Effect of antiepileptic drugs on various lipid fractions and certain liver enzymes in epileptic patients of Punjab origin

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ABSTRACT

Introduction and Aim: Epilepsy requires lifelong therapy with antiepileptic drugs (AEDs) &having medical and psychological consequence. Present study was conducted to observe the effect of different AEDs on lipid profile and certain liver enzymes on epileptic patients.

Material and Methods: 50 epileptic patients receiving AEDs for minimum 1 year were recruited as study group and 50 healthy subjects considered as control group. These subjects were recruited from general community of Punjab. Fasting blood samples were drawn from patients and healthy subjects for the evaluation of total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol, AST, ALT & ALP.

Results: Significant increase in total cholesterol, triglycerides, LDL-cholesterol, VLDL- cholesterol was observed in epileptic patients while no significant change was recorded in HDL- cholesterol. Maximum increase in total cholesterol, TGs, LDL-cholesterol, VLDL- cholesterol levels was observed in phenytoin treated patients with reference tovalproic acid, carbamazepine, levetiracetam treated patients. Levels of AST, ALT and ALP were significantly increased in epileptic patients. Maximum increase in AST & ALT was observed in levetiracetam treated epileptic patients and maximum rise in ALP levels was found in carbamazepine treated patients with reference to other AEDs treated patients.

Conclusion: Aforementioned observations suggested that epileptic patients treated with phenytoin, valproic acid, carbamazepine & levetiracetam for long time could cause dyslipidemia, might be responsible for various CHD's and hepatotoxicity in epileptic patients. Routine screening of lipid profile and hepatic enzymes during chronic use of AEDs is recommended.

Keywords: Epilepsy; anti-epileptic drugs (AEDs); aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP).

INRODUCTION

Epilepsy is the most common, chronic neurological disorder and about 65 million people affected worldwide. In India, epilepsy affects 5 to10 people's out of every 1000 people. Epilepsy usually begins in childhood, potentially impeding education, employment, social relationships and development of a sense of self-worth (1). It is estimated that there are more than 10 million peoples with epilepsy in India (2). In the elderly, epilepsy is the third most common neurologic disorder, and they are at higher risk of new onset epilepsy. Psychiatric and other

comorbidities are common among patients with epilepsy (3). The prevalence among Indian males (5.1 per 1000) was much higher than females (2.2 per 1000) (4).

Epilepsy requires long-term or lifelong therapy with antiepileptic drugs (AEDs), particularly for those patients with refractory epilepsy (5).It is a disorder of recurrent and spontaneous seizures resulting clinically into permanent alterations of normal function and morphology of neuronal cells and even cell death (6).Epilepsy imposes a large economic burden on health care systems of countries. Approximately, 70-80 % of patients who develop epilepsy may expect to have their seizures controlled with optimal antiepileptic therapy (7).

Therapy with antiepileptic drugs remains the mainstay of treatment of patients with epilepsy. The major groupings of antiepileptic drugs can be defined as: 1)Those which facilitate γ-aminobutyric acid (GABA) transmission by various mechanisms; 2) Those which block voltage-gated ion channels and thus reduce excitatory transmission; 3) Those whose mechanism of action is still open to debate(8).

Most antiepileptic drugs exert their antiepileptic effects via the Na^+ or the Ca^{2+}channel or via GABAergic transmission. In addition to the major action site, the new antiepileptic drugs tend to have several minor action sites. In the chronic phase, the incidence of adverse effects with the new antiepileptic drugs is low, but TPM and PER, drugs that potentiate glutamatergic transmission, may elicit behavioral changes and cognition disorders (9). Patients with epilepsy are often required to take antiepileptic drugs for a long period of time. Carbamazepine and phenytoin were amongst the most prescribed antiepileptic drugs as monotherapy and as combination therapy as well as valproic acid while levetiracetam and lamotrigine were found frequently prescribed amongst newer antiepileptic drugs (4).

Antiepileptic drugs act either by increasing inhibition through sustaining the release of GABA or glycineor decreasing excitation by inhibiting glutamate release. However, some antiepileptic drugs reduce membrane excitability by interrelating with neurotransmitter receptors or ion channels but the methods of action for most of them are not fully understood Many studies (10)have shown prolonged use of antiepileptic drugs is known to be associated with adverse effects such as metabolic and organ toxicity, endocrine disturbance, negative cognitive effects, and psychiatric problems. So, present study was designed to evaluate the role of AEDs like phenytoin, valproic acid, carbamazepine and Levetiracetam on various lipid fractions along with certain liver enzymes in epileptic patients of Punjab origin.

MATERIALS AND METHODS

The present case control prospective study comprising total of 100 subjects was carried out in the Department of Biochemistry, Government Medical College, Amritsar, in collaboration with Department of Medicine, Guru Nanak Dev Hospital, Amritsar. The subjects for the present study were selected from rural as well as urban community from general population of male and females. A detailed history, physical and systemic examination including measurement of height, weight, heart rate, blood pressure and body mass index (BMI) was taken and every case was thoroughly interviewed.

Ethical Issues

The study protocol is approved by the Institutional Ethics Committee. The study details and potential risks and benefits were explained to individuals taking part in the study and at least to one attendant. A written informed consent was obtained from subjects before entering into the study.

Selection of epileptic patients

Inclusion criteria:

50 epileptic patients receiving one of the following antiepileptic drugs (phenytoin, valproic acid, carbamazepine and levetiracetam) for a minimum period of 1 year were included in the study.

The epileptic patients must have five or more epileptic attacks.

The epileptic patients in the age range of 20to 50 years of both sex (male & female) from rural/urban community of Punjab origin were included.

Exclusion criteria:

Epileptic patients who had concomitant liver diseases, using other drugs causing elevation of liver enzymes (e.g. antibiotics, anti-rheumatic drugs, statins and non-steroidal anti-inflammatory drugs) or those who were alcohol drinkers were excluded from the present study.

Selection of normal healthy control subjects

50 normal healthy subjects in the age range of 20-50 years of both sexes were recruited from urban/rural general population of Punjab origin.

Measurement of anthropometric Parameters

The examination body weight was done by taking weight in kilogram (kg) and height was measured in centimeters. The Body Mass Index (BMI) was calculated from the formula as:

$$BMI = Weight/Height\ (kg/m^2)$$

Table: 1 Effect on anthropometric profile in epileptic patients on different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) and normal healthy subjects of Punjab origin

Anthropometric profile	Healthy control subjects (Mean ± S.D.)		Epileptic patients (Mean ± S.D.)	
	Male (n=28)	Female (n=22)	Male (n=31)	Female (n=19)
Age (years)	35.34±8.87	38.22±7.52	41.56±9.22	40.94±8.56
Height (cm)	156.19 ± 5.93	151 ± 4.87	146.22 ±3.09	152 ± 5.17
Body Weight (Kg.)	55.12 ± 3.11	46 ± 5.21	56.92 ± 4.14	48 ± 5.25
BMI (Kg/m^2)	25.76 ± 5.32	26.21 ± 5.01	26.75 ± 3.98	27.21 ± 3.69

Collection and processing of blood samples

Five ml of venous blood was taken from all subjects after 12 hours overnight fast in a dry disposable syringe under all aseptic conditions by venipuncture in the antecubital vein in a sterile, dry acid washed vial for biochemical assays.

Preparation of serum

The blood was allowed to stand for half an hour. After clot formation, the supernatant was centrifuged. All the samples were processed for thyroid hormones, lipid profile, and liver enzymes.

Biochemical assays

Estimation of fasting blood glucose: Fasting blood glucose levels in plasma were estimated by using the commercially available kit manufactured by Transasia Pvt. Ltd based on GOD-POD Method, End Point as described by Trinder, 1969 (11).

Estimation of lipid profile: Total cholesterol, triglycerides, HDL-cholesterol levels in serum were determined by using the commercially available kit manufactured by Transasia Pvt. Ltd based on Allian*et al.*,(12) GPO method as described by McGowan (13)and Grillo and Izzo,1985 (14) respectively. VLDL-Cholesterols were estimated by dividing triglycerides with 5 (15) and LDL-cholesterol levels were determined by using Friedwald's and Fredrickson's formula (15).

Serum Liver Enzymes:

Determination of Aspartate Aminotransferase (AST) The levels AST in serum of epileptic patients and normal healthy control subjects were determined by using commercially available standardized kits manufactured by using the commercially available kit manufactured by Transasia Pvt. Ltd based on the principle of Moss and Henderson in 1999, kinetic IFCC method (16).

Determination of Alanine Aminotransferase (ALT):

ALT levels in serum of epileptic patients and normal healthy control subjects were determined by using commercially available standardized kits manufactured by using the commercially available kit manufactured by Transasia Pvt. Ltd based on the kinetic IFCC method (17).

Determination of Alkaline Phosphatase (ALP): ALP levels in the serum of epileptic patients and normal healthy control subjects were determined by using commercially available standardized kits manufactured by using the commercially available kit manufactured by Transasia Pvt. Ltd based on the principle of Bessey, 1946(18).

Statistical Analysis:

The data was expressed as Mean ± SD. Differences between the epileptic patients and normal healthy control subjects were evaluated using the Student's independent samples "t" test. Differences were considered statistically significant at $p < 0.05$.

Table 2: Effect on fasting blood glucose and blood pressure in epileptic patients on different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) and normal healthy subjects of Punjab origin

Anthropometric Profile	Healthy Control subjects (Mean ± S.D.)		Epileptic Patients (Mean ± S.D.)	
	Male (n=28)	Female (n=22)	Male (n=31)	Female (n=19)
Fasting Blood Glucose (mg/dL)	79.67 ± 4.76	81.34 ± 3.78	81.27 ± 5.76	78.89 ± 5.78
Systolic blood Pressure (mmHg)	126.17 ± 8.32	125.22 ± 7.89	125.83 ± 8.32	126.54 ± 5.19
Diastolic blood Pressure (mmHg)	83.05 ± 4.33	81.09 ± 4.21	81.36 ± 3.33	83.22 ± 4.72

Table-3: Changes in lipid profile levels in epileptic patients on different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) and normal healthy subjects of Punjab origin

Lipid Profile	Healthy control subjects (Mean ± S.D.)			Epileptic patients (Mean ± S.D.)		
	Male (n=28)	Female (n=22)	Mean (n=50)	Male (n=31)	Female (n=19)	Mean (n=50)
Total Chol. (mg/dL) Reference Range:140-250 mg/dl	169.0±16.92	172.10±15.21	170.55±16.06	269.50± 17.11 (59.47)[b]**	272.30±15.99 (58.22) [b] **	270.90±16.55 (58.83) [b] **
Triglyceride (mg/dL) Reference <150 mg/dl]	80.70±7.23	82.20±6.99	81.45±7.11	179.60± 11.28 (122.55)[a] ***	181.40±9.93 (120.68) [a] ***	180.50±10.60 (121.60) [a] ***
VLDL- Chol. (mg/dL) Reference 2-30 mg/dl]	16.16±1.10	16.44±2.15	16.30 ± 1.62	35.92±4.21 (122.27)[a] ***	36.28 ± 3.98 (120.68)[a] ***	36.10±4.09 (121.47)[a] ***
HDL-Chol. (mg/dL) Reference Range Male: 30-65 mg/dL; Female: 35-80 mg/dL	41.10±4.21	45.11±5.04	43.10 ± 4.62	37.12±5.01 [a] (-9.68)[NS]	40.00± 3.45 (-11.32)[NS]	38.56±4.23 (-10.54)[NS]

LDL-Chol. (mg/dL) Reference Range <100 mg/dl]	112.36±9.48	110.56±8.92	111.46 ± 9.20	196.58 ± 9.59 (74.96)[a] ***	196.02 ±10.11 (56.76) [a] ***	196.30±9.83 (76.11)[a] ***

Values in parentheses represent percentage changes w. r. t. normal healthy subjects. **P≤ 0.01 ***P≤ 0.001

RESULTS

Effect on antiepileptic drugs on lipids profile

A significant increase in total cholesterol by 58.83% (from 170.55 ± 16.06mg/dL to 270.90 ± 16.55mg/dL), Triglycerides by 121.60% (from 81.45 ± 7.11mg/dL to 180.50 ± 10.60mg/dL), LDL- cholesterol by 76.11% (from 111.46 ± 9.20mg/dL to 196.30 ± 9.83mg/dL) and VLDL –cholesterol by 121.47% (from 16.30 ± 1.62mg/dL to 36.10 ± 4.09mg/dL) levels were recorded in epileptic patients on various antiepileptic drugs with respect to normal healthy control subjects. A similar trend of significant increase in total cholesterol, triglycerides, LDL- cholesterol and VLDL –cholesterol levels was also observed in male and females on antiepileptic drug treated epileptic patients of Punjab origin in comparison to normal healthy male and females while a nominal decrease by 10.54% (from 45.11 ± 5.04mg/dL to 38.56 ± 4.23mg/dL) was recorded in epileptic patients on antiepileptic drugs treatment patients with respect to normal healthy subjects (Table 2). A Maximum increase in total cholesterol (302.60 ± 12.92mg/dL), Triglycerides (189.71 ±7.84mg/dL), LDL-cholesterol (224.26 ± 10.92mg/d), VLDL-cholesterol (37.94± 3.21mg/dL) was recorded in serum of phenytoin treated epileptic patients in comparison to valproic, carbamazepine and levetiracetam drugs treated epileptic patients. A maximum fall in HDL-cholesterol (43.41 ± 5.11mg/dL) was also observed in phenytoin treated epileptic patients in comparison to valproic, carbamazepine and levetiracetam drugs treated epileptic patients (Table4).

Effect on antiepileptic drugs on certain liver enzymes

A significant increase in AST levels from 32.68±5.30 U/L to 41.80± 6.62 U/L (by 27.90%, p≤0.05); ALT from 22.08± 4.76 U/L to 41.97± 6.71 U/L (by 90.08%, p≤0.001); ALP from 75.65±8.64 U/L to 362.55±12.47 U/L (by 379.24 %, p≤0.001) were recorded in epileptic patients on various antiepileptic drugs with respect to normal healthy control subjects. A similar trend of significant increase in AST, ALT & ALP levels was also observed in male and females on antiepileptic drug treated epileptic patients of Punjab origin in comparison to normal healthy male and females (Table 4). A maximum rise in AST (41.45 ± 12.81U/L) was observed in phenytoin treated epileptic patients, ALT (43.22 ± 8.57(U/L) was recorded in levetiracetam drugs treated epileptic patients and a maximum rise in ALP (375.97 ± 41.91U/L) was also observed in carbamazepine treated epileptic patients in comparison to other antiepileptic drugs treated epileptic patients (Table 4).

Table 4: Effect of different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) on lipid profile levels in epileptic patients of Punjab origin

Lipid Profile	Phenytoin (n=12)	Valproic Acid (n=14)	Carbamazepine (n=11)	Levetiracetam (n=13)
Total Chol. (mg/dL)	302.6 ± 12.92	251.70 ± 21.19	283.10 ± 16.21	274.21 ± 14.99
Triglycerides (mg/dL)	189.71 ±7.84	167.21 ± 7.92	176.60 ± 9.37	172.21 ± 9.03
VLDL- Chol. (mg/dL)	37.94± 3.21	33.50 ± 3.13	35.32 ± 4.89	34.44 ± 3.76
HDL-Chol.	40.41 ± 5.11	40.70 ± 4.78	37.69 ± 6.52	32.16 ± 4.14

(mg/dL)				
LDL-Chol. (mg/dL)	224.26 ± 10.92	177.50 ± 10.08	210.09 ± 12.21	207.60 ± 10.82

DISCUSSION

The present case control study was conducted on 50 epileptic patients out of them 12 epileptic patients taking phenytoin; 14 patients using valproic acid; 11 epileptic patient taking carbamazepine and 13 epileptic patients on levetiracetam therapy. In present study a significant increase was found in total cholesterol, triglycerides, LDL-cholesterol & VLDL cholesterol in epileptic patients in comparison to normal healthy subjects and a nominal decrease in HDL-cholesterol was observed in epileptic patients in comparison to normal healthy subjects. A maximum increase in lipid fraction was recorded in phenytoin treated patients with respect to valproic acid, carbamazepine & levetiracetam treated patients (Table 4). The literature reports(19 -20)exploring the effects of different antiepileptic drugs on lipid metabolism are inconsistent and reported either increased, decreased or no change in the levels of serum total cholesterol or triglycerides or LDL –cholesterol or HDL-cholesterol level on drug monotherapy.

The previous studies (21) revealed an increase in total cholesterol, triglycerides, VLDL- cholesterol, LDL-cholesterol epileptic patients on long-term treatment with phenytoin. So, particular attention has been paid on the effect of pancreatic β-cells, where it inhibits the release of insulin and suppresses the response of plasma insulin to various stimuli, thereby increasing the serum lipid levels. The effect of phenytoin may also be due to the induction of CYP enzyme. They are the inducers of CYP51 enzyme. CYP51 is a housekeeping gene of the Cytochrome- P450 super family, which is involved in cholesterol biosynthesis in humans. The CYP450 enzyme system is involved in the synthesis and metabolism of cholesterol. In particular; CYP51A1 plays a key role in cholesterol synthesis (22). A significant increase in various lipid fraction levels like total cholesterol, triglycerides, LDL-cholesterol and VLDL- cholesterol levels suggested that treatment of epileptic patients with phenytoin for long time could cause dyslipidemia in epileptic patients which in turn initiate various cardiovascular diseases like atherosclerosis.

A significant increase was recorded in liver enzymes like AST, ALT and ALP in epileptic patients treated with antiepileptic drugs such as phenytoin, valproic acid, carbamazepine, levetiracetam with respect to normal healthy subjects (Table5). Liver enzymes such as AST, ALT, and ALP can serve as markers of hepatocellular injury or of an obstruction in the bile flow cholestasis. The significant elevations of liver enzymes are usually transitory or dose-related and might be associated with hepatocellular injury hence could lead to death or an acute liver failure which could imperatively require liver transplantation. The hepatotoxicity induced by antiepileptic drug occurs either because of production of reactive toxic metabolite/s or because of induction of immune-allergic reactions (23).

A maximum rise in the levels of AST was recorded in phenytoin treated epileptic patients in comparison to valproic acid, carbamazepine, levetiracetam treated antiepileptic treated drugs and maximum increase in the levels of ALT and ALP was seen in levetiracetam and carbamazepine respectively with respect to other antiepileptic drug treated patients (Table 6). The rise in liver enzymes in different antiepileptic drugs treated patients suggested that hepatotoxicity might be associated with other clinical manifestations of drug allergy (fever, rash and eosinophilia). This reaction is typical of carbamazepine and phenytoin. Another idiosyncratic hepatotoxic reaction comes from hepatotoxic metabolites because of aberrant metabolism (24). In 2007,

Bjornsson *et al.*, (25) reported there was no correlation between the duration of therapy of carbamazepine and elevated liver enzymes.

Table 5: Effect on certain liver enzymes in epileptic patients on different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) and normal healthy subjects of Punjab origin

Liver Enzymes	Healthy control subjects (Mean ± S.D.)			Epileptic patients (Mean ± S.D.)		
	Male (n=28)	Female (n=22)	Mean (n=50)	Male (n=31)	Female (n=19)	Mean (n=50)
AST (U/L) [Reference Range: Male: <35U/L Female: <31 U/L]	31.11 ± 5.47	34.26 ± 5.13	32.68±5.30	41.50 ± 7.09 (33.39) [a**]	42.11 ± 6.16 22.91[a] *	41.80± 6.62 (27.90)[a] *
ALT (U/L) [Reference Range: Male: <45 U/L]	21.98 ± 4.25	22.19 ± 5.27	22.08± 4.76	41.91 ± 7.99 (90.67)[a] ***	42.03 ± 5.43 (89.40)[a]***	41.97± 6.71 (90.08)[a] ***
ALP (U/L) [Reference Range: Male: 53-128 U/L Female: 42-98U/L]	74.68 ± 8.11	76.63 ± 9.18	75.65± 8.64	358.50±12.98 (380.04)[a] ***	366.60±11.96 (378.40)[a] ***	362.55±12.47 (379.24)[a] ***

Values in parentheses represent percentage changes w. r. t. normal healthy subjects. *P≤ 0.05, **P≤ 0.01 ***P≤ 0.001

Table-6: Effect of different epileptic drugs (Phenytoin, Valproic acid, Carbamazepine, Levetiracetam) on certain liver enzymes in epileptic patients of Punjab origin

Liver Enzymes	Phenytoin (n=12)	Valproic Acid (n= 14)	Carbamazepine (n= 11)	Levetiracetam (n= 13)
AST (U/L)	41.45 ± 12.81	36.37 ± 15.32	39.42 ± 11.83	40.29 ± 14.06
ALT (U/L)	40.22 ± 11.01	36.43 ± 16.46	38.71 ± 14.35	43.22 ± 8.57
ALP (U/L)	363.03 ± 58.50	339.64 ± 38.21	375.97 ± 41.91	356.45 44.40

CONCLUSION

Aforementioned observations suggested that epileptic patients treated with phenytoin, valproic acid, carbamazepine & levetiracetam for long time could cause dyslipidemia, might be responsible for the pathophysiology of various cardiovascular diseases like atherosclerosis and increases in liver enzymes could cause hepatotoxicity in epileptic patients. So, Routine screening of complete lipid profile and hepatic enzymes level during the chronic use of antiepileptic drugs is recommended. The controlled studies with larger samples size should be carried out to reveal the further frequency and the risk factors of serious hepatotoxicity.

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