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A Study of Serum Glycolytic Enzymes and Serum B Hepatitis in relation to Liv.52 therapy

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ABSTRACT

Out of the 300 cases of hepatitis studied, 48 were found positive for Australia antigen, in which males predominated (2:1), with low socio-economic status in the young middle age group, the majority coming from urban areas. The complications included acute fulminant hepatitis, arthritis, glomerulonephritis, hepatosplenomegaly, ascites and portal cirrhosis in 4.2-25% cases. Investigations showed that Hb was moderately reduced and E.S.R. raised. Liver function tests showed increased prothrombin time, thymol flocculation and serum bilirubin and the A/G ratio was reversed. Among the glycolytic enzymes, maximum increase was noted in SGPT, ICDH and LDH5 levels.

Random allocation of Liv.52 therapy along with symptomatic treatment in all cases of hepatitis and cirrhosis, irrespective of Australia antigen status, brought about a significant decrease in all the glycolytic enzymes after one month. These were the results of the whole group though there were individual variations. When HBsAg positive cases were analysed for Liv.52 therapy improvement at fortnightly intervals, it was found that 58.3% of cases showed progressive improvement, clinically as well as biochemically. 25% cases failed to show any response to Liv.52.

Non-responsers to Liv.52 showed partial improvement on addition of steroids, but the levels never returned to normal. On withdrawing steroids, after initial marginal deterioration, there was slow, steady improvement with normalcy restored in 4-6 weeks. Liver biopsy of these cases showed chronic active hepatitis with nodular regeneration. Nearly 16.6% were admitted in hepatic coma and showed satisfactory response to Liv.52 drops.

16.7% cases were severely ill with advanced cirrhosis, jaundice and ascites and were treated by steroids at other centres with no significant improvement. When put on Liv.52 they showed steady improvement. Two deaths occurred at the beginning of admission as a result of paracentesis. Two remained unwell but 4 improved satisfactorily (50%).

INTRODUCTION

Liv.52 is the proprietary name for a compound tablet and liquid preparation containing 8 different indigenous ingredients, which are reported to have marked stimulant effects on the functional efficiency of the liver and spleen. Besides, they have a protective action on the histological architecture of the liver and a salutary effect on liver glycogen and serum protein. They also have a diuretic effect, especially in hepatic cirrhosis and act as haematinic tonics. All these actions are obviously of great importance in liver disorders arising from infection and toxins. Among the present day drugs, culminating from Western research, no single drug has been able to show these pharmacological effects and even steroids tend to fail in the management of various types of hepatitis and cirrhosis.

Type A hepatitis despite being much more common, fortunately only rarely progresses to chronic hepatitis and cirrhosis. On the other hand, Type B hepatitis with a long incubation period occasionally fails to regress and passes on to fulminant hepatitis, chronic hepatitis and even cirrhosis. There are no known drugs which can arrest this process and the role of steroids in the management is controversial besides having several side-effects. In the present study, it was planned to evaluate the effect of Liv.52 on cases of type B hepatitis.

MATERIAL AND METHODS

Three hundred cases of hepatitis were studied and 25 normal persons served as controls. Out of these, 48 were found positive for Australia antigen. The diagnosis was made by counter-current electrophoresis on agar media and by positive latex agglutination test. The following biochemical tests were done on each case before and after therapy.

(1) Routine liver function tests.

(2) Estimation of serum glycolytic enzymes.
   (i) SGOT and SGPT by the modified Karman method; ICDH by the Burn and Bell method (1960)
   (ii) Total lactic acid dehydrogenase (LDH) by the calorimetric method of King modified by Varley (1969)
   (iii) Heat labile isoenzyme of LDH (LDH-5) by heat stability of Wroblewski (1961) and agar gel electrophoresis.

Twenty four patients having +ve test for HBsAg in serum were randomly allocated for one of the following therapies:

1) Group A – Symptomatic treatment only (Oral glucose and vitamins)
2) Group B – Symptomatic treatment and Liv.52
3) Group C – Symptomatic treatment and Liv.52+steroids.

OBSERVATIONS

Out of a total of 300 clinically diagnosed cases of hepatitis, 48 were found positive for Australia antigen, thus giving an incidence of 16% in the present series.

Tables 1 and 2 depict the age, sex, socio-economic status and place of origin of type B cases. The maximum number of cases occurred in the 2nd and 3rd decades, with a male to female ratio of 2 : 1. 66.6% belonged to a low socio-economic status and there was a higher incidence of patients from urban areas.

Table 1: Showing age and sex distribution of the Australia antigen positive cases

Sex	Age in years			Percentage
	0-20	21-40	41-60	
1. Male (32)	8	16	8	66.66
2. Female (16)	4	8	4	33.4
Total (48)	12	24	12	100.0
Male: Female = 2 : 1				

Table 2: Distribution of cases according to their socio-economic status and place of origin			
Socio-economic status		No. of cases	Percentage
1.	Low	32	66.6
2.	Middle	12	25.0
3.	High	4	8.4
Place of origin			
1.	Rural	20	41.6
2.	Urban	28	58.4

Table 3 shows the clinical complications. The cases presented with persistent jaundice with hepatosplenomegaly and ascites (16.6 to 25%), portal cirrhosis in 25.0% and internal haemorrhage with fulminant hepatitis in 8.3%.

Table 3: Complications in Australia antigen positive cases			
Sl. No.	Complications	No. of cases	Percentage
1.	Chronic jaundice	12	25.0
2.	Hepatomegaly	12	25.0
3.	Hepatosplenomegaly	8	16.6
4.	Ascites	8	16.6
5.	Portal cirrhosis	12	25.0
6.	Internal haemorrhage + fulminant hepatitis	4	8.3
7.	Arthritis / Arthralgia	12	25.0
8.	Associated glomerulonephritis	2	4.2
9.	Encephalopathy	6	12.5

Tables 4 and 5 demonstrate the results of haematological and liver function tests. Haemoglobin was reduced at 9.7 gm% and the ESR raised at 42.1±15.8 mm in the 1st hour. Leucocyte count did not show any specific abnormality. Serum bilirubin was raised at 2.3 mg% and the prothrombin time at 24.7 sec. All serum glycolytic enzymes were abnormally raised, specially SGPT and ICDH. The average A/G ratio was reversed.

Table 4: Haematological findings in Australia antigen positive cases			
Sl. No.	Haematological findings	Range	Mean ± S.D.
1.	Haemoglobin (gm%)	6.0 - 12.0	9.7 ± 2.8
2.	E.S.R.	30.0 - 68.0	42.1 ± 15.8
3.	TLC	3200 - 10200	7811 ± 292
4.	DLC		
	(a) Polymorphs	15.0 - 67.0	55.6 ± 18.6
	(b) Lymphocytes	25.0 - 75.0	39.0 ± 12.5
	(c) Eosinophils	0.0 - 23.0	5.7 ± 2.88

Table 5: Liver function tests of HBsAg positive cases			
Sl. No.	Liver function (mg%)	Range	Mean±S.D.
1.	Serum bilirubin(mg%)	0.70 - 5.7	2.3 ± 1.84
2.	SGOT (IU/L)	17.00 - 114	44.0 ± 20.80
3.	SGPT(IU/L)	22.00 - 216	61.0 ± 27.3
4.	ICDH(IU/L)	25.30 - 54.6	38.6 ± 5.1
5.	TLDH(IU/L)	195.5 - 401	239.0 ± 56.87
6.	LDH-5(IU/L)	54.4 - 75.2	61.8 ± 11.8
7.	Alkaline phosphatase (KA units)	9.0 - 17.0	11.6 ± 3.90
8.	Serum Protein:		
	A.G. ratio Total	6.07 - 7.90	6.52 ± 1.10
	Albumin	2.41 - 3.69	2.94 ± 0.86

	Globulin	2.83 - 4.50	3.52 ± 1.05
9.	Prothrombin time	17 - 37	24.7 ± 8.6
10.	Thymol flocculation	+ - ++	Abnormal in all cases

Table 6 compares the levels of serum glycolytic enzymes in controls, non-type B and type B chronic hepatitis and cirrhosis cases. Type B hepatitis cases showed 2 to 3 times increased serum gllycolytic enzymes as compared to non-type B chronic hepatitis cases and 3 to 4 times higher as compared to controls.

Table 6: Comparative levels of serum glycolytic enzymes in controls, non-type B and type B chronic hepatitis/cirrhosis cases.

Status	SGOT (IU/L)	SGPT (IU/L)	ICDH (IU/L)	TLDH (IU/L)	LDH (IU/L)
Controls	17.2 ±4.3	16.5 ±4.5	5.9 ±2.3	128.5 ±44.5	18.5 ±6.4
Non type B chronic hepatitis/cirrhosis	21.5 ±6.2	27.3 ±10.3	21.5 ±4.2	189.5 ±86.9	23.8 ±12.4
Type B chronic hepatitis/cirrhosis	32.8 ±10.0	51.3 ±28.3	24.4 ±5.6	288.0 ±70.1	60.0 ±11.0

RESULTS OF TREATMENT

All the cases of chronic hepatitis and cirrhosis, irrespective of results of immune counterelectrophoresis, were randomly allocated to treatment with usual supportive drugs with or without Liv.52 (Group B, Group A) or with Liv.52+steroids (Group C). The usual steroid used was prednisolone, 0.5 mg/kg body weight. The treatment was continued in each case for one month and investigations repeated.

Table 7 compares the levels of serum glycolytic enzymes in all cases of viral hepatitis (type B and others) without and with Liv.52 therapy. There is a fall in all the enzymatic levels after treatment but the mean figures for treatment with Liv.52 show a bigger fall and the difference is significant with Liv.52 therapy than without, for SGPT, ICDH and LDH-5.

Table 7: Levels of serum glycolytic enzymes in all cases of chronic hepatitis/cirrhosis before and after treatment. (without and with Liv.52 therapy)

Treatment	SGOT (IU/L)	SGPT (IU/L)	ICDH (IU/L)	TLDH (IU/L)	LDH-5 (IU/L)
Before treatment	28.6 ± 9.7	40.8 ± 30.8	25.0± 6.1	238.2 ± 77.0	41.8 ± 10.6
Symptomatic treatment without Liv.52	27.1 ± 8.4	36.3 ± 20.3	20.9 ± 5.0	208.7 ± 75.1	35.9 ± 11.3
Symptomatic treatment with Liv.52	20.2 ± 8.1	19.8 ± 14.7	14.0 ± 3.0	180.0 ± 66.6	25.4 ± 8.4

Table 8 shows serum bilirubin and glycolytic enzyme levels in HBsAg positive cases before and after treatment with Liv.52 at 15 days and 1 month intervals. A progressive decline was seen of the mean figures in all the cases when considered together. On analysis of individual cases, it was found that 12 cases did not show any response to Liv.52 alone. Steroids were added to Liv.52 in these 12 cases for 1 month and the enzyme assays were repeated after this period, and again after 15 and 30 days of Liv.52 therapy alone.

Table 8: Serum glycolytic enzyme levels in HBsAg positive cases before and after treatment with Liv.52 therapy.

Sl. No.	Serum enzymes	Before treatment (Mean ± SD)	After treatment lasting	
			15 days (Mean ± SD)	1 month (Mean ± SD)
1.	Serum bilirubin (mg%)	1.6 ± 0.22	1.20 ± 0.20	1.1 ± 0.2
2.	SGOT(IU/L)	26.0 ± 7.2	23.0 ± 7.0	20.0 ± 5.4
3.	SGPT(IU/L)	29.0 ± 8.1	21.0 ± 7.0	18.0 ± 4.8

4.	ICDH (IU/L)	24.0 ± 5.7	20.0 ± 4.8	12.8 ± 2.0
5.	TLDH (IU/L)	190.6 ± 28.1	162.0 ± 23.8	149.2 ± 20.1
6.	LDH-5(IU/L)	28.8 ± 4.6	22.6 ± 3.0	20.1 ± 3.0

Table 9 shows the results in these 12 cases. Serum bilirubin and glycolytic enzymes deteriorated with only Liv.52 therapy. Partial improvement occurred after 1 month on steroids; however, serum levels never become normal. After withdrawing steroids for 15 days, the level deteriorated marginally. Liver biopsy of these cases showed chronic active hepatitis in 4 cases, portal cirrhosis in 2 cases and persistent hepatitis in 6 cases. Two cases expired due to gastrointestinal haemorrhage during steroid therapy. However, continued Liv.52 therapy for another 30 days resulted in a slow decline to normal levels with clinical improvement.

Table 9: Serum glycolytic enzyme levels in 12 HBsAg positive cases before and after treatment with Liv.52, then Liv.52 + steroids followed by Liv.52 alone

Sl. No.	Serum bilirubin	Before treatment	After treatment lasting		15 days (Liv.52 alone)	1 month (Liv.52 alone)
			15 days (Liv.52)	1month (steroids) (Liv.52+)		
1.	Serum bilirubin (mg%)	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.06	0.9 ± 0.08	0.8 ± 0.4
2.	SGOT (IU/L)	23.6 ± 5.8	27.0 ± 6.4	17.0 ± 3.0	18.0 ± 3.2	16.3 ± 2.8
3.	SGPT (IU/L)	33.3 ± 10.4	43.0 ± 11.0	21.5 ± 4.1	32.5 ± 8.5	20.4 ± 3.2
4.	ICDH (IU/L)	24.2 ± 3.1	24.0 ± 3.0	10.2 ± 2.1	16.6 ± 2.8	11.5 ± 2.7
5.	TLDH(IU/L)	208.0 ± 40.7	210.0 ± 40.0	147.0 ± 22.0	178.0 ± 32.6	141.3 ± 21.5
6.	LDH (IU/L)	34.0 ± 7.7	33.8 ± 7.4	21.9 ± 6.0	28.5 ± 52.0	20.4 ± 5.4

Another 8 cases were severely ill at the beginning of treatment. Four had features of acute fulminant hepatitis with gastrointestinal haemorrhage and the other 4 had clinical features of advanced cirrhosis with portal hypertension.

They had already received steroids at the time of admission and failed to respond.

Table 10 demonstrates the serum glycolytic enzyme levels in these 8 HBsAg positive cases on steroids (at admission) and after treatment with Liv.52 preparations. They showed rapid and progressive improvement in liver function tests and glycolytic enzymes after withdrawal of steroids and administration of Liv.52. However, two cases expired during the first 15 days of therapy due to hepatic coma following paracentesis of tense ascites. Liver biopsy showed advanced portal cirrhosis in these cases. Three out of four cases of fulminant hepatitis were saved and returned to normal liver function from initial, severely disordered liver function when on steroids.

Table 10: Serum glycolytic enzyme levels in HBsAg positive cases before and after treatment with Liv.52 plus steroids given from the beginning (8 cases)

Sl. No.	Serum enzymes	Before treatment	After treatment lasting	
			15 days	1 month
1.	Serum bilirubin (mg%)	4.3 ± 1.16	2.1 ± 0.9	0.85 ± 0.09
2.	SGOT (IU/L)	89.0 ± 21.6	47.2 ± 7.0	28.5 ± 4.1
3.	SGPT (IU/L)	149.0 ± 26.2	63.2 ± 10.2	24.3 ± 6.2
4.	ICDH (IU/L)	43.6 ± 9.8	21.7 ± 5.6	14.9 ± 2.9
5.	TLDH (IU/L)	298.0 ± 46.7	214.4 ± 50.4	172.0 ± 41.6
6.	LDH-5 (IU/L)	65.8 ± 10.7	50.6 ± 6.3	42.0 ± 5.8

DISCUSSION

The development of immunologic methods for the detection of HBsAg has been a major advance in the management of hepatitis. The continued presence of HBsAg antigen in the blood, bridging necrosis and massive hepatic necrosis of liver biopsy in progressive hepatitis, failure of

transaminase levels to return to normal within a few months and lack of complete resolution of symptoms with persistent hepatomegaly suggest progression of acute hepatitis to the chronic type and this occurs in approximately 10% of type B hepatitis, while virtually all others recover completely. This risk of progressive hepatitis in type B infection tends to be more in elderly patients, in diabetics and in patients with severe illness. Another complication of Type B infection is fulminant hepatitis (Dingstag *et al*., Harrison 1964) occurring in 1.2% of patients. The high incidence of type B hepatitis in tropical and subtropical countries is believed to be due to poor socio-economic status, unhygienic living, malnutrition and probably mosquitoes. Blumberg *et al*. (1969) have shown that susceptibility to persistence of infection depends on the immune response of the patient and autosomal recessive trait.

The incidence of HBsAg in cases of hepatitis in our series was 16%. Gooke and Kavey (1969) and Blumberg *et al*. (1970) reported the incidence of HBsAg in acute hepatitis as varying from 13 to 17%. However, Sama *et al*. (1973) and Tandon (1973) reported negative results for HBsAg in epidemics of acute hepatitis in Baroda and South Delhi.

Six out of 48 cases of HBsAg were in hepatic coma representing an incidence of 12.5%. Forty-four cases of the 252 cases of non-type B hepatitis, i.e. 17%, presented to us in hepatic coma. They were of the younger age group (Between 20-25 years) and 70% were females while the rest were males. Hepatic coma was more frequent among pregnant women.

Out of 48 HBsAg positive cases, ascites was present in 16.6% hepatosplenomegaly in 41.6% and chronic hepatitis and portal cirrhosis in 25% cases. Blood examination in these cases showed lowered Hb and raised ESR and urine examination showed presence of bile salts and bile pigments. Serum bilirubin was raised to 2.30±1.84 mg%. Among the glycolytic enzymes SGOT, SGPT were at 44.0±20.8 IU/L and 61.0±27.3 IU/L respectively. The other glycolytic enzymes ICDH, TLDH and LDH-5 were also significantly raised. All these are indicative of inflammation in the liver. The age group of these cases was older than in acute infectious hepatitis cases with HAA and cases were more often seen in elderly males. The findings are in agreement with other workers, *viz*. Sarin *et al*. (1974), Sherlock *et al*. (1972). The mean age was 52.6 years. The presentation of Australia antigen positive cases was with mild fever, arthralgia and urticaria, and rarely in fulminant hepatitis and hepatorenal syndrome, palpable liver and spleen in more than half the cases and ascites in 25% of cases. The negative cases were afebrile with only marginal liver enlargement and insignificant spleen enlargement. It was clear that Australia antigen leads to an inflammatory process within the liver along with immune complex formation which is responsible for the typical clinical picture and hypergammaglo-bulinaemia.

Liv.52 with its well known anti-inflammatory and hepatic regenerative property was considered a suitable agent for treatment of these cases. It was the added advantage of non-toxicity even when given in large doses and for a long time. Dasgupta and Mukherjee (1970) reported good clinical response with improvement of liver function tests at the end of 4½ months' treatment with Liv.52 in cases of chronic hepatitis Histological evidence of improvement in piecemeal necrosis and arrest of the progressive lobular degeneration was evident in these cases.

In the present series, the serum glycolytic enzymes in cases of chronic hepatitis non-type B were significantly raised as compared to controls. Similarly glycolytic enzyme levels for type B chronic hepatitis were 2.5 times more as compared to controls.

All the cases of chronic hepatitis and cirrhosis, irrespective of the results of electrophoresis, were randomly allocated for treatment with the usual supportive drugs and Liv.52. Observations were made at the end of one month. In controls, the fall of glycolytic enzymes was insignificant but in the Liv.52 group (Group B) there was a significant decline in serum glycolytic enzymes after one

month. Although the enzyme levels were still increased as compared to normal controls, the decline in enzyme levels was statistically significant.

In hepatitis and cirrhosis with HBsAg, Liv.52 therapy brought about a significant decline in serum glycolytic enzymes in 36 out of 48 cases. Two of these cases were in hepatic coma. Twelve non-responsive cases, when treated with added steroids, showed some improvement. The liver function tests and glycolytic enzyme levels showed progressive improvement once steroids were added, but then never returned to normal. On withdrawing steroids after one week to 1 month due to complications, there was some initial deterioration but finally, on continuation of Liv.52 with other supportive treatment and without steroids, patients made progressive improvement. Two cases expired from gastrointestinal haemorrhage after starting steroids. The remaining 10 cases improved subsequently on Liv.52 therapy.

Eight cases which severely ill from the beginning with jaundice and ascites were treated with Liv.52 plus steroids by the attending physicians before the patients were sent to this hospital. Two of them expired following paracentesis of tense ascites. The remaining cases improved satisfactorily when steroids were stopped and Liv.52 plus supportive drugs administered. Liver biopsy in two of these showed advanced portal cirrhosis.

Thus, this study shows clearly that cases of acute or chronic hepatitis with positive HBsAg respond satisfactorily to Liv.52, especially those who have not yet developed portal hypertension and other features of advanced parenchymal failure. Neuropsychiatric complications like hepatic precoma and coma can be most beneficially reversed by Liv.52 drops in this group. Dasgupta and Mukherjee reported uniformly good response to Liv.52 in chronic active hepatitis, as judged by biochemical and histological criteria. Most noticeable was the arrest in the progression of chronic active hepatitis towards cirrhosis.

Mukherjee and Dasgupta (1971) reported significant results with Liv.52 studies by biopsy in adult cirrhosis, after a period of 9 months, but insignificant improvement after 3 and 6 months. Histological results showed good evidence of hepatic regeneration with improvement in hepatic functions. In placebo treatment there was advancement in the degree of hepatofibrosis and cirrhosis. Our observations although of lesser duration, show statistically significant improvement in cases of chronic hepatitis and cirrhosis as far as their serum glycolytic enzymes and clinical data are concerned. The liver biopsy could not be repeated at the end of treatment it is also clear that the addition of steroids is positively harmful in these critically ill patients and these should be managed on usual supportive, symptomatic measures and prevention of the hepatorenal syndrome. The addition of Liv.52 is a definite further advantage in their management.

CONCLUSION

Hitherto, no specific drug was known to benefit Australia antigen positive hepatitis, but Liv.52 tablets or drops can now bestow clinical and biochemical improvement by virtue of a direct, hepatocellular, regenerative and stimulating effect. About 60% cases of acute and chronic hepatitis of this nature benefited substantially with Liv.52 only. In others, the addition of steroids could not help and led to death in 16.6% due to G.I. haemorrhage. Liv.52 therapy, after withdrawing steroids, showed improvement in another nearly 30% cases. No improvement could be obtained in 10% cases, most probably due to their advanced state of portal cirrhosis, while steroids were positively harmful. Liv.52 provides beneficial therapy in type B hepatitis especially in the earlier stages.

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