being treated with VPA, CBZ or both.

The prevalence of NAFLD is increasing in children and adolescents (Patton *et al*., 2006; Manco *et al*., 2008) and is recognized as part of the Metabolic Syndrome (MS) (Kelishadi *et al*., 2009; Treeprasertsuk *et al*., 2011). The diagnosis of fatty liver is based on the presence of steatosis or steatohepatitis (Oliva *et al*., 2006). Liver steatosis can be assessed by ultrasound that is not sensitive because it can detect liver steatosis only when it is greater than 30% (Ryan *et al*., 2002), Magnetic Resonance Imaging (MRI) (Fishbein *et al*., 2005) or spectroscopy (MRS) (Szczepaniak *et al*., 2002) which are quite expensive, or on liver biopsy, an invasive procedure also associated with some risks (Wieckowska *et al*., 2007). Therefore, CT scan L/S ratio was used to measure the attenuation of liver which is according to Kodama *et al*. (2007) the best predictor of pathologic fat content.

In the present study, NAFLD was demonstrated in 42.8% of VPA, 21.4% of CBZ and 60% of combination therapy treated patients. All of them were overweight and 53.3% had IR. It is worth mentioning that TG levels were significantly increased in the overweight group when compared to lean group. Similarly, elevated triglycerides have been found to be associated with hepatic steatosis in various series of children (Chan *et al*., 2004a; Kocak *et al*., 2000; Bedogni *et al*., 2006).

NAFLD shows a wide spectrum of histological abnormalities and clinical outcome (Cotrim *et al*., 2000; Angulo, 2002), with insulin resistance as the most important underlying disorder (Haque and Sanyal, 2002; Schwimmer *et al*., 2003; Tominaga *et al*., 2009). As a result of impaired suppression of triglyceride hydrolysis in adipose tissue (Reaven, 2002), plasma levels of free fatty acids increase in an insulin-resistant state and enhance intrahepatic synthesis of triglyceride rich lipoproteins and fat accumulation (Mulhall *et al*., 2002) as shown in our clinical series.

As evidenced by prior studies VPA related weight gain was associated with hyperinsulinemia in women with epilepsy (Isojarvi *et al*., 1996, 1998). However, subsequent reports have shown that VPA is associated with elevated serum insulin levels in lean subjects as well (Pylvanen *et al*., 2002). It was suggested that either VPA may inhibit the metabolism of insulin in the liver which then results in elevated serum insulin concentrations in the peripheral circulation (Pylvanen *et al*., 2006) or may directly induce insulin secretion from the pancreatic beta cells (Luef *et al*., 2003). However, it seems that VPA induced hyperinsulinemia is independent of the drug-related weight gain and may actually precede weight gain (Pylvanen *et al*., 2006).

In our patients liver enzymes were significantly higher in the VPA and combination therapy treated patients when compared to controls. Elevated liver enzymes may be a surrogate measure of NAFLD; however, liver steatosis can be present with normal transaminase levels (Mofrad *et al*., 2003; Ansari *et al*., 2011).

CBZ therapy was associated with increased TC, LDL-C and Apo A1levels. Similar results were obtained in previous reports (Zeitlhofer *et al*., 1993; Yalcin *et al*., 1997; Eiris *et al*., 2000). The alterations thus demonstrated in serum lipids and the significant positive correlation between Apo B levels and CBZ cumulative dose, as well as a possible modulation of enzyme activity (Fichsel, 1980) might explain the increased rate of fatty liver infiltration in CBZ treated group.

Apolipoprotein B-100 is the chief protein component constituent of the atherogenic very low-density lipoprotein, of intermediate-density lipoprotein and of low-density lipoprotein particles, each particle including 1 apolipoprotein B molecule. Hence, plasma apolipoprotein B levels reflect the total number of atherogenic particles. On the other hand, Apolipoprotein A1 is the major apolipoprotein constituent of the anti-atherogenic high-density lipoproteins (Chan *et al*., 2004b). Therefore, the balance between their levels is very critical in determining the risk for coronary heart disease.

Patients on combination therapy demonstrated the highest risk of dyslipidemia, atherogenic ratio, IR as well as NAFLD. Our results support previous studies, reporting increased incidence of Cardiovascular Disease (CVD) (Targher *et al*., 2006; Targher and Arcaro, 2007) and carotid atherosclerosis (Volzke *et al*., 2005), (Schindhelm *et al*., 2007; Khashab *et al*., 2008) among patients with NAFLD.

This might be explained by the fact that VPA is a fatty acid derivative that competes with Free Fatty Acids (FFA) for albumin binding and act as a Gamma Aminobuteric Acid (GABA) agonist which causes an increase in the levels of (FFA) (Johannessen, 2000) and consequently TG levels (Vorum *et al*., 1993). At the same time, CBZ induces liver microsomal enzymes that lead to alteration in the metabolism of bile acids, cholesterol and other lipids, bilirubins and many other endogenous molecules and endogenous drugs that are metabolized by these enzymes Luoma *et al*. (1982) which collectively explain the synergetic effect of both drugs on lipid metabolism.

The L/S attenuation ratio showed significant negative correlation with the duration of AED therapy, VPA and CBZ cumulative doses (i.e., liver fat content increased). Also, it was negatively correlated with (BMI, all fractions of lipid profiles and HOMA-IR) and positively correlated with (G/I ratio) but it didn't reach statistical significance. This agrees with the "multiple hit" hypotheses. The first "hit" is the accumulation of the fat in the liver. Once the liver is infiltrated by fat, a second hit triggers the progression from steatosis to steatohepatitis. Initially the first hit was considered to be insulin resistance but it was also expanded to include unknown genetic factors and obesity (Day, 2002). Several potential "second hits" include oxidative stress from reactive oxygen species in the mitochondria and cytochrome P450 enzymes which might be involved during the AED therapy. Other second hits include the presence of endotoxins, cytokines, adipokines and environmental factors (Edmison and McCullough, 2007).

The main limitation of the study is that we didn't study overweight controls. This was very difficult because abdominal CT scan was done only for those who were indicated to do abdominal CT scan (e.g., traumatic injuries or to rule out malignancy).

Patients on long term AED therapy are at increased risk for developing various metabolic changes especially with poor weight control. These metabolic changes were expressed differently among the three applied groups, with the highest risk among combination therapy group. Such metabolic derangements are well described as the link for the development of metabolic syndrome with a possible risk of cardiovascular disease later in life (Desouky *et al*., 2007; Mahajan *et al*., 2010). Moreover, Almenabbawy *et al*. (2009) reported that adjustment of dietary intake by balanced diet in epileptic children and adolescents can help in adjusting not only the dose of the anti-epileptics but also improving the general condition as well as reduce the recurrence of epileptic fits. Therefore, careful choice of the AED should be based on the patient's weight status and life style as well as providing regular follow up especially for those on combination therapy.

REFERENCES

Abdel-Wahab, B.A. and M.E. Metwally, 2011. Ginkgo biloba enhances the anticonvulsant and neuroprotective effects of sodium valproate against kainic acid-induced seizures in mice. J. Pharmacol. Toxicol., 6: 679-690.

Ahmad, M., 2011. Epilepsy: Stigma and management. Curr. Res. Neurosci., 1: 1-14.

Akbar, D.H. and A.H. Kawther, 2006. Non-alcoholic fatty liver disease and metabolic syndrome: what we know and what we don't know? Med. Sci. Monit., 12: RA23-RA26.

Almenabbawy, K.M., S.I. Helal, S.T. Zaki, O.M. Said, M.M. Salam, M.M. Abdelmoneim and L.M. Aboismaiel, 2009. Is balanced diet has effect on epileptics? J. Med. Sci., 9: 234-239.

Angulo, P., 2002. Non-alcoholic fatty liver disease. New Engl. J. Med., 346: 1221-1231.

Ansari, J.A., M. Sayyed and F. Sayeed, 2011. Management of non alcoholic fatty liver diseases and their complications. Int. J. Pharmacol., 7: 579-588.

Assmann, G., H. Schriewer, G. Schmitz and E.O. Hagele, 1983. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/$MgCl_2$. Clin. Chem., 29: 2026-2030.

Bedogni, G., S. Bellentani, L. Miglioli, F. Masutti, M. Passalacqua, A. Castiglione and C. Tiribelli, 2006. The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. B.M.C. Gastroenterol., 6: 33-33.

Carl, A., E.R. Ashwood and D.E. Bruns, 2006. Lipids, Lipoproteins, Apolipoproteins and other Cardiovascular Risk Factors. In: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Carl, A., E.R. Ashwood and D.E. Bruns (Eds.). Saunders, Philadelphia, USA., Pages: 2448.

Chan, D.C., P.H. Barrett and G.F. Watts, 2004a. Lipoprotein transport in the metabolic syndrome: methodological aspects of stable isotope kinetic studies. Clin. Sci., 107: 221-232.

Chan, D.F., A.M. Li, W.C. Chu, M.H. Chan and E.M. Wong *et al*., 2004b. Hepatic steatosis in obese Chinese children. Int. J. Obes. Relat. Metab. Disord., 28: 1257-1263.

Commission on Classification and Terminology of the International League Against Epilepsy, 1989. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia, 30: 389-399.

Cook, S., M. Weitzman, P. Auinger, M. Nguyen and W.H. Dietz, 2003. Prevalence of a metabolic syndrome phenotype in adolescents: Findings from the third national health and nutrition examination survey, 1988-1994. Arch. Paediatr. Adolesc. Med., 157: 821-827.

Cotrim, H.P., R. Parana, E. Braga and L. Lyra, 2000. Nonalcoholic steatohepatitis and hepatocellular carcinoma: Natural history. Am. J. Gastroenterol., 95: 3018-3019.

Daniel, W.W., 1995. Biostatistics: A Foundation for Analysis in the Health Sciences. 6th Edn., John Wiley and Sons Inc., New York.

Day, C.P., 2002. Non-alcoholic steatohepatitis: Where are we now and where are we going?. Gut, 50: 585-588.

De Onis, M., A.W. Onyango, E. Borghi, A. Siyam, C. Nishida and J. Siekmann, 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Org., 85: 660-667.

Demircioolu, S., A. Soylu and E. Dirik, 2000. Carbamazepine and valproic acid: Effects on the serum lipids and liver functions in children. Pediatr. Neurol., 23: 142-146.

Desouky, A.M., M. Raafat, A.A. Metwaly, A. El-Shamaa and E. Abdallah, 2007. Arterial compliance, renal, cardiac, endocrine and metabolic disorders as a predictors of hypertension syndrome. J. Med. Sci., 7: 503-515.

Dietschy, J.M., L.E. Weeks and J.J. Delento, 1976. Enzymatic assessment of free and estrified cholesterol levels using the oxygen electrode in a modified glucose analyzer. Clin. Chem. Acta., 73: 407-407.

Edmison, J. and A.J. McCullough, 2007. Pathogenesis of non-alchoholic steatohepatitis: Human data. Clin. Liver Dis., 11: 75-104.

Eiris, J., M.I. Novo-Rodriguez, M. Del-Rio, P. Meseguer, M.C. Del-Rio and M. Castro-Gago, 2000. The effects on lipid and apolipoprotein serum levels of long-term carbamazepine, valproic acid and phenobarbital therapy in children with epilepsy. Epilepsy Res., 41: 1-7.

Eiris, J.M., S. Lojo, M.C. Del-Rio, I. Novo, M. Bravo, P. Pavon and M. Castro-Gago, 1995. Effects of long-term treatment with antiepileptic drugs on serum lipid levels in children with epilepsy. Neurology, 45: 1155-1157.

Engel, Jr. J. and International League Against Epilepsy (ILAE), 2001. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: Report of the ILAE task force on classification and terminology. Epilepsia, 42: 796-803.

Fichsel, H., 1980. Effects of different anticonvulsive drugs on liver enzymes. Acta Neurol. Scand, 62: 108-108.

Fishbein, M., F. Castro, S. Cheruku, S. Jain, B. Webb, T. Gleason and W.R. Stevens, 2005. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis and ultrasound. J. Clin. Gastroenterol., 39: 619-625.

Franzoni, E., M. Govoni, S. D'Addato, S. Gualandi, Z. Sangiorgi, G.C. Descovich and G.P. Salvioli, 1992. Total cholesterol, high-density lipoprotein cholesterol and triglycerides in children receiving antiepileptic drugs. Epilepsia, 33: 932-935.

Gastaldelli, A., M. Kozakova, K. Hojlund, A. Flyvbjerg, A. Favuzzi, A. Mitrakou And B. Balkau, 2009. Fatty liver is associated with insulin resistance, risk of coronary heart disease and early atherosclerosis in a large European population. Hepatology, 49: 1537-1544.

Haque, M. and A. Sanyal, 2002. The metabolic abnormalities associated with non-alcoholic fatty liver disease. Best Pract. Res. Clin. Gastroenterol., 16: 709-731.

Isojarvi, J.I., T.J. Laatikainen, M. Knip, A.J. Pakarinen, K.T. Juntunen and W. Myllyla, 1996. Obesity and endocrine disorders in women taking valproate for epilepsy. Ann. Neurol., 39: 579-584.

Isojarvi, J.I., J. Rattya, V.V. Myllyla, M. Knip and R. Koivunene *et al*., 1998. Valproate, lamotrigine and insulin mediated risks in women with epilepsy. Ann. Neurol., 43: 446-451.

Johannessen, C.U., 2000. Mechanisms of action of valproate: A commentatory. Neurochem. Intern., 37: 103-110.

Kelishadi, R., S.R. Cook., A. Adibi, Z. Faghihimani and S. Ghatrehsamani *et al*., 2009. Association of the components of the metabolic syndrome with non-alcoholic fatty liver disease among normal-weight, overweight and obese children and adolescents. Diabetol. Metab. Syndr., 1: 29-29.

Keskin, M., S. Kurtoglu, M. Kendirci, M.E. Atabek and C. Yazici, 2005. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics, 115: 500-503.

Khashab, M.A., S. Liangpunsakul and N. Chalasani, 2008. Nonalcoholic fatty liver disease as a component of the metabolic syndrome. Curr. Gastroenterol. Rep., 10: 73-80.

Kocak, N., A. Yuce, F. Gurakan and H. Ozen, 2000. Obesity: a cause of steatohepatitis in children. Am. J. Gastroenterol., 95: 1099-1100.

Kodama, Y., C.S. Ng, T.T. Wu, G.D. Ayers and S.A. Curley *et al*., 2007. Comparison of CT methods for determining the fat content of the liver. Am. J. Roentgenol., 188: 1307-1312.

Luef, G.J., M. Lechleitner, G. Bauer, E. Trinka and P. Hengster, 2003. Valproic acid modulates islet cell insulin secretion: a possible mechanism of weight gain in epilepsy patients. Epilepsy Res., 55: 53-58.

Luef, G.J., M. Waldmann, W. Sturm, A. Naser and E. Trinka *et al*., 2004. Valproate therapy and non-alcoholic fatty liver disease. Ann. Neurol., 55: 729-732.

Luoma, P.V., V.V. Myllyla and E. Hokkanen, 1982. Relationship between plasma high density lipoprotein cholesterol and anticonvulsant levels in epileptics. J. Cardiovasc. Pharmacol., 4: 1024-1027.

Mahajan, R., K. Gupta and V. Kapoor, 2010. A systematic account of pathogenesis, diagnosis and pharmacotherapy of metabolic syndrome: Things we need to know. Int. J. Pharmacol., 6: 338-345.

Manco M, G. Bottazzo, R. De-Vito, M. Marcellini, G. Mingrone and V. Nobili, 2008. Non alcoholic fatty liver disease in children. J. Am. Coll. Nutr., 27: 667-676.

McGowan, M.W., J.D. Artiss, D.R. Strandbergh and B. Zak, 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29: 538-542.

Mofrad, P., M.J. Contos, M. Haque, C. Sargeant and R.A. Fisher *et al*., 2003. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology, 37: 1286-1292.

Mulhall, B.P., J.P. Ong and Z.M. Younossi, 2002. Non-alcoholic fatty liver disease: an overview. J. Gastroenterol. Hepatol., 17: 1136-1143.

NHBPEP, 2004. The fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents. Pediatrics, 114: 555-576.

National Cholesterol Education Program (NCEP), 1994. National Cholesterol Education Program: Report of the expert panel on blood cholesterol levels in children and adolescents. Pediatrics, 89: 495-501.

Nejad, S.E.M., M.R.A. Nikpour, F. Rahim, S.N. Naghibi and M.A. Bahrammi, 2009. A randomized open-label comparison of lamotrigine and valproate in patients with juvenile myoclonic epilepsy. Int. J. Pharmacol., 5: 313-318.

Nikalje, A.P.G., M. Ghodke and A. Girbane, 2011. GABA modulating agents: A brief review. Asian J. Biol. Sci., 4: 201-220.

Oliva, M.R., K.J. Mortele, E. Segatto, J.N. Glickman, S.M. Erturk, P.R. Ros and S.G. Silverman, 2006. Computed tomography features of nonalcoholic steatohepatitis with histopathologic correlation. J. Comput. Assist. Tomogr., 30: 37-43.

Park, H.S., J.H. Han, K.M. Choi and S. Kim, 2005. Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. Am. J. Clin. Nutr., 82: 1046-1051.

Patton, H.M., C. Sirlin, C. Behling, M. Middleton, J.B. Schwimmer and J.E. Lavine, 2006. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J. Pediatr. Gastroenterol. Nutr., 43: 413-427.

Pylvanen, V., A. Pakarinen, M. Knip and J. Isojarvi, 2006. Insulin-related metabolic changes during treatment with valproate in patients with epilepsy. Epilepsy Behav., 8: 643-648.

Pylvanen, V., M. Knip, A. Pakarinen, M. Kotila, J. Turkka and J.I. Isojarvi, 2002. Serum insulin and leptin levels in valproate-associated obesity. Epilepsia, 43: 514-517.

Ramzan, M., I. Ali and A. Matin, 2009. Sonographic assessment of hepatic steatosis (fatty liver) in<school children of Dera Ismail Khan City (NWFP) Pakistan. Pak. J. Nutr., 8: 797-799.

Reaven, G., 2002. Metabolic syndrome. Pathophysiology and implication for management of cardiovascular disease. Circulation, 106: 286-288.

Rockall, A.G., S.A. Sohaib, D. Evans, G. Kaltsas and A.M. Isidori *et al*., 2003. Hepatic steatosis in Cushing's syndrome: a radiological assessment using computed tomography. Eur. J. Endocrinol., 149: 543-548.

Ryan, C.K., L.A. Johnson, B.I. Germin and A. Marcos, 2002. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. Liver Transpl., 8: 1114-1122.

Schindhelm, R.K., J.M. Dekker, G. Nijpels, L.M. Bouter, C.D. Stehouwer, R.J. Heine and M. Diamant. 2007. Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the hoorn study. Atherosclerosis, 191: 391-396.

Schwimmer, J.B., R. Deutsch, J.B. Rauch, C. Behling, R. Newbury and J.E. Lavine, 2003. Obesity, insulin resistance and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J. Pediatr., 143: 500-505.

Sonmez, F.M., E. Demir, A. Orem, S. Yildirmis, F. Orhan, A. Aslan and M. Topbas, 2006. Effect of antiepileptic drugs on plasma lipids, lipoprotein (a) and liver enzymes. J. Child Neurol., 21: 70-74.

Szczepaniak, L.S., P. Nurenberg, D. Leonard, J.D. Browning and J.S. Reingold *et al*., 2002. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am. J. Physiol. Endocrinol. Metab., 288: 462-468.

Targher, G., L. Bertolini, R. Padovani, F. Poli and L. Scala *et al*., 2006. Non-alcoholic fatty liver disease is associated with carotid artery wall thickness in diet-controlled type 2 diabetic patients. J. Endocrinol. Invest., 29: 55-60.

Targher, G. and G. Arcaro, 2007. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis, 191: 235-240.

Tominaga, K., F. Fujimoto, K. Suzuki, M. Hayashi, M. Ichikawa and Y. Inaba, 2009. Prevalence of non-alcoholic fatty liver disease in children and relationship to metabolic syndrome, insulin resistance and waist circumference. Environ. Health Prev. Med., 14: 142-149.

Treeprasertsuk, S., F. Lopez-Jimenez and K.D. Lindor, 2011. Nonalcoholic fatty liver disease and the coronary artery. Dig. Dis. Sci., 56: 35-45.

Verrotti, A., S. Domizio, B. Angelozzi, G. Sabatino, G. Morgese and F. Chiarelli, 1997. Changes in serum lipids and lipoproteins in epileptic children treated with anticonvulsants. J Paediatr. Child Health, 33: 242-245.

Verrotti, A., F. Basciani, M. De-Simone, D. Trotta, G. Morgese and F. Chiarelli, 2002. Insulin resistance in epileptic girls who gain weight after therapy with valproic acid. J. Child Neurol., 17: 265-268.

Verrotti, A., R. La-Torre, D. Trotta, A. Mohn and F. Chiarelli, 2009. Valproate-induced insulin resistance and obesity in children. Horm. Res., 71: 125-131.

Verrotti, A., S. Agostinelli, P. Parisi, F. Chiarelli and G. Coppola, 2011. Nonalcoholic fatty liver disease in adolescents receiving valproic acid. Epilepsy Behav., 20: 382-385.

Volzke, H., D.M. Robinson, V. Kleine, R. Deutscher and W. Hoffmann *et al*., 2005. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. World J. Gastroenterol., 11: 1848-1853.

Vorum, H., L. Gram and B. Honore, 1993. Valproate and palmitate binding to serum albumin in valproate-treated patients, Relation to obesity. Epilepsy Res., 16: 55-64.

WHO, 2006. Who child growth standards: Length/height-forage, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. Methods and Development. Geneva, Switzerland, http://www.who.int/childgrowth/standards/.

Warnick, G.R., R.H. Knopp and V. Fitzpatrick, 1990. Estimating low density lipoprotein cholesterol by the friedwald equation is adequate for classifying patients on the basis of nationally recommended cut points. Clin. Chem., 36: 15-19.

Wieckowska, A., A.J. McCullough and A.E. Feldstein, 2007. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. Hepatology, 46: 582-589.

Yalcin, E., A. Hassanzadeh and K. Mawlud, 1997. The effects of long-term anticonvulsive treatment on serum lipid profile. Acta Paediatr. Jpn., 39: 342-345.

Zeitlhofer, J., A. Doppelbauer, G. Tribl, T. Leitha and L. Deecke, 1993. Changes of serum lipid patterns during long-term anticonvulsive treatment. Clin. Invest., 71: 574-578.