Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 4 (4): 318-322
October-December, 2004

Comparison of Liver Function Tests After 2/3 Hepatectomy and Liver Biopsy in Experimental Rats

[1]Siddique M., [2]S.A. Malik, [3]A.S. Khan, [3]G. Nazir and [3]J.A. Chawla

Previous studies have shown that changes in liver function profile may vary with postoperative time interval and may be related to the extent of hepatic resection. This study describes the characteristic profiles in parenchymal liver enzymes and other serum liver function tests over a one-week course comparing 2/3 hepatectomy with liver biopsy (<10% resection) in experimental rats. A group of 6 animals was used for this purpose, separately for hepatectomy and biopsy, respectively. The hepatic venous blood samples were drawn at 4, 8, 16, 24, 36, 48, 72, 96 and 168 h, respectively. The tests included were alanine amino transferase (ALT) aspartate amino transferase (AST) alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transpeptidase, total protein, serum albumin and bilirubin. The results were compared with normal control group of animals. Both serum ALT and AST showed early increase (8 h) after both operations. ALT returned to near control level between 48-72 h, while AST regained normal level by 72 h in both groups. Noticeable increase in ALP occurred only in the partially hepatectomized group with a delay between 24-96 h before ultimate normalization. No significant changes for ALP occurred in the biopsy group. LDH increased early in both experimental groups and the level tended to normalize by 168 h of surgery in the biopsy group while in hepatectomized group there was a steady decline towards control level. In contrast alkaline phosphatase and γ GT showed little change in partially hepatectomized group but in biopsy group noticeable increase occurred in comparison with control and hepatectomized group. The total protein dropped below the control level by about 25% at 4 h after partial hepatectomy. The decrease in biopsy group was little in the same period. Serum bilirubin remained above normal initially for 16 h and then became normal after 7 days in partial hepatectomy group. Interestingly the change in biopsy group was opposite to hepatectomy group point by point. The only tests that differ in two groups and seem to play a role in a clinical setting are serum bilirubin, total protein and γ GT.

Key words: Partial hepatectomy, liver biopsy, liver function tests

[1]National Institute of Health, Islamabad, Pakistan
[2]Department of Biology, Quaid-e-Azam University, Islamabad, Pakistan
[3]Women Medical College, Abbottabad, Pakistan

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Dr. Muhammad Siddique
National Institute of Health,
Islamabad, Pakistan

E-mail: nutnih@isb.paknet.com.pk

ANSInet
Asian Network for Scientific Information

INTRODUCTION

Liver has a considerable capacity to regenerate after surgical or toxic insult. Partial Hepatectomy (PH) is routinely employed in treating selected patients with primary liver cancer. Liver biopsy is used for the investigation of etiology of a number of clinical conditions. Moreover partial hepatectomy has also served as a valuable experimental tool to study liver regeneration and function[1].

Changes in laboratory values after hepatic resection can be summarized in three major categories. First the destruction of hepatocytes results in the release of liver enzymes like alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase. These tests are commonly called liver function tests. These tests undergo transient elevations after major hepatic resection in many studies[2-4]. Postoperative consequence of the hepatic resection is a change in the capacity of remaining liver to perform normal physiologic function such as the clearance of bilirubin or the ability to support gluconeogenesis. Finally, the remaining hepatic parenchyma has an altered protein synthetic capacity in producing substances such as total protein level, albumin and coagulation factors.

Changes in postoperative liver function, hepatic regeneration and changes in individual liver enzymes after hepatic resection have been previously described[5-7]. The purpose of this study was to compare the difference in the composition of hepatic venous blood after 2/3 partial hepatectomy and a small biopsy from the liver after major and minor resection. Such comparative study has not so far been reported in literature. These findings will greatly help in monitoring the status of the liver after major and minor resection.

MATERIALS AND METHODS

Male albino rats (six in each, hepatectomy, biopsy and control group) of the Sprague-Dawly strain weighing between 180-220 g were used. They were obtained from the animal house of National Institute of Health Islamabad. Partial hepatectomy and biopsy was performed by the method of Higgins and Anderson[15] under ether anesthesia. Hepatic venous blood samples were obtained from posterior vena cava, with 21-gauge needle. Blood samples were allowed to clot and centrifuged at 3000 rpm. The sera was separated and stored in stoppered tubes in a refrigerator at 5°C. The serum samples obtained at 4, 8, 16, 24, 36, 48, 72, 96 and 168 h after (hepatectomy and biopsy) operation were analysed for ALT, AST, ALP, LDH, γ GT, TP, albumin and bilirubin. The data for individual analyses were used to calculate means and standard deviations.

RESULTS

It is note worthy that normal level of ALT and AST (Table 1 and 2) is reached earlier in biopsy group as compared to hepatectomized group. Table 3 shows that the pattern of change in two experimental groups is similar with the only difference that the level of ALP remained

Table 1: Changes in the concentration of Alanine aminotransferase in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	82.54	40.46	61.50	82.54	40.46	61.50
4	310.92	167.74	239.33	467.44	262.56	365.00
8	977.70	215.64	596.67	1189.94	636.40	913.17
16	805.96	347.04	576.50	1140.43	572.23	856.33
24	414.98	173.36	294.17	341.30	216.46	278.83
36	378.26	193.40	285.83	233.48	100.52	167.00
48	165.90	113.10	139.50	128.63	37.71	83.17
72	91.62	38.38	65.00	74.05	32.61	53.33
96	105.08	60.26	82.67	71.64	40.70	56.17
168	43.67	48.64	38.70	89.85	40.49	65.17

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 2: Changes in the concentration of Aspartate aminotransferase in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	165.85	125.49	145.67	165.85	125.49	145.67
4	899.08	583.26	741.17	626.96	374.70	500.83
8	1664.56	1047.78	1306.17	1271.42	768.24	1019.83
16	1000.83	483.83	742.33	883.34	612.00	747.67
24	1052.07	554.93	803.50	492.64	389.36	441.00
36	795.45	425.89	610.67	351.12	293.22	322.17
48	609.80	283.20	446.50	185.44	155.90	170.67
72	178.44	110.90	144.67	132.78	78.56	105.67
96	244.24	106.76	175.50	161.15	99.51	130.33
168	139.85	126.49	133.17	172.96	121.70	147.33

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 3: Changes in the concentration of Alkaline phosphatase in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	596.23	362.43	479.33	596.23	362.43	479.33
4	918.26	615.74	767.00	651.93	510.41	581.17
8	967.70	495.30	731.50	597.56	343.10	470.33
16	1166.86	643.80	905.33	612.78	308.22	460.50
24	1877.24	1023.10	1450.17	1038.20	737.14	887.00
36	1454.90	1364.44	1409.67	926.54	659.12	792.83
48	1858.88	1217.46	1538.17	881.53	320.47	601.00
72	1333.63	900.03	1116.83	458.42	339.58	399.00
96	2397.12	1274.54	1835.83	864.56	526.44	695.50
168	1033.70	675.96	854.83	841.14	578.86	710.00

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 4: Changes in the concentration of Lactate dehydrogenase in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	2645.77	615.89	1630.83	2645.77	615.89	1630.83
4	4372.25	2425.09	3398.67	3378.21	2126.45	2752.33
8	3702.70	2475.64	3089.17	3473.75	1903.59	2688.67
16	3436.46	2321.88	2879.17	2936.91	758.75	1847.83
24	3060.56	1006.98	2033.67	3071.52	2118.48	2595.00
36	2653.32	924.68	1789.00	3110.95	2405.05	2758.00
48	2309.03	1559.63	1934.33	3427.58	1629.08	2528.33
72	1967.71	678.95	1323.33	1327.47	697.19	1012.33
96	983.11	369.23	676.17	2788.10	1551.50	2169.83
168	1174.67	801.33	988.00	1993.74	1400.26	1697.00

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 5: Changes in the concentration of Gamma Glutamyl Transpeptidase in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	23.59	7.67	15.63	23.59	7.67	15.63
4	20.52	12.62	16.57	39.16	32.84	36.00
8	18.21	7.13	12.67	32.31	24.89	28.60
16	16.64	12.70	14.67	32.68	23.44	28.56
24	11.81	5.39	8.60	29.32	27.68	28.50
36	15.12	11.28	13.20	29.21	28.20	28.70
48	18.74	7.26	13.00	3027.00	27.70	29.00
72	12.61	6.73	9.67	24.11	21.89	23.00
96	17.82	15.18	16.50	22.55	21.45	22.00
168	23.71	16.63	20.17	17.79	16.21	17.00

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 6: Changes in the concentration of Total Protein in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	7.16	5.58	6.37	7.16	5.58	6.37
4	5.06	4.38	4.72	5.53	5.15	5.34
8	5.62	4.62	5.12	6.56	5.94	6.25
16	6.18	4.48	5.33	5.76	4.58	5.17
24	5.91	3.95	4.93	5.85	5.65	5.75
36	5.58	4.58	5.08	6.10	5.58	5.48
48	6.11	4.45	5.28	6.86	5.54	6.20
72	6.74	5.60	6.17	6.11	4.95	5.53
96	5.21	4.29	4.75	5.82	5.44	5.63
168	5.46	4.80	5.13	6.35	6.15	6.25

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 7: Changes in the concentration of Albumin in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	3.23	2.71	2.97	3.23	2.71	2.97
4	3.07	2.59	2.83	3.70	3.46	3.58
8	3.20	2.80	3.00	4.34	3.52	3.93
16	2.82	2.34	2.58	3.62	3.42	3.52
24	3.36	2.44	2.90	3.90	3.16	3.53
36	2.93	2.09	2.75	3.93	3.67	3.80
48	3.05	2.09	2.57	4.15	3.69	3.92
72	4.72	3.54	4.13	3.77	3.19	3.48
96	3.54	3.02	3.28	4.18	3.72	3.95
168	3.98	3.52	3.75	4.35	4.19	4.27

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

Table 8: Changes in the concentration of Bilirubin in the hepatic venou blood of rats at various time intervals after 2/3rd hepatectomy and a liver biopsy

Hours	PH+1SD	PH-1SD	PH mean	B+1SD	B-1SD	B mean
0	0.98	0.30	0.64	0.98	0.30	0.64
4	0.41	0.27	0.34	1.49	0.41	0.95
8	0.21	0.11	0.16	1.17	0.49	0.83
16	0.33	0.29	0.31	1.10	0.70	0.90
24	0.76	0.00	0.66	0.81	0.19	0.50
36	0.50	0.20	0.35	1.25	0.63	0.94
48	0.65	0.33	0.49	0.81	0.55	0.68
72	0.61	0.07	0.34	1.25	0.63	0.64
96	0.40	0.20	0.30	0.53	0.49	0.51
168	0.48	0.18	0.33	0.36	0.24	0.30

PH = Partial Hepatectomy B = Biopsy SD = Standard Deviation

well below the level for hepatectomized group. Table 4 shows that the level of LDH fluctuated after liver biopsy as compared to hepatectomy group. It is interesting that γ GT level (Table 5) after partial hepatectomy fluctuated throughout the observation period and remained close to the normal at various time intervals. In biopsy group the γ GT level increased sharply above the normal between 8 and 16 h. Table 6 shows that the TP level in biopsy group remained slightly above that noted for hepatectomy group. The serum albumin level (Table 7) is noticeably higher in biopsy group than in hepatectomy and control group at nearly all time intervals. The serum bilirubin level (Table 8) in biopsy group is notably lower throughout than in hepatectomized group.

DISCUSSION

A review of the literature shows that a comprehensive comparison of liver function profile in hepatectomized and simple liver biopsy procedure in animals has not been carried out. This study describes the comparison between liver function profile after simple liver biopsy and 2/3 hepatectomy in rats at different time intervals.

Partial hepatectomy is followed by extensive changes in the activity of a variety of enzymes as part of liver regenerative process[6,7]. In the present study several enzymes (ALT, AST, ALP, LDH, γGT) were checked in the hepatic venous outflow after partial hepatectomy and biopsy and compared with their levels in control group. ALT and AST both are useful indicators of liver function. Both ALT and AST (Table 1 and 2) showed early increase in hepatectomized and biopsy group of animals. In both groups AST regained normal level little late (72 h) as compared to ALT level (48-72 h). The most likely explanation for this transaminase elevation is the release of enzymes from damaged liver parenchymal cells.

Alkaline phosphatase is another enzyme important in liver function. It is indicative of cholestasis. In the present study noticeable increase in ALP occurred only in hepatectomy group (Table 3) and the enzyme level came to the base line in 24-96 h.

No significant change occurred in biopsy group. Normally alkaline phosphatase occurs in the cells of bile canaliculi. Partial hepatectomy causes increased production of bile canalicular cells[8] leading to increased level of enzyme in the serum. The profile of a progressive rise in the enzyme level and lack of acute elevation in hepatectomized group is consistent with a mechanism of bile ductule proliferation in regenerating liver (Table 3). Lactic dehydrogenase (LDH) and γ GT are also known to respond to hepatectomy in rat, increasing above control level, 6 h later[9]. In the rabbit, increase in serum LDH occurs by 87% during the first 10 days of partial hepatectomy[10]. In the present study LDH increased early in both experimental groups and the level tend to normalize by 168 h of surgery. LDH enzyme also increases above normal in human beings after partial hepatectomy and wedge resection[11]. In contrast to this enzyme γ GT showed little change in the hepatectomy group but in biopsy group significant increase occurred in comparison with both hepatectomized and control group. This is probably because the enzyme γ GT is concentrated in the cell membranes instead of cytosol and secondly due to enhanced biosynthesis of this enzyme due to the release of hepatic stimulator substance.

In the present study total protein dropped below the control level about 25% at 4 h after partial hepatectomy but the decrease was comparatively less in biopsy group. Pelton *et al.*[11] have reported hypoproteinemia in human beings after hepatic resection and gradual fall to control level by 2nd week[12]. Since liver is the major site of synthesis of plasma proteins and albumin. A fall is expected postoperatively due to hepatic insufficiency of remaining liver. In rabbit Shakoori and his associates failed to see any effect on total proteins during the first 20 days after partial hepatectomy[13]. In the present study in rats the value of total protein rose between 48-72 h. However, a look at the level of albumin during this period shows that albumin rises by about 30% above normal. This indicates that the liver remnant synthesizes/releases 30% more albumin than is normal, raising the total protein level to its normal value. Thus the level of total proteins

at 72 h as suggested by some workers[14] is not due to lack of utilization of globulins but due to 30% above normal synthesis of albumin. Similarly, the 25% decrease in total proteins after 72 h (72-96 h) seems to be due to the decrease in albumin level.

Bilirubin content of blood and liver is one of the patent indicator of RBC destruction and physiological state of the liver. Its level in serum has been reported to elevate 4 fold but transiently in the first 12 h of post hepatectomy period in the rat. In contrast Shakoori *et al.*[13] observed only a slight (20%) increase in serum bilirubin during the first 20 days following partial hepatectomy in rabbit. In human beings Pelton *et al.*[11] found several fold increase in serum bilirubin during the first 48 h of partial hepatectomy.

In the present study on rat, serum bilirubin remained above normal during the initial 16 h after partial hepatectomy and then declined to near normal level by the end of 1 week. Interestingly, the change in biopsy group was the exact opposite of the pattern for the hepatectomy group point by point. It is not possible to account for these opposite trends as the basis of merely surgical trauma or stress. Diminished clearance of bilirubin following partial hepatectomy is understood to result from increased load of cell destruction and accumulation of bilirubin on substantially reduced liver cell mass. Since the reduction in cell mass in biopsy group is far less than in hepatectomised rats, the observed change in bilirubin in this group is difficult to explain.

Concluding the present study reveals that both partial hepatectomy and biopsy in rats leads to disturbance in liver function tests. In most cases the change in liver function profile is transient and normal levels of all biochemical parameters studied are restored within one week after surgery. This suggests that the liver regenerative power/process is quite advanced. The paradox regarding the level of γ GT and bilirubin in biopsy groups needs further research otherwise as expected, relatively early restoration of normal levels of many of the biochemical parameters studied in the biopsy group is commensurate with quantitative difference in the liver tissue compared to the substantially reduced liver mass in partially hepatectomized rats.

REFERENCES

1. Bucher, N.L.R., 1991. Liver regenertion: An Overview. J. Gastroentrol. Hepathol., 6: 615-624.
2. Shakoori, A.R., A. Rani, A.I. Chema and S.H. Ali, 1985. Some biochemical and histological changes in regenerating liver in partially hepatectomised rabbits. Pak. J. Zool., 17: 271-288.
3. Assy, N. and G.Y. Minuk, 1997. Liver regeneration methods for monitoring and their application. J. Hepatol., 26: 945-952.
4. Michalopoulos, G. and M.C. Defrowces, 1997. Liver regeneration. Science, 276: 60-66.
5. Sekas, G. and R.T. Cook, 1979. The evaluation of liver function after partial hepatectomy in rat. Br. J. Exp. Pathol., 60: 447-452.
6. Shakoori, A.R. and M.S. Ahmad, 1973. Studies on liver function, gallus, liver growth and Nucleic acid content. Pak. J. Zool., 5: 111-117.
7. Rao, K.N., Virjima and S.M. Schneider, 1993. Role of serum markers for liver function and liver regeneration in management of choloform poisoning. J. Analytical Toxicol., 17: 99-102.
8. Allalouf, D., Schwarz man, J. Hart and R. Menache, 1986. Lactate dehydrogenase and alkaline phosphatase isoenzymes in regenerating liver. Res. Exp. Med., 186: 103-107.
9. Pinkerton, J.A. and J.L. Sawyers, 1971. A study of postoperative course after hepatic lobectomy. Ann. Surg., 173: 800-11.
10. Almersjo, O., S. Beng mark and Engenik, 1968. Liver serum enzyme changes after hepatic deartenalization in man. Ann. Surg., 167: 9-17.
11. Pelton, J.J., J.P. Hoffman and B.L. Eisenberg, 1998. Comparison of Liver function tests after hepatic lobectomy and hepatic wedge resection. American Surgeon, 64: 408-414.
12. Nagas, N., H. Yukaha and Y. Ogawa, *et al.*, 1987. Human liver regeneration after major hepatic resection. A study of normal liver and liver with chronic hepatitis and cirrhosis. Ann. Surg., 206: 30-39.
13. Shakoori, A.R., R. Kokab and G. Mah Jabeen, *et al.*, 1995. Effect of mercuric chloride on the activity of some hepatic enzyme of regenerating liver following partial hepatectomy. Apptla, 45: 177-184.
14. Schreiber, G., J. Urban and W. Reuter, *et al.*, 1971. The secretion of serum protein and synthesis of albumin and total protein in regenerating rat liver. J. Biol. Chem., 14: 4531-38.
15. Higgins, G.M. and R.M. Anderson, 1931. Restoration of the liver of the white rat following partial surgical removal. Arch. Pathol., 12: 186.