Biochem. Lett., Vol.3, PP 15-40 (2007)

EVALUATION OF ALPHA-GLUTATHIONE-S-TRANSFERASE AS A MARKER OF HEPATOCELLULAR DAMAGE

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ABSTRACT

Alpha-glutathione-S-transferase (α-GST) are the cytoplasmatic class of enzymes responsible for cellular detoxifying processes. The serum α-GST activity in relation to chronic infection caused by hepatitis C virus (HCV) and acute infection caused by hepatitis A, B and C viruses. 15 anti-HCV negative and negative for HBV and HAV healthy subjects served (controls), 35 patients with chronic hepatitis [8/35 are anti-HCV positive subjects with normal alanine aminotransferases (ALT) and 27/35 chronic hepatitis are with high ALT levels]; 17 patients with acute liver disease (1 acute HCV infection, 3 acute HBV infections and 13 acute HAV infections were studied. Serum (α-GST) alpha glutathione-S-transferase, (aspartate aminotransferase, alkaline phosphatase (ALP), gamma glutamyl transferase GGT, total protein, albumin, total and direct bilirubin were assayed in all subjects, and after 0 week, 2 weeks and 4 weeks in the acute group.

INTRODUCTION

The glutathione-S-transferase (EC 2.5.1.18) form a complex family of multifunctional proteins displaying various biological functions, their main purpose being the phase II detoxification of xenobiotics. This family of enzymes consists of cytosolic isoforms (alpha, mu, pi, and theta) as well as isolated microsomal form ***(Giffen et al, 2002)***. In

humans, 80% of all alpha-glutathione-S-transferase (α-GST) is found within the liver ***(Clarke et al, 1997)*** where it comprises 5 – 10% of the soluble hepatic protein ***(Giffen et al, 2002)***. This has resulted in much interest in the measurement of α-GST, via the use of species specific enzyme immunoassays (EIA) ***(Kilty et al, 1998)***, as a superior marker of hepatotoxicity. Further advantages, such as its cytocolic localization, small size (MW 50,000), and short half-life (approximately 90 min), potentially offer more information regarding acute changes to hepatocellular integrity than other markers such as alanine amintrasferase (ALT) (EC 2.6.1.2) and aspartare aminotransferase (AST) (EC 2.6.1.1). Traditionally, ALT and AST are the enzymes most commonly used in the assessment of hepatocellular status. Nevertheless, it is recognized that both ALT and AST are more abundant within the preportal region of the liver lobule ***(Giffen et al, 2002)***, and as such, their utility in the assessment of centrilobular or midzonal hepatotoxicity may be questioned. AST has a ubiquitous distribution with significant activities in the heart, liver, kidney, and skeletal muscle, respectively. It should therefore not solely be used as an indicator of liver damage unless other supporting enzymes are measured ***(Giffen et al, 2002 and Mazur et al, 2003)***. ***Maxwell, P.R. and Flisiak, R. (2006)*** provide information on the potential use of (GST as an additional prognostic biomarker in chronic hepatitis patients. The aim of this work is to investigate the diagnostic value of alpha glutathione-S-transferase as a biochemical marker and monitoring hepatitis disease compared to the other liver function.

SUBJECTS AND METHODS

This study was performed on 52 individuals aged (13–47years) of both sexes classified as follows.

Group I served as control consists of 15 healthy individuals with normal abdominal ultrasonography, normal liver function tests (ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin) and negative hepatitis markers and HCV PCR.

Group II 35 chronic hepatitis patients collected from specialized medical hospital – Mansoura University, diagnosed since ≥ 6 months with positive PCR. This group was exposed to the

following investigations liver function tests (ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin), hepatitis markers and abdominal ultrasonography.

Group III 40 patients exhibiting hepatitis infection under treatment (support of liver). This group was from Manzala fever hospital exposed to the following investigations. Liver functions were done after 0 week, 2 weeks and 4 weeks: hepatitis markers, and abdominal ultrasonography were done after 0 week and 4 weeks (from symptoms and signs appearance), and PCR after 4 weeks from symptoms appearance. 23 cases are excluded because they give negative PCR, hepatitis markers, and the liver functions returned to normal levels. The rest 17 cases were found to be one case hepatitis C, 3 cases hepatitis B and 13 cases hepatitis A. Alpha glutathione-S-transferase (α-GST) was measured in all groups as a hepatitis marker.

Venous blood samples were collected and kept 30 min, at room temperature. The samples were centrifuged at 3000 r.p.m. for 15 min, and serum was separated. All samples were kept at - 70°C for 2 months if not analyzed immediately.

PCR was performed according to ***(Williams, 1989).*** Using Applied Biosystems 2720 Thermal Cycler**.** HAV IgM and anti-HAV ***Zachoval et al (1984)***. HBs Ag ***Boniolo et al (1982)***. HBc Ab IgM and anti-HBc ***Pawlotsky et al (2000)***. HBe Ag/Ab ***Mushawar et al (1981)***. HCV Ab ***Wilbur (1993)***. Glutathione-S-transferase ***Mannervik (1985)***. ALT and AST ***Tietz (1987)***. GGT ***Szasz (1974)***. ALP ***Szasz et al (1971)***. Total and direct bilirubin ***Dacie and Lewis (1984)***. Albumin ***Doumas et al (1971)***. Total protein ***Henry (1964)***. α-GST kits produced by ILB Immunobiological Laboratories. Anti-HCV, anti-HBc, anti-HBs and HBe Ab kits produced by Diaclone, Besancon, France. Albumin kits produced by Biomerieux Company, France. ALP kits produced by Quimica Clinica Aplicada S.A. ALT and AST kits produced by Randox Laboratories Ltd, Ardmore, Diamond Road, Co Antrim., U.K. Bilirubin (total and direct) kits produced by Diamond Diagnostics Company. GGT kits produced by Boehringer Mannheim, France, SA. HAV IgM, HBe (Ag/Ab) and HCV kits produced by International Immunodiagnostics, (USA). HBs Ag kits produced by Biotec Laboratories Ltd., France. Total protein

kits produced by Egyptian American Company (EAC). HCV RNA was extracted from serum samples using ultraspec kit (Biotecx Laboratories Inc. 6023 South Loop East, Houston, Texas 77033. USA). Stratagen kit was used (Stratagen Diagnostic kit Inc., 11011 N Torrey prines Rood do Jolla, CA 92037 1.800 - 424 - 5444).

STATISTICAL ANALYSIS

Statistical analysis was performed using Spss statistical package for social sciences (Spss) according to ***(Narusis, 2006)*** P value was considered statistically significant at $P<0.05$. Numerical data were expressed as mean ± S.D. The levels of parameters were analyzed by ANOVA, Mann-Whitney T-test was used for comparison between groups. The correlations were evaluated by person's correlation coefficient P value <0.05 was considered significant.

RESULTS

Table 1 showed the result of hepatitis markers [HAV Ab (IgM and IgG), HBsAg, HBsAb, HBeAg, HBeAb, HBcAb (IgM and total) and HCV] in patients with acute hepatitis after 0 weeks.

Table 2 showed the result of hepatitis markers [HAV Ab (IgM and IgG), HBsAg, HBsAb, HBeAg, HBeAb, HBcAb (IgM and total), HCV antibody and HCV PCR] in patients with acute hepatitis after 4 weeks.

Table 3 showed the mean values and the significance test of α-GST, ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin in the sera of the controls and the patients with acute hepatitis after 0 week.

Table 4 showed the mean values and the significance test of α-GST, ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin in the sera of the controls and the patients with acute hepatitis after 2 weeks.

Table 5 showed the mean values and the significance test of α-GST, ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin in the sera of the controls and the patients with acute hepatitis after 4 weeks.

Table 6 showed the mean values and the significance test of α-GST, ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin in the sera of the controls and the patients with chronic hepatitis.

This study revealed that, all liver enzymes were highly significantly increased (α-GST by 1775.6%, 548%, 402% and 207.1%), (ALT by 1652%, 98.92%, 19.33% and 159.7%), (AST by 1918%, 180.25%, 73.35% and 269.1%), (GGT by 1534%, 172.3%, 69.2% and 212.6%), and (alkaline phosphatase by 259.2%, 114.5%, 63.51% and 71.29%) respectively in both acute (0, 2, 4 weeks) and chronic cases ($P< 0.001$).

Total protein and albumin were decreased significantly (total protein by 8.65% and albumin by 10.03%) in chronic cases while it showed non-significant results in acute cases.

Total and direct bilirubin were highly significantly increased by (1512%, 229%, 101.7% and 177.5% for total bilirubin) and (3928%, 315.2%, 61.25% and 260.5% for direct bilirubin) respectively in both acute and chronic cases.

Statistical analysis for correlation showed no correlation between α-GST and other studied parameters in both acute and chronic hepatitis patients.

Table (1) showed the result of hepatitis markers [HAV Ab (IgM and IgG), HBsAg, HBsAb, HBeAg, HBeAb, HBcAb (IgM and total) and HCV] in patients with acute hepatitis after 0 weeks.

No.	HAV Ab		HBs Ag	HBs Ab	HBe		HBcAb		HCV anti-body
	IgM	IgG			Ag	Ab	IgM	IgG total	
1	- ve	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve	- ve
2	+ ve	+ ve	- ve	- ve					- ve
3	+ ve	+ ve	- ve	- ve					- ve
4	+ ve	+ ve	- ve	- ve					- ve
5	+ ve	+ ve	- ve	- ve					- ve
6	- ve	- ve	+ ve	- ve	- ve	- ve	+ ve	+ ve	- ve
7	- ve	- ve	- ve	- ve					- ve
8	+ ve	+ ve	- ve	- ve					- ve
9	+ ve	+ ve	- ve	- ve					- ve
10	+ ve	+ ve	- ve	- ve					- ve
11	+ ve	+ ve	- ve	- ve					- ve
12	- ve	- ve	+ ve	- ve	- ve	- ve	+ ve	+ ve	- ve
13	+ ve	+ ve	- ve	- ve					- ve
14	+ ve	+ ve	- ve	- ve					- ve
15	+ ve	+ ve	- ve	- ve					- ve

16	+ ve	+ ve	- ve	- ve					- ve
17	+ ve	+ ve	- ve	- ve					- ve

Table (2) showed the result of hepatitis markers [HAV Ab (IgM and IgG), HBsAg, HBsAb, HBeAg, HBeAb, HBcAb (IgM and total), HCV antibody and HCV PCR] in patients with acute hepatitis after 4 weeks.

No.	HAV Ab		HBs Ag	HBs Ab	HBe		HBcAb		HCV Anti-body	HCV PCR
	IgM	IgG			Ag	Ab	IgM	IgG total		
1	- ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve
2	+ ve	+ ve	- ve	- ve					- ve	- ve
3	+ ve	+ ve	- ve	- ve					- ve	- ve
4	+ ve	+ ve	- ve	- ve					- ve	- ve
5	+ ve	+ ve	- ve	- ve					- ve	- ve
6	- ve	- ve	- ve	- ve	- ve	- ve	+ ve	+ ve	- ve	- ve
7	- ve	- ve	- ve	- ve					+ ve	+ ve
8	+ ve	+ ve	- ve	- ve					- ve	- ve
9	+ ve	+ ve	- ve	- ve					- ve	- ve
10	+ ve	+ ve	- ve	- ve					- ve	- ve
11	+ ve	+ ve	- ve	- ve					- ve	- ve
12	- ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve
13	+ ve	+ ve	- ve	- ve					- ve	- ve
14	+ ve	+ ve	- ve	- ve					- ve	- ve
15	+ ve	+ ve	- ve	- ve					- ve	- ve
16	+ ve	+ ve	- ve	- ve					- ve	- ve
17	+ ve	+ ve	- ve	- ve					- ve	- ve

Table (3) showing the mean levels ± S.E of αGST, ALT, AST, GGT, alkaline phosphotase, albumin, total protein, total and direct bilirubin in patients with acute hepatitis (after 0 weeks) compared with the normal controls.

Parameters	Cases	Mean±S.E	S.D	%change	T	P
αGST (ng/ml)	Controls(N=15) Patients(N=17)	3.94 ± 0.27 73.90 ± 3.39	1.05 13.98	1775.60	20.57	<0.001
ALT (U/L)	Controls(N=15) Patients(N=17)	24.89 ± 0.87 436.5 ± 25.18	3.36 97.51	1652.00	16.34	<0.001
AST (U/L)	Controls(N=15) Patients(N=17)	18.84 ± 0.69 380.13±31.90	2.67 123.50	1918.00	11.32	<0.001
GGT (U/L)	Controls(N=15) Patients(N=17)	17.14 ± 0.622 280.1 ± 19.88	2.41 77.010	1534.00	13.22	<0.001
Alkaline Phosphatase (U/L)	Controls(N=15) Patients(N=17)	119.33 ± 1.74 428.6 ± 29.25	6.75 113.27	259.20	10.56	<0.001
Albumin (gm/dL)	Controls(N=15) Patients(N=17)	4.57 ± 0.04 4.44 ± 0.07	0.145 0.292	2.78	1.59	N.S.
Total Protein (gm/dL)	Controls(N=15) Patients(N=17)	7.83 ± 0.05 7.94 ± 0.07	0.195 0.274	1.3	1.23	N.S.
Total Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.39 ± 0.03 6.34 ± 0.37	0.116 1.447	1512.00	15.87	<0.001

Direct Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.12±0.004 4.83±0.300	0.016 1.164	3928.00	15.69	<0.001

Table (4) showing the mean levels ± S.E of αGST, ALT, AST, GGT, alkaline phosphotase, albumin, total protein, total and direct bilirubin in patients with acute hepatitis (after 2 weeks) compared with the normal controls.

Parameters	Cases	Mean±S.E	S.D	%change	T	P
αGST (ng/ml)	Controls(N=15) Patients(N=17)	3.94 ± 0.27 25.53 ± 1.58	1.05 6.501	548.0	13.50	<0.001
ALT (U/L)	Controls(N=15) Patients(N=17)	24.89 ± 0.87 49.51 ± 2.20	3.36 9.053	98.92	10.43	<0.001
AST (U/L)	Controls(N=15) Patients(N=17)	18.84 ± 0.69 52.80 ± 3.17	2.67 13.075	180.25	10.46	<0.001
GGT (U/L)	Controls(N=15) Patients(N=17)	17.14 ± 0.62 46.72 ± 3.29	2.41 13.558	172.30	8.84	<0.001
Alkaline Phosphatase (U/L)	Controls(N=15) Patients(N=17)	119.33±1.74 255.90±9.04	6.747 37.260	114.50	14.85	<0.001
Albumin (gm/dL)	Controls(N=15) Patients(N=17)	4.57 ± 0.04 4.47 ± 0.07	0.145 0.293	2.117	1.205	N.S.
Total Protein (gm/dL)	Controls(N=15) Patients(N=17)	7.83 ± 0.05 7.86 ± 0.05	0.195 0.187	0.39	0.454	N.S.
Total Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.39 ± 0.03 1.29 ± 0.10	0.116 0.421	229.00	8.47	<0.001
Direct Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.12 ± 0.004 0.50 ± 0.101	0.016 0.418	315.20	3.73	<0.001

Table (5) showing the mean levels ± S.E of αGST, ALT, AST, GGT, alkaline phosphatase, albumin, total protein, total and direct bilirubin in patients with acute hepatitis (after 4 weeks) compared with the normal controls.

Parameters	Cases	Mean±S.E	S.D	%change	T	P
αGST (ng/ml)	Controls(N=15) Patients(N=17)	3.94 ± 0.27 19.76 ± 0.91	1.05 3.734	402.00	16.74	<0.001
ALT (U/L)	Controls(N=15) Patients(N=17)	24.89 ± 0.87 29.07 ± 2.15	3.36 8.85	19.33	1.80	<0.050
AST (U/L)	Controls(N=15) Patients(N=17)	18.84 ± 0.69 32.66 ± 1.53	2.670 6.304	73.35	8.24	<0.001
GGT (U/L)	Controls(N=15) Patients(N=17)	17.14 ± 0.62 28.97 ± 2.03	2.41 8.375	69.02	2.62	<0.050
Alkaline Phosphatase (U/L)	Controls(N=15) Patients(N=17)	119.33±1.74 195.12±4.34	6.747 17.874	63.51	16.2	<0.001
Albumin (gm/dL)	Controls(N=15) Patients(N=17)	4.57 ± 0.04 4.52 ± 0.07	0.145 0.301	0.935	0.521	N.S.
Total Protein (gm/dL)	Controls(N=15) Patients(N=17)	7.83±0.05 7.79 ± 0.06	0.195 0.225	0.497	0.53	N.S.
Total Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.39 ± 0.03 0.79 ± 0.05	0.116 0.212	101.70	7.4	<0.001

Direct Bilirubin (mg/dL)	Controls(N=15) Patients(N=17)	0.12 ± 0.004 0.19 ± 0.008	0.016 0.032	61.25	8.37	<0.001

Table (6) showing the mean levels ± S.E of αGST, ALT, AST, GGT, alkaline phosphotase, albumin, total protein, total and direct bilirubin in patients with chronic hepatitis compared with the normal controls.

Parameters	Cases	Mean±S.E	S.D	%change	T	P
αGST (ng/ml)	Controls(N=15) Patients(N=35)	3.94 ± 0.27 14.58 ± 0.62	1.05 3.66	270.1	15.74	< 0.001
ALT (U/L)	Controls(N=15) Patients(N=35)	24.89 ± 0.87 64.63 ± 4.87	3.36 28.82	159.7	8.03	< 0.001
AST (U/L)	Controls(N=15) Patients(N=35)	18.84 ± 0.69 69.54 ± 3.89	2.670 23.012	269.1	12.84	< 0.001
GGT (U/L)	Controls(N=15) Patients(N=35)	17.14 ± 0.62 53.57 ± 1.77	2.409 10.481	212.6	19.4	< 0.001
Alkaline Phosphatase (U/L)	Controls(N=15) Patients(N=35)	119.33±1.74 204.40±2.18	6.747 12.903	71.29	30.5	< 0.001
Albumin (gm/dL)	Controls(N=15) Patients(N=35)	4.57 ± 0.04 4.11 ± 0.12	0.145 0.715	10.031	3.62	< 0.001
Total Protein (gm/dL)	Controls(N=15) Patients(N=35)	7.83 ± 0.05 7.17 ± 0.06	0.195 0.312	8.65	8.71	< 0.001
Total Bilirubin (mg/dL)	Controls(N=15) Patients(N=35)	0.39 ± 0.03 1.09 ± 0.50	0.116 0.264	177.5	12.99	< 0.001
Direct Bilirubin (mg/dL)	Controls(N=15) Patients(N=35)	0.12 ± 0.004 0.43 ± 0.047	0.016 0.279	260.5	6.61	< 0.001

Fig. (1 – 9) showed individual values of α-GST, ALT, AST, GGT, ALP, albumin, total protein, total and direct bilirubin, in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis compared to the normal control.

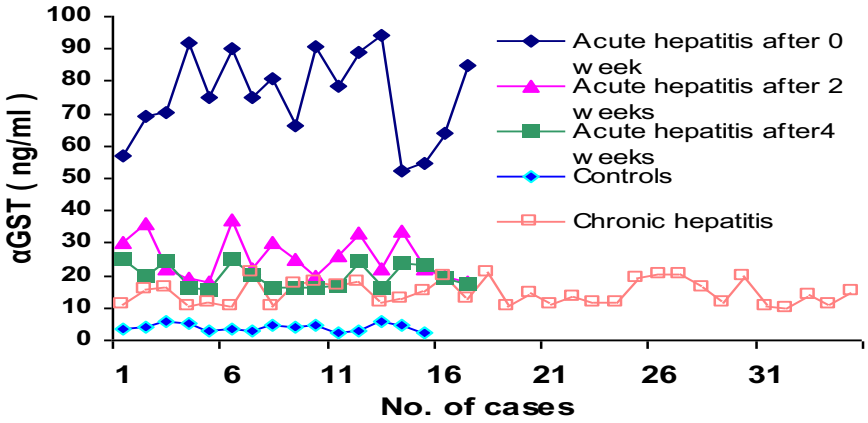

Fig. (1) Individual values of αGST (ng/ml) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

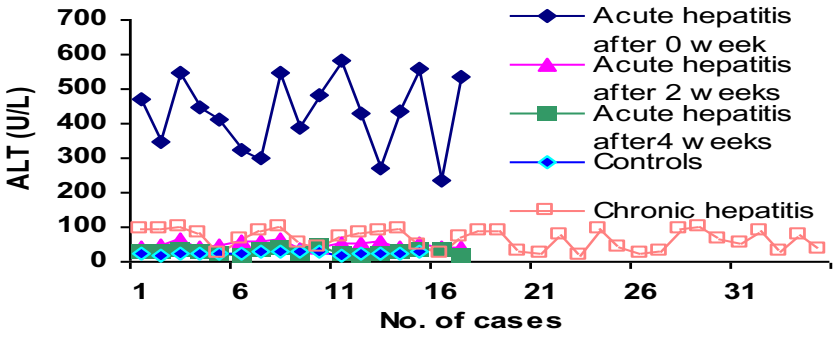

Fig. (2) Individual values of ALT (U/L) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

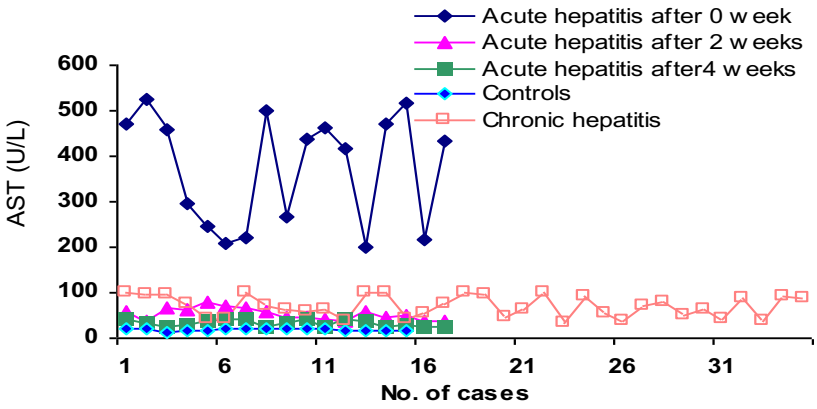

Fig. (3) Individual values of AST (U/L) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

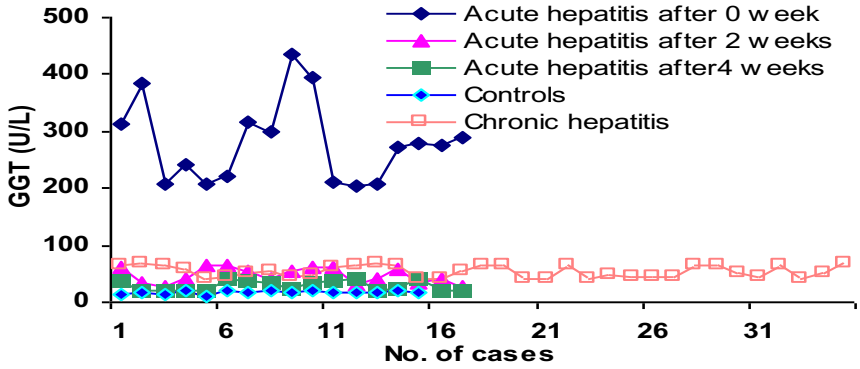

Fig. (4) Individual values of GGT (U/L) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

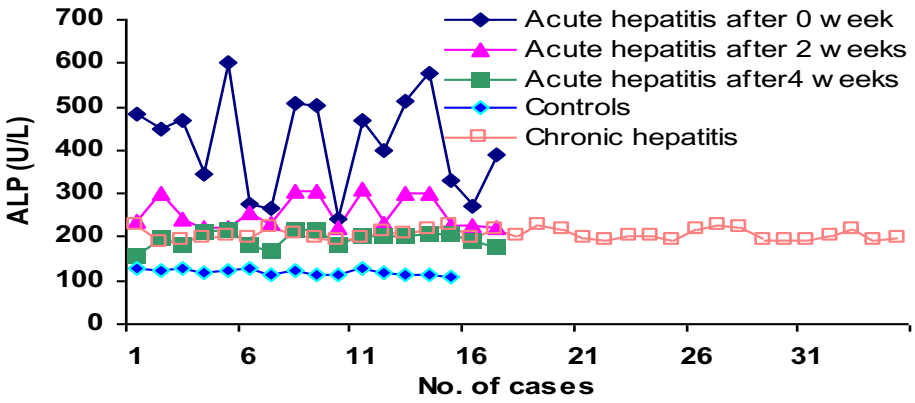

Fig. (5) Individual values of ALP (U/L) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

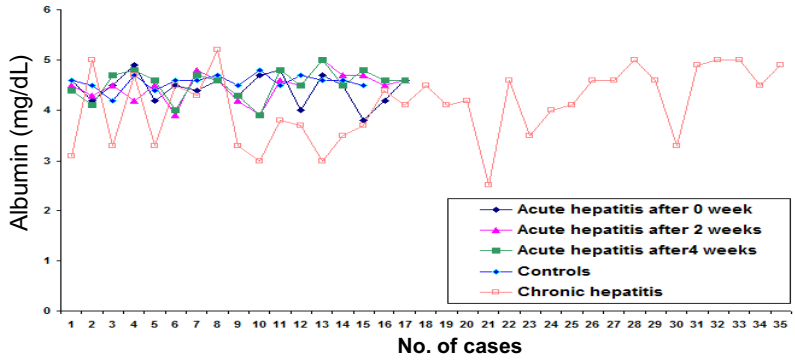

Fig. (6) Individual values of albumin (mg / dL) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

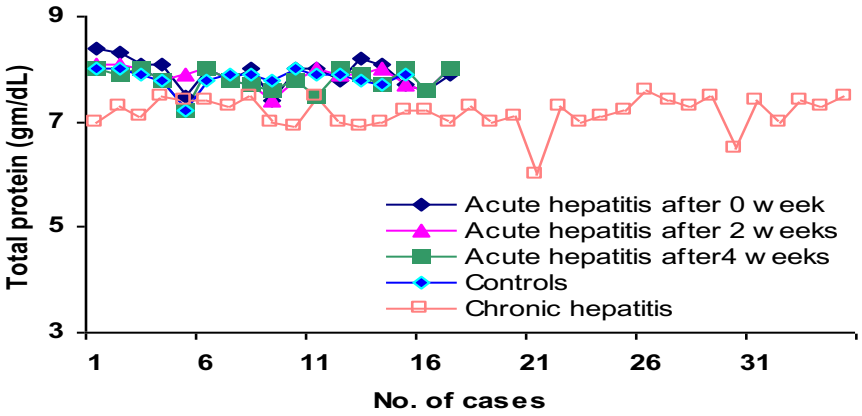

Fig. (7) Individual values of total protein (gm/dL) in patients with acute hepatitis (after (0, 2 and 4 weeks), chronic hepatitis and normal (controls)

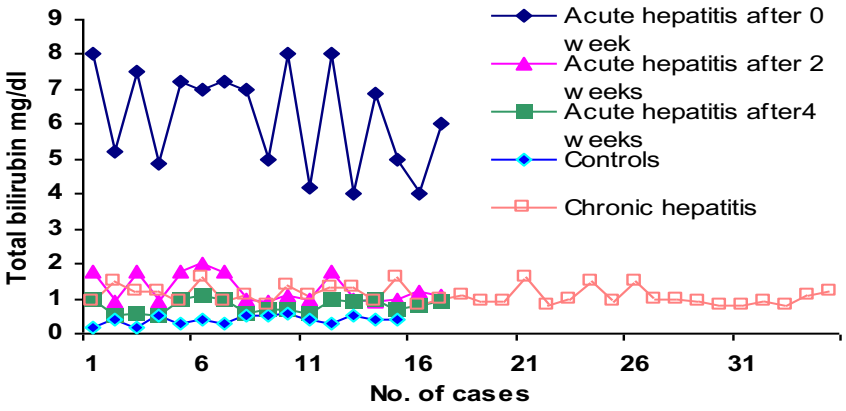

Fig. (8) Individual values of total bilirubin (mg/dL) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

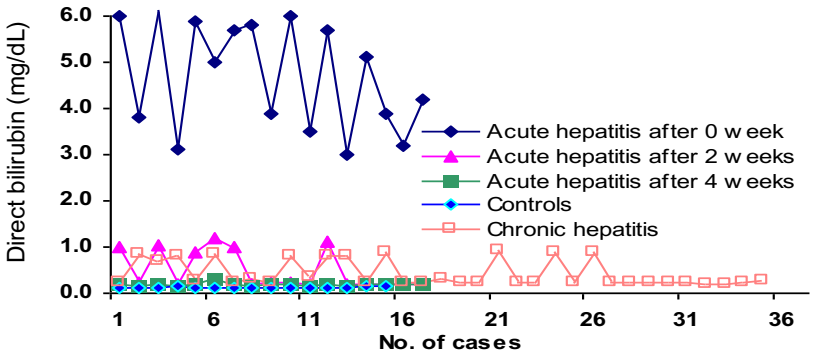

Fig. (9) Individual values of direct bilirubin (mg/dL) in patients with acute hepatitis (after 0, 2 and 4 weeks), chronic hepatitis and normal (controls).

DISCUSSION

The aim of this work is to study the enzymes alpha glutathione-s-transferase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, Albumin, total protein, total and direct bilirubin in the serum of a group of patients with acute hepatitis (17 cases) after 0, 2, 4 weeks of symptoms appearance and another group of patients with chronic hepatitis (35 cases) hoping that these results will provide a useful tool for using α-GST in the early diagnosis and follow up in the treatment of acute and chronic hepatitis patients, using α-GST as a marker of hepatocellular damage.

α-glutathione-S-transferase may be a good serologic marker of hepatocellular damage because of its low molecular weight, uniform hepatic distribution, high cytosolic concentration, and short half-life. Therefore, it provides a more accurate reflection of the activity of hepatocellular damage. In contrast to ALT that has a predominately periportal distribution, α-GST has been shown by immunohisto-chemical studies to be distributed uniformly in the liver lobule.

These characteristics suggests that α-GST might be a more sensitive and specific indicator of hepatocellular damage, particularly

damage due to chronic hepatitis, which has both lobules as well as portal and periportal patterns of injury***. (Nelson et al.,1995)***

The mean value of serum αGST of patients with acute hepatitis was highly significantly increased to: (i) 73.9 ± 13.98 ng/ml by 1775.6 % , $P < 0.001$ after 0 week (Table 3), (ii) 25.529 ± 6.501 ng/ml by 548 %, $P < 0.001$ after 2 weeks (Table 4) and (iii) 19.76 ± 3.734 ng/ml by 402 %, $P < 0.001$ after 4 weeks (Table 5). Our results are in accordance with those of ***(Koo et al.,2000)*** reported that plasma αGST increased earlier than liver transaminase levels, αGST may be a more sensitive indicator of early liver injury and should be used in monitoring hepatocellular damage during the progression of sepsis. ***Kumtepe et al., (2002)*** concluded that measurement of plasma GST might provide an earlier and much more sensitive indicator of hepatocellular damage than other liver function tests. ***Sidlova et al., (2003)*** who found significantly higher levels in patients with cystic fibrosis, he suggested that a raised serum αGST level might be a marker of possible pathological changes of the hepatobiliar system in cystic fibrosis (CF). Serum α-GST seems to be a more sensitive marker than transaminase for the monitoring of hepatocellular integrity and as an early predictor of hepatic damage. In chronic hepatitis: the patient mean value of serum αGST was increased to 14.58 ± 3.66 ng/ml by 270.1 % , $P < 0.001$ (Table 6). These results are in agreement with ***(Williams and Marks, 1998)*** reported that GST is more frequently elevated than AST in chronic active hepatitis. ***Giffen et al., (2002)*** reported that αGST in the Wistar Han rat was shown to be a valid marker of these types of induced hepatotoxicity. However, the measurement of αGST offered no additional information in detecting either the time of onset/ recovery or the severity of each type of hepatic injury induced, in comparison to the panel of markers already established within this laboratory. ***Mazur et al., (2003)*** stated that the activity of alpha - GST is reported to reflect interstitial liver damage better than that of aminotransferase, especially in patients with auto-immune hepatitis, and according to some authors, has also been proposed to be indirectly involved in hepatocellular damage due to hepatitis C virus (HCV) infection.

The mean value of serum ALT of patients with acute hepatitis was increased to: (i) 436.5 ± 97.5 U/L by 1652 %, $P < 0.001$ after 0 week (Table 3) (ii) 49.51 ± 9.05 U/L by 98.92 %, $P < 0.001$ after 2 weeks (Table 4) and (iii) 29.07 ± 8.85 U/L by 19.33 %, $P < 0.05$ after 4 weeks (Table 5). Our results are in accordance with ***(Herve et al.,2001)*** during acute HCV infection, ALT levels in the blood may rise to twenty times above normal. Excess ALT leaks into the bloodstream when liver cell are injured or dying. ***Lewandrowski; (2002)*** reported that in acute hepatocellular injury such as infectious and toxic hepatitis, ALT and AST levels may increase 20 to 50 fold (even up to 100 fold) of the upper limit of normal range. ***Sidlova et al., (2003)*** reported that serum ALT and AST were increased, at least to some extent, in most liver disorders. In general, serum aminotransferase increase reflects the relative extent of active hepatocellular damage but not necessarily its aggregate severity. In acute liver damage abnormalities in serum aminotransferase concentration often lag behind the change in hepatocellular integrity. ***Bishop et al., (2005)*** reported that higher elevation are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory condition of the liver, ALT elevation are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum. While the mean value of serum ALT in patients with chronic hepatitis was increased to 64.63 ± 28.82 U/L, by 159.7%, $P<0.001$ (Table 6). The results are in agreement with ***(Burtis et al.,2001)*** with viral hepatitis and other forms of liver disease associated with hepatic necrosis, serum AST and ALT levels are elevated even before the clinical signs and symptoms of disease such as jaundice. ***Bishop et al., (2005)*** reported that elevated aminotransferase suggest hepatocellular damage. In severe viral hepatitis that causes extensive acute necrosis, significantly elevated serum aminotransferase levels may be found whereas only moderate increases are found in less severe cases. ***Mehta et al., (2005)*** suggested that liver enzyme elevation > 5 times ULN (Upper Limit of Normal) occur frequently in course of chronic hepatitis C .

The mean value of serum AST of patients with acute hepatitis was increased to: (i) 380.13 ± 123.5 U/L by 1918 %, P < 0.001 after 0 week (Table 3), (ii) 52.8 ± 13.08 U/L by 180.3 %,P < 0.001 after 2 weeks (Table 4) and (iii) 32.659 ± 6.304U/L by 73.35%, P<0.001 after 4 weeks (Table 5). Our results are in accordance with ***(Dajani et al.,2001)*** who reported that serum ALT and AST are increased at least to some extent, in most liver disorders. An additional limitation of using aminotransferases as markers for hepatocellular injury is their comparatively long plasma half-lives (17h for AST ; 47 h for ALT). Thus during acute liver damage, abnormalities in serum aminotr-ansferase concentration often lag behind changes in hepatocellular integrity. ***Bishop et al., (2005)*** reported that AST levels are highest in acute hepatocellular disorders. In viral hepatitis , levels may reach 100 times. The mean value of serum AST of patients with chronic hepatitis was increased to 69.54 ± 23.01 U/L by 269.1 %, P < 0.001 (Table 6) these results are in accordance with ***(Marshall; 2000)*** reported that increased aminotransferase activities reflect cell damage; plasma levels may be 20 times the upper limit of normal in patients with hepatitis. ***Herve et al., (2001)*** found that liver enzyme levels in people with chronic HCV infection can be normal, periodically elevated, or persistently elevated. In chronic hepatitis C , the elevation of aminotransferases ranging from 0 to 20 times (usually <5 times) the upper limit of normal AST levels may rise to twenty times above normal during acute HCV infection , and remain elevated during chronic HCV infection. ***Mehta et al., (2005)*** reported that HCV infections were recognised by elevation in liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The mean value of serum GGT in patients with acute hepatitis was significantly increased to: (i) 280.1 ± 77 U/L by 1534 %, P < 0.001 after 0 week (Table 3), (ii) 46.72 ± 13.56 U/L by 172.3 %, P < 0.001 after 2 weeks (Table 4) and (iii) 28.97 ± 8.38 U/L by 69.02 %, P < 0.001 after 4 weeks (Table 5). These results are in accordance with many authors. ***Burtis et al., (2001)*** and ***Lewandrowski; (2002)*** reported that in acute infectious hepatitis, GGT elevation is moderate (two to five fold increase) and is less useful than ALT levels. ***Bishop***

et al., (2005) reported that GGT is elevated in virtually all hepatobiliary disorders, GGT is a useful test to confirm hepatic disease in patients with elevated alkaline phosphatase. In chronic hepatitis, the mean value of serum GGT in patients with chronic hepatitis was significantly increased to 53.57 ± 10.48 U/L by 212.6 %, P < 0.001 (Table 6) these results are in line with many authors. ***Burtis et al., (2001)*** and ***Lewandrowski; (2002)*** reported that GGT activity is elevated in all forms of liver diseases.

The mean value of serum alkaline phosphatase in patients with acute hepatitis was increased to (i) 428.6 ± 113.27 U/L by 259.2 %, P <0.001 after 0 week (Table 3), (ii) 255.9 ± 37.26 U/L by 114.5 %, P < 0.001 after 2 weeks (Table 4), (iii) 195.1 ± 17.87 U/L, by 63.51%, P<0.001 after 4 weeks (Table 5). These results are parallel to those of ***Pratt and Kaplan, (2003)*** found that an increase in ALP activity of three times the upper limit of normal may occur in all types of hepatic disorder including viral hepatitis, chronic hepatitis. ***(Bishop et al., 2005)*** found that in acute viral hepatitis, alkaline phosphatase is usually either normal or moderately raised but up to 40 % of patients have levels two and-a- half times the upper reference limit. Hepatitis A infection may cause a cholestatic picture, with pruritus and elevation of alkaline phosphatase and cholestatic features may also be seen with hepatitis A and B infection, very high alkaline phosphatase may be found in Epstein Barr virus infection. In chronic hepatitis, the mean value of alkaline phosphatase in patients with chronic hepatitis was increased to 204.4 ± 12.9 U/L by 71.29% , P<0.001 (Table 6). These results are in good agreement with those of: ***(Bishop et al.,2005)*** who reported that slight to moderate increases in ALP activity occur in many patients with hepatocellular disorders, such as hepatitis and cirrhosis, chronic hepatitis and transient increases may occur in all types of liver disease.

With regard to albumin the mean value of serum albumin in patients with acute hepatitis was insignificantly changed. While, in chronic hepatitis; the mean value of serum albumin in patients with chronic hepatitis was significantly decreased to 4.109 ± 0.72 gm/dl by 10.03 %, P < 0.001 (Table 6) compared to the controls.

The mean value of serum total protein in patients with acute hepatitis showed insignificant results (Table 5). In chronic hepatitis; the mean value of serum total protein in patients with chronic hepatitis was significantly decreased to 7.174 ± 0.312 gm/dl by 8.41 %, P < 0.001 (Table 6). The high significance of the results of total protein and albumin is explained by the presence of 12/35 cases which have lower levels of total protein and albumin (34.2%). ***Marshall; (2000)*** found that the serum albumin has remained normal in acute illness (acute hepatitis). Plasma albumin concentration tends to decrease in chronic liver disease, but is usually normal in the early stages of acute hepatitis due to its long half-life (approximately 20 days). ***Fontana and lok , (2002)*** reported that a seriously damaged liver is unable to produce sufficient albumin. Albumin levels usually remain normal until late-stage disease. ***Lewandrowski; (2002)*** found that the changes in the total serum protein may result from changes in albumin, globulins or both. As mentioned earlier a change in one protein may be offset by a change in the opposite direction of another. For this reason , it may be useful to determine the ratio of the albumin concentration to the globulin concentration (A/G ratio). The ratio may be markedly abnormal in spite of a normal total protein , such as associated with a decreased albumin concentration. However, albumin has a relatively long half-life (19 days) and a large body pool and typically takes some times to fall after the onset of liver injury. Therefore ,patients with acute hepatocellular disorders may exhibit normal albumin levels. ***Bishop et al., (2005)*** found that most proteins are produced by the liver, A decreased serum albumin may be a result of decreased liver protein synthesis. The albumin level correlates well with the severity of functional impairment and is found more often in chronic rather than acute liver disease.

The mean value of serum total bilirubin of patients with acute hepatitis was increased to: (i) 6.34 ± 1.45 mg/dl, by 1512 %, P < 0.001after 0 week (Table 3), (ii) 1.29 ± 0.42 mg/dl, by 229 %, P < 0.001 after 2 weeks (Table 4), and (iii) 0.79±0.21 by 101.7%, P < 0.001 after 4 weeks (Table 5). While the mean value of serum total bilirubin of patients with chronic hepatitis was increased to 1.091 ± 0.26 mg/dl, by 177.5 %, P < 0.001 (Table 6).

The mean value of serum direct bilirubin of patients with acute hepatitis was increased to: (i) 4.83 ± 1.16 mg/dl, by 3928 %, $P < 0.001$ after 0 week (Table 3), (ii) 0.498 ± 0.418 mg/dl, by 315.2 %, $P < 0.001$ after 2 weeks (Table 4) and (iii) 0.194 ± 0.032 mg/dl, by 61.25 %, $P < 0.001$ after 4 weeks (Table 5). While the mean value of serum direct bilirubin of patients with chronic hepatitis was increased to (0.433 ± 0.279 mg/dl), by 260.5 %, $P < 0.001$(Table 6). Our results are in accordance with ***(Lehmann; 1998)*** who reported that ,in acute viral hepatitis, total bilirubin is slight persistent elevation. ***Williams and Marks, (1998)*** found that infulminant liver failure the peak bilirubin is a prognostic sign; and in chronic liver diseases a gradual and pronounced increase in serum bilirubin occur. ***Henry, (2001)*** reported that in acute liver diseases, the most dramatic symptoms are related to abnormal bilirubin handling. In most cases, jaundice is noted by the patients, usually as a yellow discoloration of the eyes, within a few days of onset of these symptoms. Because jaundice is much more common in acute liver disease than in chronic liver disease. The hallmark of acute hepatocellular injury. In children, only 1% have peak bilirubin greater than10 mg/dl. In adults, jaundice develops in 70 % of cases of acute hepatitis A, 33% of cases of hepatitis B, and about 20 % of acute hepatitis C cases. In the most common form, damage to hepatocytes is primarily related to an immune response. In viral and some forms of drug induced hepatocellular injury. There is a gradual rise in cytoplasmic enzyme (AST and ALT), followed after some days by an increase in bilirubin. Alkaline phosphatase is usually normal or mildly elevated , while GGT is increased in most cases of acute hepatocellular injury, AST and ALT reach their peak values (usually 8 to 40 times the upper reference limit)at or before the onset of jaundice, then gradually decline. The rate of fall in AST and ALT average 10% to 12% per day , much slower than predicted by their half- life and indicating ongoing hepatic injury. Levels return to normal an average of four weeks after clinical diagnosis, but with wide variation in duration of elevated values. Bilirubin typically peaks at about one to two weeks, usually at less than 15 mg/dl, then falls slowly. ***Swan; (2004)*** reported that liver cell injury is indicated when the total bilirubin level is high while indirect bilirubin is low. Hepatitis

C infection can slow the processing of bilirubin in the liver and bilirubin levels can become elevated, causing jaundice. ***Bishop et al., (2005)*** reported that largest percentage of patients has hepatic jaundice. Hepatic Jaundice may result from impaired cellular uptake, defective conjugation or abnormal secretion of bilirubin by the liver cell.

CONCLUSION

The increment of serum α-GST indicates a liver involvement even when ALT levels are normal. This may be clinically relevant to apparently "healthy carriers" whose α-GST values, when increased, might need further evaluation.

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