STUDY ON SOME AMINO ACID METABOLISING ENZYMES AND ASSOCIATED CHANGES IN CERTAIN HUMAN LIVER DISORDERS

R J Chhabra[1], C P Kamariya[2], Ketan Mangukiya[3,*]

[1]Professor and Head, [2]Assistant professor, Department of biochemistry,
PDU Govt. Medical College and Civil Hospital, Rajkot, Gujarat, India
[3]Assistant Professor, Department of Biochemistry,
Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India

***Corresponding Author:**
E-mail: dr.ketan.mangukiya@gmail.com

ABSTRACT:

Background: *The estimation of enzymes in serum is useful aids to detect and confirm a diagnosis of various hepatic disorders and their importance in clinical assessment of liver pathology is now well established. During the last three decades, the importance of trace elements in human health and disease has been receiving increasingly greater attention.*

Aims and objectives: *To study and compare the peak diagnostic elevations and normalization patterns of the four serum enzymes ALT(Alanine transaminase) and AST(Aspartate transaminase),OCT(Ornithine carbomyl transferase), ARG(Arginase) and the disease associated changes in serum copper and bilirubin during liver disorders. To see a statistically correlation between various circulating elevated enzymes and the associated change in serum concentration of Copper and Bilirubin.*

Material and Method: *The clinical material for present study comprised of 46 normal healthy subjects serving as a control group(Group 1) and a study group of 186(Group 2) patients suffering from liver disorders as diagnosed by attending physicians. Blood sample from all participants was analyzed for various parameter like HBsAg, ALT, AST, OCT, ARG, S.bilirubin and S.copper at time of admiision,2 nd day after admission, After 1 week, After 2 week, After 3 week(on discharge) and during first and second followup.*

Results & **Conclusion:** *Amongst the enzymatic parameters OCT activity was probable the most useful indicator of the severity of illness. The comparative usefulness in the order is OCT>AST>ALT>ARG.Total bilirubin was found to be very good indicator of severity, hepatic damage and prognosis. Seum copper level were indicative of severity of disease to some extent. High serum peak arginase activity were observed in post necrotic cirrhosis and hepatic encephalopathy. High and significant elevation of serum copper level were observed in all liver disorder during acute phase of disease except alcoholic hepatitis.*

Key words: *Liver disease, OCT, ARG, Copper*

INTRODUCTION

The Human liver is the largest and from the metabolic stand point the most complex internal organ discharging its diverse functions even in adverse circumstances till 15% of its parenchymal cells remain functional. It plays central role in the maintenance of the interior milieu or metabolic homeostatis.The estimation of enzymes in serum are useful aids to detect and confirm a diagnosis of various hepatic disorders and their importance in clinical assessment of liver pathology is now well established[1].

Due to establishment of precise rapid and well-studied on enzyme assay techniques with good correlation to the diseased states[2].physicians have begun to rely more heavily on these enzymes values and patterns in order to detect liver disease, mark the progression, severity and monitor follow up of the therapy.

Conventially ALT (Alanine transaminase) and AST(Aspartate transaminase) became accepted marker for liver injury but in subsequent year it was realised that the true markers happen to be the liver specific enzymes OCT(Ornithine carbomyl transferase), ARG(Arginase) etc[3].The major disadvantage of the ALT is that its lack of specificity[4]..Haemolysed sera cannot be used for AST and ALT assay since it has been shown by King[5].. The well-defined different half-lives of enzymes in plasma or serum are of major diagnostic importance. The Enzymes with longer half-life such as ALT(47±5 hr) often reach their maximum level later and persist with decreasing level in comparison to those with shorter half-life such as AST(17±2.5 hr).

Two such enzyme OCT and ARG which is liver specific and they have a high hepatoplasmatic ratio or gradient similar to aminotransferase. Both enzymes OCT and ARG have 2-5 times short half-life as compared to aminotransferase. Since all enzymes ALT, AST, OCT and ARG of this study act on amino acid substrates they have been collectively called as amino acid metabolising enzymes in this study.

Both copper and Zinc are intimately concerned with many liver disease and their major function in metabolism appear to be enzymatic, thus it is reasonable to speculate that their level in cells controls the physiological processes through the formation and regulation of activity of those enzymes dependant up on them[6].. This study of serum copper was also conducted with view to reveal whether any

statistical correlation exist between serum trace metal concentration and diagnostic seum enzymes ALT, AST, ARG, OCT and Bilirubin.

MATERIAL AND METOD

The present study was conducted at the B.J.Medical College and attached civil hospital, Ahmedabad, Gujarat, India. The clinical material for present study comprised of 46 normal healthy subjects serving as a control group (Group 1) and a study group of 186(Group 2) patients suffering from liver disorders as diagnosed by attending physicians.

Exclusion criteria- Patients having Diabetes malitus, Hypertension, COPD, Thyroid disorder, Cardiac disease and any other major illness apart from liver disease were excluded from study.

Sample collection- informed consent was obtained from each patient before sample collection. 10 ml blood sample was collected from each subjects in a plain vial by vein puncture and centrifuged at 2000 RPM for 10 min after allowing the blood to clot at room temperature. The serum will be separated into proper aliquots and will be analyzed for various parameter like HBsAg, ALT, AST, OCT, ARG, S.bilirubin and S.copper etc.at time of admiision, 2nd day after admission, After 1 week, After 2 week, After 3 week(on discharge) and during first and second followup.

Serum HBSAg was done by card test. Estmation of Trace metal like serum copper was done by Flame atomic absorption spectrophotometry (FAAS) and rest of the parameter was measured by spectrophotometry. All samples were repeated twice along with QC sera for Accuracy purpose.

Obtained data were analysed statistically by calculating P-value and correlation coefficient.

RESULT

The study group consist of total 186 participants between 14-52 year age group. Among them 133 were male and 53 female. The number of participant in control group is 46 having age between 11-50 year.

Obtained data are compared with control group by calculating P-value(Online student t-test calculator).P-value less than 0.05 was consider as a significant.

Table 1: Distribution of study group patients according to Diagnosis, Sex and Range

Diagnosis	Number(n)	Sex M:F	Age range(year)
Acute viral hepatitis(HBsAg –ve)	91	58:33	14-47
Acute viral hepatitis(HBsAg +ve)	36	26:10	16-44
Toxic drug induced hepatitis(HBsAg –ve)	12	8:4	34-49
Acute alcoholic hepatitis without cirrhosis(HBsAg –ve)	18	18:0	36-52
Chronic active hepatitis(HBsAg +ve)without cirrhosis	10	7:3	30-45
Post necrotic cirrhosis with Jaundice(HBsAg –ve)	11	11:0	38-50
Hepatic encephalopathy viral aetiology(HBsAg +ve)	08	5:3	31-39
Total	**186**	**133:53**	**14-52**

Table 2: concentration of various parameter determined serially during hospital stay and follow up in total cases of Acute viral hepatitis

Inter-val	Total no. of case	Cases discharged in period	Casedied	ALT (U/L) Mean± SD	AST (U/L) Mean ± SD	OCT (U/L) Mean ± SD	ARG (U/L) Mean± SD	T.Bilirubin (Mg/dl)) Mean ± SD	S.copper (Microgm/dl) Mean ± SD
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
On admission	36	-	-	427.44 ±29.32*	265.38 ±25.46*	60.15 ±10.24*	15.80 ±4.86*	7.44 ±2.25*	148.14 ±10.40*
2 day after admission	35	-	1	304.82 ±23.50*	241.52 ±20.11*	52.32 ±8.66*	18.11±6.17*	10.82 ±3.15*	145.65 ±5.33*
After 1 week	35	-	-	236.18 ±30.72*	177.43 ±23.18*	43.86 ±14.9*	16.34±4.10*	9.17 ±2.32*	133.82 ±3.87*
After 2 week	33	-	2	170.92 ±21.46*	125.28±16.83*	26.45 ±5.80*	12.63±2.37*	6.78 ±1.15*	132.74 ±4.08*
After 3 week(on discharge)	33	33	-	101.28 ±12.24*	68.32±10.74*	8.91 ±3.33	7.42 ±3.06	3.34 ±1.30*	128.9 ±3.37*
Follow- up									
1(1wk)	26	-	-	83.66 ±8.12*	51.29 ±10.12*	8.52 ±2.42	6.35 ±2.55	3.18 ±1.84*	128.12 ±4.82
2(3wk)	21	-	-	51.83 ±7.76*	30.70 ±6.60*	6.12 ±2.80	5.85 ±1.83	1.81 ±1.33	120.48 ±5.28

*** -- indicate P-value less than 0.05(significant)**

Table 3: concentration of various parameter determined serially during hospital stay and follow up in total cases of toxic drug hepatitis

Inter-val	Total no. of case	Cases discharged in period	Casedied	ALT (U/L) Mean± SD	AST (U/L) Mean ± SD	OCT (U/L) Mean ± SD	ARG (U/L) Mean± SD	T.Bilirubin (Mg/dl)) Mean ± SD	S.copper (Microgm/dl) Mean ± SD
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
On admission	12	-	-	98.23 ±14.56*	123.81 ±18.66*	20.86 ±6.82*	8.62 ±3.44*	3.72 ±1.52*	149.44 ±8.69*
2 day after admission	12	-	1	84.84 ±12.35*	92.43 ±12.87*	15.38 ±4.64*	9.80 ±3.52*	4.06 ±1.35*	138.62 ±7.48*

After 1 week	10	2	-	71.31 ±8.27*	78.52 ±8.15*	9.22 ±2.60	7.46±2.85	3.36 ±1.27*	140.82 ±4.72*
After 2 week	7	3	2	45.86 ±2.94*	42.15 ±6.83*	5.83 ±2.82	4.86±1.13	2.88 ±1.08	122.61 ±6.33
After 3 week(on discharge)	7	7	-	33.82 ±1.83*	28.42 ±2.61	4.26 ±2.88	3.18 ±1.50	1.24 ±0.62	118.82 ±6.59
Follow- up									
1(1wk)	6	-	-	28.55 ±2.84	26.24 ±3.10	4.52 ±2.88	3.25 ±2.10	0.95 ±0.45	118.48 ±7.15
2(3wk)	4	-	-	26.78 ±4.12	25.36 ±2.72	4.28 ±2.26	3.12 ±2.16	0.76 ±0.28	114.28 ±7.23

*** -- indicate P-value less than 0.05(significant)**

Table 4: concentration of various parameter determined serially during hospital stay and follow up in total cases of Acute alcoholic hepatitis without cirrhosis

Inter-val	**Total no. of case**	**Cases discharged in period**	**Casedied**	**ALT (U/L) Mean± SD**	**AST (U/L) Mean ± SD**	**OCT (U/L) Mean ± SD**	**ARG (U/L) Mean± SD**	**T.Bilirubin (Mg/dl)) Mean ± SD**	**S.copper (Microgm/dl) Mean ± SD**
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
On admission	18	-	-	72.54 ±14.22*	116.28 ±12.81*	30.48 ±6.85*	8.31 ±2.64*	4.82 ±1.77*	124.15 ±4.22
2 day after admission	18	-	-	65.26 ±10.34*	118.56 ±10.74*	25.26 ±6.64*	7.25±2.35*	4.74 ±1.82*	125.26 ±4.36
After 1 week	17	-	1	54.62 ±8.68*	97.51 ±11.82*	22.82 ±5.67*	6.82±2.15*	3.62 ±1.56*	124.36 ±4.04
After 2 week	15	2	-	50.86 ±7.38*	83.53±12.65*	16.42 ±7.18*	6.31±2.06	2.70 ±1.42*	120.85 ±3.62
After 3 week(on discharge)	15	15	-	42.75 ±6.23*	58.57±9.26*	12.9 ±2.12*	5.25 ±1.26	2.02 ±0.85*	114.68 ±5.12
Follow- up									
1(1wk)	12	-	-	34.26 ±2.89	50.37 ±10.22*	10.33 ±2.36	4.36 ±1.10	1.86 ±0.76*	113.55 ±4.88
2(3wk)	10	-	-	22.85 ±2.30	42.28 ±7.76*	9.28 ±3.45	4.20 ±1.36	1.70 ±0.62*	114.23 ±3.36

*** -- indicate P-value less than 0.05(significant)**

Table 5: concentration of various parameter determined serially during hospital stay and follow up in total cases of Chronic active hepatitis(HBsAg +ve)without cirrhosis

Inter-val	Total no. of case	Cases discharged in period	Casedied	ALT (U/L) Mean± SD	AST (U/L) Mean ± SD	OCT (U/L) Mean ± SD	ARG (U/L) Mean± SD	T.Bilirubin (Mg/dl)) Mean ± SD	S.copper (Microgm/dl) Mean ± SD
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
On admission	10	-	-	168.66 ±11.82*	97.80 ±8.90*	17.28 ±2.73*	21.64 ±5.72*	4.37 ±1.15*	148.14 ±6.85*
2 day after admission	10	-	-	149.12 ±13.46*	84.51 ±7.66*	15.42 ±2.95*	22.37±6.14*	4.16 ±1.22*	144.72 ±5.78*
After 1 week	9	-	1	108.77 ±10.66*	63.08 ±8.11*	13.36 ±1.75*	14.80±5.46*	3.27 ±1.08*	131.48 ±4.82*
After 2 week	9	-	-	83.45 ±10.11*	54.83±6.44*	9.84 ±1.82*	8.33±4.58*	2.26 ±0.95*	128.65 ±5.40*
After 3 week(on discharge)	9	-	-	76.44 ±11.73*	41.67±5.15*	7.95 ±2.14*	5.29 ±1.83	1.82 ±0.55*	120.30 ±6.26
Follow- up									
1(1wk)	8	-	-	65.52 ±8.94*	37.24 ±5.09*	7.19 ±1.57	4.75 ±2.15	1.38 ±0.30*	118.78 ±6.71
2(3wk)	6	-	-	44.35 ±6.05*	28.69 ±4.28	5.86 ±1.62	4.40 ±1.88	1.12 ±0.46	118.25 ±7.25

*** -- indicate P-value less than 0.05(significant)**

Table 6: concentration of various parameter determined serially during hospital stay and follow up in total cases of Post necrotic cirrhosis with Jaundice

Inter-val	Total no. of case	Cases discharged in period	Casedied	ALT (U/L) Mean± SD	AST (U/L) Mean ± SD	OCT (U/L) Mean ± SD	ARG (U/L) Mean± SD	T.Bilirubin (Mg/dl)) Mean ± SD	S.copper (Microgm/dl) Mean ± SD
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
On admission	11	-	-	73.82 ±16.38*	102.35 ±12.20*	20.22 ±2.86*	26.46 ±6.31*	3.92 ±1.16*	169.72 ±6.88*
2 day after admission	11	-	-	69.34 ±14.77*	86.42 ±11.63*	18.28 ±2.65*	25.38±6.85*	3.55 ±1.24*	160.44 ±7.00*

After 1 week	10	-	1	55.64 ±15.26*	62.81 ±10.88*	12.72 ±2.15*	18.29±5.77*	2.62 ±1.88*	148.38 ±6.27*
After 2 week	10	-	-	48.80 ±11.73*	52.42±10.20*	8.13 ±3.72*	11.34±2.15*	1.66 ±1.73*	142.24 ±6.45*
After 3 week(on discharge)	10	10	-	42.35 ±5.15	48.44±7.96*	6.84 ±1.28	8.58 ±2.12*	1.21 ±0.86	131.52 ±6.30*
Follow- up									
1(1wk)	8	-	-	36.62 ±2.48	34.48 ±2.62	5.86 ±1.62	6.11 ±1.96	1.16 ±0.76	128.48 ±4.22*
2(3wk)	4	-	-	32.46 ±3.08	30.66 ±2.10	5.12 ±1.47	5.06 ±2.16	1.20 ±0.68	129.62 ±5.29*

*** -- indicate P-value less than 0.05(significant)**

Table 7: concentration of various parameter determined serially during hospital stay and follow up in total cases of Hepatic encephalopathy viral aetiology(HBsAg +ve)

Inter-val	Total no. of case	Cases discharged in period	Casedied	ALT (U/L) Mean± SD	AST (U/L) Mean ± SD	OCT (U/L) Mean ± SD	ARG (U/L) Mean± SD	T.Bilirubin (Mg/dl)) Mean ± SD	S.copper (Microgm/dl) Mean ± SD
Control	-	-	-	14.84 ±4.77	18.22 ±3.60	4.08 ±1.90	2.96 ±1.72	0.60 ±0.18	112.92 ±7.06
Onset of hepatic encephalopathy Grade 1-2	8	-	-	165.25 ±85.80*	206.80 ±62.4*	78.05 ±24.4*	32.27 ±8.75*	15.22 ±6.72*	181.56 ±15.28*
Hepatic encephalopathy Grade 3-4	5	-	3	181.30 ±96.6*	376.45 ±55.7*	96.32 ±20.81*	37.50±9.20*	17.86 ±7.18*	166.33 ±21.50*
Last blood sample before death	4	-	4	231.95 ±54.5*	428.63 ±39.8*	90.47 ±31.6*	43.41±8.16*	16.34 ±6.90*	171.70 ±20.42*

*** -- indicate P-value less than 0.05(significant)**

Table 8: Statatical correlation between S.Enzymes ALT, AST, OCT, ARG and S.Copper and total bilirubin in patients with acute viral hepatitis

Correlation Between	**Correlation Coefficient(r)**	**Significance of correlation 'p'**
S.copper and		
S.ALT	+0.193	NS
S.AST	+0.165	NS
S.OCT	+0.103	NS
S.ARG	-0.571	NS
S.Total bilirubin	+0.214	<0.05
S.ALT and		
S.AST	+0.575	<0.05
S.OCT	+0.182	NS
S.ARG	+0.256	NS
S.Total bilirubin	+0.509	<0.05
S.AST and		
S.OCT	+0.422	
S.ARG	+0.253	NS
S.Total bilirubin	+0.341	NS
S.OCT and		
S.ARG	+0.260	NS
S.Total bilirubin	+0.279	NS
S.ARG and		
S.Total bilirubin	+0.417	<0.05

DISCUSSION

A study was conducted to find out whether the initial admission values of the four enzymes(ALT,AST,OCT,ARG)and associated factors like s.copper and T.bilirubin were able to indicate severity or distinguish the sever from moderate and mild hepatitis cases.

It can be noted that no significant statistical difference was found between the mean admission values of serum ARG in these subgroups, hence amongst the enzymes, serum ARG was the poorest indicator of severity. Serum OCT and AST on the other hand indicated severity quite well between these various subgroup. Therefore it can be said that amongst these enzymes, serum OCT and AST indicated severity better than ALT and ARG and by comparison the serum level of OCT were found statistically superior to AST as indication of severity between these subgroup. This confirms the report of chausha et al[7].,ceriotti[8]. and Gitlin[9]. and Ceausa et al[10]. that serum OCT is a good indicator of severity in liver disorders.

Among the associated factors serum copper and bilirubin was the poorest indicator, since statically significant difference was not observed in its levels between these subgroups. It was observed that mortality increased with increasing S.OCT activities beyond 40 U/L and that amongst the 3 cases of HBsAg positive viral hepatitis who died due to the disease, two had peak S.OCT activities greater than 80 U/L while one patients had peak S.OCT activity 71.3 U/L. Also seen from the table that, there was notable difference between average duration of hospitalization between these three catagories. Hence it can be stated that a high peak S.OCT activity indicates a delayed recovery and uncertain prognosis whereas a low S.OCT activity indicates a speedy and good prognosis.

In this study it was observed that between moderate and sever hepatitis cases the heights of S.ARG elevation though did not differ significantly, yet in the severe cases the abnormal S.ARG activity persisted for a longer duration. It can be observed that the mean serum copper level were elevated significantly in all disorder except in the alcoholic hepatitis. Highest elevation were observed in hepatic encephalopathy cases and post necrotic cirrhosis. Highest serum copper levels were observed in HBsAg positive acute viral hepatitis compared to HBsAg negative acute viral hepatitis. These results are agreement with the report of Sharma et al[11]., Goswami and bhattacharya[12].The highest serum total bilirubin levels were observed in hepatic coma patients followed by acute viral hepatitis patients.

Statically significant direct proportional correlation were obtained between ALT and AST and also between ALT and T.bilirubin. A significant linear positive correlation(r = +0.442,p-0.05) was obtained between serum AST and OCT.A good directly proportional correlation(r= +0.417,p-0.05) was obtained between S.ARG and T.bilirubin.

CONCLUSION

Amongst the enzymatic parameters OCT activity was probable the most useful indicator of the severity of illness. The comparative usefulness in the order is OCT>AST>ALT>ARG. Total bilirubin was found to be very good indicator of severity, hepatic damage and prognosis. Seum copper level were indicative of severity of disease to some extent. High serum peak arginase activity were observed in post necrotic cirrhosis and hepatic encephalopathy. High and significant elevation of serum copper level were observed in all liver disorder during acute phase of disease except alcoholic hepatitis.

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