Evaluation of Liver function in sera of patients with Epilepsy

Dr*. Sura Ahmed Al-Emami / Al-Mustansiryia Univirsity / College of Science / Department of Chemistry
Dalia Mohammed Redaa / Al-Mustansiryia Univirsity / College of Science / Department of Chemistry.
Haider Ridha Mohammed Saeed / Al-Mustansiryia Univirsity / College of Science / Department of Chemistry.
* e-mail:- sura742003@yahoo.com.

Abstract:-

Background: patients with epilepsy on antiepileptic drug therapy deserve special consideration for the adverse effect on hepatic functions. Aim:the present study aims to evaluate the hepatotoxicity by drugs of patients with epilepsy through determination of liver function tests such as total protein, albumin, bilirubin, cholesterol, and liver enzymes (alkaline phosphatase, and amino transferases). Subjects: twenty four patients with epilepsy were studied and compared with twenty six healthy individuals. Results: significant elevation of liver enzymes activity{(ALP ($p<0.001$), SGPT($p<0.01$),SGOT($p<0.001$)}, protein($p<0.01$) , albumin ($p<0.01$), bilirubin($p<0.001$) and cholesterol($p<0.05$) concentrations were observed in the sera of patients with epilepsy in comparison with that of the control group. Also a significant positive correlation between the specific activity of alkaline phosphatase and albumin concentration was found in the present study in the sera of patients with epilepsy.
Keywords:- epilepsy, drug, liver , protein , bilirubin ,enzyme.

الخلاصة:-

ان مرضى الصرع الذين يتناولون الادوية المضادة للصرع يستحقون مراعاة خاصة نتيجة لتأثير العلاج على وظائف الكبد.الهدف: تهدف الدراسة الحالية الى تقييم تأثر الكبد بالادوية المعطاة لمرضى الصرع من خلال تقدير الفحوص الخاصة بوظائف الكبد مثل البروتين الكلي, الالبومين,انزيمات الكبد (انزيم الفوسفاتيز القاعدي,انزيمات الامينو ترانسفيريز),الكولستيرول والبيليروبين. العينات: تم دراسة 24 عينة من مرضى الصرع ومقارنتها مع 26 عينة من الاشخاص الاصحاء. النتائج: اظهرت النتائج وجود ارتفاع معنوي في فعالية انزيمات الكبد بفارق احصائي معنوي, وايضا ارتفاع معنوي في مستوى كل من البروتين الكلي, الالبومين, الكولستيرول والبيليروبين بفارق احصائي معنوي في مصول دم المصابين بالصرع مقارنة بالاصحاء. كذلك تبين وجود علاقة موجبة معنوي بين الفعالية الخصوصية لانزيم الفوسفاتيز القاعدي مع مستوى الالبومين.الاستنتاج: يستنتج من الدراسة الحالية الى امكانية استخدام الالبومين والفعالية الخصوصية لانزيم الفوسفاتيز القاعدي للكشف عن الخلل الوظيفي للكبد الناتج من تناول الادوية العلاجية لمرض الصرع.

Introduction:-

Epilepsy might be considered a symptom of an underlying neurological disorder which did not represent a single disease entity[(1)]. The term epilepsy , meaning a seizure is derived from the Greek term lopsis [(2)]. An epileptic seizure could be described by abnormal, paroxysmal electrical discharge within the gray matter of the brain[(3)(4)]. A recurrent of epileptic seizures in the absence of extraordinary provocation would be lead to epilepsy ,that in widespread, forceful, and repetitive contraction of the body musculature is termed a convulsion. Convulsions are not necessarily specific for epileptic seizures [(3)(5)(6)]. The successful management of epilepsy requires a thorough and individualized approach that accurately establishes the patient's seizure type(s) and, when appropriate, epilepsy syndrome[(7)]. Several 'new' antiepileptic drugs (AEDs), i.e., oxcarbazepine, vigabatrin, lamotrigine, zonisamide, gabapentin, tiagabine, topiramate and levetiracetam have been

introduced into clinical practice within the last decade. Most of these new drugs are at least as effective as the 'old' AEDs (phenytoin, phenobarbital, sodium valproate and carbamazepine); and, in general, they seem to be better tolerated than the old drugs[8].The liver is the site where the waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea[9] . In the liver the drugs are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are excreted in urine or bile[10][11]. After further metabolic steps, which usually include conjugation to a glucuronide or a sulfate or glutathione, the hydrophilic product is exported into plasma or bile by transport proteins located on the hepatocyte membrane, and it is subsequently excreted by the kidney or the gastrointestract[11]. Serum liver function tests are important but often problematic in evaluating patients with and without symptoms of hepatic disease. The common term "liver function tests" is misleading because most tests used in clinical practice measure hepatocellular damage not function. True liver function tests are those that measure synthesis of proteins made by the liver (albumin) or the liver's capacity to metabolize drugs. A commonly ordered panel of automated tests includes bilirubin, aminotransferases, alkaline phosphatase, and gamma-glutamyl transpeptidase[12][13] .

There are contradictory reports on the influence of antiepileptic drugs on serum lipids [14],[15],[16],[17]. Therefore the present study aims to evaluate the hepatocellular damage by drugs in patients with epilepsy through determination of liver function tests not only by liver enzymes activity but also by albumin, cholesterol, and bilirubin levels.

Subjects and Methods

Subjects: - Twenty four patients with Temporal lobe epilepsytheir age (8-44 year) within mean 26 year were involved in this study. The patients were referred to Neuroscience Hospital, Baghdad, Iraq. They were all underwent drug treatment (carbamazepine in dosage 500 mg and valproic acid in dosage 200mg). As a control, 26 age and gender matches healthy individual were included in the present study.

Serum Sampling: - Five milliliters of samples of venous blood were taken and left for 10 minutes at room temperature. After blood coagulation , the sera were separated by centrifugation at 3000 rpm for 10 minutes and then sera stored at -20^{o}C until being used . Hemolyzed samples were discarded .

Methods:-

Determination of protein concentration: - The total serum protein concentration was determined by Biuret method using total protein kit , Biomarieux[18].

Determination of albumin: - The serum concentration of albumin was determined colorimetrical using bromocresol green method, Biolab kit[19].

Determination of amino transferases (SGOT-SGPT) activities :- The activites of amino transferases were determined by colorimetric method using Biomarieux Kit[20],[21],and the specific activity of enzymes were expressed in U per mg of protein.

Determination of alkaline phosphatase activity:- Serum activity of ALP was determined by Kind and King method using Syrbio Kit [22], and the specific activity of enzymes were expressed in U per mg of protein.

Determination of total bilirubin: - The serum concentration of bilirubin was determined by colorimetric method using Biomaghreb Kit [23].

Determination of total cholesterol:- The total cholesterol was determined by enzymatic colorimetric method using Biomarieux Kit [24].

Statistical Analysis:- Data were analyzed using spss (version 19.0). The results are expressed as mean ± standard deviation (SD) . Statistical and correlation analysis were undertaken using t-test , and pearson's correlation coefficients. Values of $P < 0.05$ were considered significant.

Results:-

The mean values of both activities and specific activities of ALP, SGPT, and SGOT in the sera of patients with Epilepsy were compared with that of the control group. The results reflected presence of a significant increase of ALP ($P < 0.001$), SGPT ($p<0.01$), SGOT ($p< 0.001$) activities, and significant increase of ALP ($P < 0.001$), SGPT ($P < 0.05$), SGOT($P < 0.05$) specific activities. (Table I).

Table I:- Mean values of ALP,SGPT,&SGOT activities and specific activities in the sera of control and patients with Epilepsy.

	Group	Samples number	Range	Mean	± Standard deviation	P Value
ALP activity U/L	Control	26	21-96	42.1920	20.5616	<0.001
	Epilepsy	24	36-236.9	106.7238	41.9745	
ALP specific activity U/mg	Control	26	0.1-1.6	0.6290	0.3816	<0.001
	Epilepsy	24	0.47-4.3	1.4958	0.8775	
SGPT activity U/L	Control	26	2-12	5.84	3.1712	<0.001
	Epilepsy	24	3-39	16.875	9.786	
SGPT specific activity U/mg	Control	26	0.03-0.23	0.0964	0.0567	<0.05
	Epilepsy	24	0.01-0.62	0.2141	0.1728	
SGOT activity U/L	Control	26	7-27	14.4615	4.8845	<0.001
	Epilepsy	24	10-67	30.4458	14.5800	
SGOT specific activity U/mg	Control	26	0.1-0.45	0.2581	0.1014	<0.05
	Epilepsy	24	0.13-0.82	0.3745	0.2013	

Table (II) shows the mean values of protein, albumin, total cholesterol, total bilirubin , and conjugated bilirubin concentrations in the sera of control and patients with Epilepsy. As it is clear from the results, a significant differences are present in protein ($P <0.05$), albumin ($P <0.01$),total cholesterol ($P <0.05$), conjugated bilirubin ($P <0.001$) and total bilirubin ($P <0.001$) concentrations between the control and their corresponding patients group.

A correlation between the liver enzymes activity and specific activity with the other parameters (total protein, albumin, total cholesterol, conjugated bilirubin , and total bilirubin) were studied in the present study in the sera of patients with epilepsy and it were demonstrated as shown in Table (III) that there were a significant negative correlation between the total protein and albumin with the specific activity of ALP. While a non- significant correlation were found between the activity and specific activity of the liver enzymes(ALP, SGPT, SGOT) with the cholesterol , conjugated bilirubin, and total bilirubin in sera of patients with epilepsy.

Table II:-Mean values of total protein, albumin, total cholesterol, total bilirubin ,and conjugated bilirubin concentrations in the sera of control and patients with epilepsy

	Group(samples number)	Range	Mean	±Standard deviation	P Value
Total protein (g/L)	Control(26) Epilepsy(24)	4.3-7.9 5.1-18.4	6.3143 8.3182	1.0379 3.5516	< 0.05
Albumin (g/L)	Control(26) Epilepsy(24)	3.4-8 1.5-7.5	5.2731 4.2167	0.9543 1.3830	< 0.01
Total Cholesterol (mg/dL)	Control(26) Epilepsy(24)	40-228 71-469	134.3462 194.2917	46.9603 86.8649	< 0.05
Total bilirubin (mg/dL)	Control(26) Epilepsy(24)	0.195-1.37 0.49-2.86	0.6702 1.5482	0.3573 0.6576	< 0.001
Conjugated bilirubin (mg/dL)	Control(26) Epilepsy(24)	0.06-0.7 0.21-1.79	0.2287 0.798	0.1894 0.4990	< 0.001

Table (III):- The correlation coefficient (r) between serum liver enzymes activity and specific activity with the total protein, albumin, total cholesterol, total bilirubin ,and conjugated bilirubin, and their p value.

		Total protein g/L	Albumin g/L	Total Cholesterol mg/dL	Total bilirubin mg/dL	Conjugated bilirubin mg/dL
ALP activity (U/L)	r	-0.254	-0.262	0.109	0.022	-0.175
	p	0.293	0.251	0.639	0.934	0.503
ALP specific activity(U/mg)	r	-0.612	-0.587	0.271	-0.062	-0.06
	p	0.005	0.008	0.261	0.825	0.819
SGPT activity (U/L)	r	-0.113	0.357	0.325	-0.197	-0.183
	p	0.617	0.087	0.121	0.449	0.482
SGPT specific activity(U/mg)	r	-0.37	0.195	0.365	-0.188	0.387
	p	0.083	0.385	0.095	0.503	0.139
SGOT activity (U/L)	r	0.297	0.283	-0.108	-0.147	0.141
	p	0.18	0.18	0.614	0.572	0.589
SGOT specific activity(U/mg)	r	-0.35	0.02	0.089	0.027	0.231
	p	0.11	0.929	0.694	0.925	0.373

Discussion:-

In the present study liver enzymes activity (ALP, SGPT, SGOT) were measured in the sera of patients with epilepsy and it was found presence of a significantly elevated in comparison to that found in control group. In order to determine the specific activity of the liver enzymes, the total protein was measured in the present study, and the results reflect presence a significant increase in the specific activity of liver enzymes. This results were agreement with others that obtained by Wall M &et.al. who reported that serum alkaline phosphatase was elevated in 29.7% and alanine aminotransferase in 25.2% of cases[25]. Also Nau *et al*(2009) reported that approximately 47% of patients with seizure had preexisting liver disease, and 25% had elevated serum creatinine[26]. Aggarwal *et al*(2005) reported that blood levels of alkaline phosphatase were significantly more in patients with epilepsy receiving carbamazepine compared to controls, while SGPT were not significantly different in two groups[27]. On the other hand Komatsu *et al*(1996) reported that the patients with epilepsy showed no abnormal values either in hepatic (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin) and renal (blood urea nitrogen, creatinine) function tests postoperatively[28]. The Increased in serum ALP activity is due to the drugs that can cause increased ALP activity. Meanwhile increased levels of SGPT are an indicator of damage to liver cells, as this enzyme is contained within the liver cell itself, where when the cell is injured, the enzyme is released into the bloodstream and the increased level can be measured. SGOT an enzyme seen in the liver, heart, kidney, skeletal muscle and brain have a half life much shorter than that of SGPT in the blood stream is, therefore the values of SGOT tend to drop more rapidly once liver function is resumed. SGOT elevations and SGPT elevations should parallel each other in liver disease.

Throughout this study a significant increase of total protein level was shown in the sera of patients with epilepsy in comparison to that of the control group. This increase is indicted that patients have chronic inflammatory conditions.

Albumin is a common blood protein produced by the liver. Although tests that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function[29],[30]. A change in serum albumin level may be associated with a decrease in liver functioning mass, although neither is specific for liver disease[29][30]. This fact was in line with the results obtained in the present study where a significant decrease in albumin level was shown in patients with epilepsy in comparison to that of the control group which indicated that patients have liver dysfunction. This result was agreement with the results that obtained by Rugino *et al(2003)* who reported that divalproex administration was a contributing factor in the development of reversible hypoalbuminemia in this population of severely disabled, neurologically injured children and young adults of epilepsy[31].

Throughout this study a significant increase in cholesterol level was observed in sera of patients with epilepsy in comparison to the control group. This result was agreement with the result that obtained by Dewan. *et al*(2008) ,who reported that the mean total cholesterol in children receiving phenytoin was15.9% higher as compared to children receiving valproic acid who had 5.5% higher mean total cholesterol, than controls. [32]. Also Aggarwal *et al*(2005) reported that the mean + SD of cholesterol 162 ± 25.8mg/dL in patients with epilepsy receiving carbamazepine was significantly more ($P<0.001$) than controls 131±25.2 mg/dL[27]. While this result was disagreement with the result that obtained by Grosso *et al*(2009) who reported that no significant change in cholesterol was observed in children with epilepsy receiving valproic acid[33].

Total bilirubin is a component of bile; bilirubin is secreted by the liver as conjugated bilirubin into the intestinal tract. High levels can lead to jaundice and indicate destruction in the liver and bile duct. Throughout this study a significant increase in total bilirubin level was observed in sera of patients with epilepsy. This result was agreement with the results that obtained by Tutor-Crespo *et al*(2002) who reported that bilirubin and its conjugate and non conjugate fractions were found to offer values that to a large extent matched those of the control group[34]. Also the present study was

agreement with the result that obtained by Antoniuk *et al(1996)* who reported that the bilirubin levels in children epilepsy patients receiving valproic acid ranged from 5.5 to 19.8 mg%[35]. While the present study was disagreement with the results that obtained by Gough *et al*(1989) who reported that highly significant (p less than 0.001) reductions in average bilirubin levels were noted for carbamazepine, phenytoin , phenobarbital , and multiple drug groups[36].

A positive correlation was found in the present study between total protein and albumin with the specific activity of ALP.

Conclusions:-

From the results that had been obtained it was concluded that the uses of the albumin together with the specific activity of ALP are more specific and marker to diagnosis for liver dysfunction in patients with epilepsy.

Reference:

1- Ramaratnam S., & Satishchandra P. Guidelines for epilepsy management in India classification of seizures and epilepsy syndromes. Annals of Indian Academy of Neurology.2010;13(4):247-249.
2- Desai J. Epilepsy and cognition. Journal of Pediatric Neurosciences.2008;3,(1):16-29.
3- Walker HK, Hall WD, Hurst JW. Clinical Methods: The History, Physical, and Laboratory Examinations. 1990, 3rd edition,Boston: Butterworths; chap.56.
4- Blumenfeld H. Consciousness and epilepsy: why are patients with absence seizures absent? Prog Brain Res. 2005;150: 271-286.
5- Englot DJ, Blumenfeld H. Consciousness and epilepsy: why are complex-partial seizures complex? Prog Brain Res. 2009; 177:147-170.
6- Danielson NB, Guo JN, Blumenfeld H. The default mode network and altered consciousness in epilepsy. Behav Neurol. 2011 Jan 1;24(1):55-65
7- Beyenburg S, Bauer J, Reuber M. New drugs for the treatment of epilepsy: A practical approach. Postgrad Med J 2004; 80:581-7.
8- Brunbech L, Sabers A. Effect of antiepileptic drugs on cognitive function in individuals with epilepsy: A comparative review of newer versus older agents. Drugs 2002;62:593-604
9- Gianni E., Testa R.,& Savarino V. Liver enzyme alteration: a guide for clinicians.2005; 172(3): 367–379.
10- Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348:529-537.
11- Lee W. Drug-Induced Hepatotoxicity**.** N Engl J Med 2003; 349:474-485.
12- Rochling FA . Evaluation of abnormal liver tests. Clin Cornerstone. 2001; 3(6):1-12.
13- Schoch L, Whiteman K. Monitoring liver function. Nursing. 2007 Nov;37(11):22-23.
14- Demircioglu S, Soylu A, Diric E.Carbamazepine and valproic acid: Effects on theserum lipids and liver functions in children.Pediatr Neurol 2000, 23: 142-146.
15- Eris JM, Lojo S, Del Rio MC, Novo I. Effect of long term treatment with antiepileptic drugs on serum lipid levels in children with epilepsy. Neurology 1995; 45: 1155-1157.
16- Yilmaz E, Dosan Y, Gurgoze MK, Gungor S.Serum lipid changes during anticonvulsivetreatment in epileptic children. Acta NeurolBelg 2001; 101: 217-220.
17- Nikolaos T, Stylianos G, Chryssoula N, Irini P,Christos M, Dimitrios T, *et al.* The effect oflong-term antiepileptic treatment on serumcholesterol (TC, HDL, LDL) and triglyceridelevels in adult epileptic patients onmonotherepy. Med Sci Monit 2004; 10: MT50-MT52.
18- Gornall AG, Bardawill CJ,& David MM. Determination of serum proteins by means of the biuret reaction . J Biol Chem. 1949;177(2):751-66.

19- Doumas BT., Watson WA., & Briggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chem Acta. 1971;31:87-96.
20- Britman S.,Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases.Am J Clin Path.1957;28:56-63.
21- Karmen A.,Wroblewski F., & Ladue JS. Transaminase activity in human blood. Clin Invest. 1955;34(1):126-131.
22- Kind PRN., & King EG. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine.J Clin Path. 1954;7:322-326.
23- Walters MI. , Gerarde RW. Standardizations in bilirubin assays Microchem J. 1970;15: 231.
24- Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 1973 Dec;19(12):1350-1356.
25- Wall M, Baird-Lambert J, Buchanan N,& et.al. Liver function tests in persons receiving anticonvulsant medications. Seizure. 1992;1(3):187-190.
26- Nau KM, Divertie GD, Valentino AK ,& et.al. Safety and efficacy of levetiracetam for critically ill patients with seizures. Neurocrit Care. 2009;11(1):34-37.
27- Aggarwal A., Kumar M. & Faridi M.M.A .Effect of Carbamazepine on Serum Lipids and Liver Function Tests. Indian Pediatrics .2005;42(17):914-918.
28- Komatsu H, Ueki M, Morita J, & et.al. Clinical characteristics and biotransformation of sevoflurane in paediatric patients during antiepileptic drug therapy. Paediatr Anaesth. 1996;6(5):379-382.
29- Dufour DR, Lott JA, Nolte FS, & et.al. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin Chem*2000;46(12):2027-49.
30- Dufour DR, Lott JA, Nolte FS, &et.al. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clin Chem*2000;46(12):2050-68.
31- Rugino TA, Janvier YM, Baunach JM,& et.al. Hypoalbuminemia with valproic acid administration. Pediatr Neurol. 2003 Nov;29(5):440-444.
32- Dewan P. , Aggarawal A. & Faridi MMA .Effect of Phenytoin and Valproic Acid Therapy on Serum Lipid Levels and Liver Function Tests. Indian Pediatrics .2008;45(17):855-858.
33- Grosso S, Mostardini R, Piccini B,&et.al. Body mass index and serum lipid changes during treatment with valproic acid in children with epilepsy. Ann Pharmacother. 2009;43(1):45-50.
34- Tutor-Crespo MJ, Hermida-Ameijeiras J,& Tutor-Valcarce JC. A study of some indirect biochemical markers in the evaluation of enzymatic induction caused by antiepileptic drugs. Rev Neurol. 2002;35(8):711-716.
35- Antoniuk SA, Bruck I, Hönnicke LR. & et.al. Acute hepatic failure associated with valproic acid in children. Report of 3 cases. Arq Neuropsiquiatr. 1996 Dec;54(4):652-654.
36- Gough H, Goggin T, Crowley M, &et.al. Serum bilirubin levels with antiepileptic drugs. Epilepsia. 1989;30(5):597-602.