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BIOCHEMICAL ASSESSMENT OF LIVER CELL REGENERATION IN NORMAL VERSUS BILHARZIAL LIVERS AFTER PARTIAL HEPATECTOMY

By

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The liver exhibits a remarkable regenerative capacity after major resection provided it is normal. It is commonly assumed that a normal liver tolerates 70-80% resection safely. On the other hand, the capacity for regeneration after partial hepatectomy in chronic liver disease such as produced by schistosomiasis mansoni, is still unknown. The aim of this work is to assess DNA, thymidine kinase activity and liver function tests after partial hepatectomy in normal hamsters compared to those infected with schistosoma mansoni, this is to show the effect of schistosomal infestation on the regenerative ability of the liver. In the bilharzial groups there was a significant increase in the liver DNA level (P<0.005). In the nonbilharzial group there was a highly significant increase in the liver DNA level (P<0.005). In the bilharzial groups there was a significant increase in the liver thymidine kinase activity (P<0.005), in the non-bilharzial group there was a highly significant increase in the liver thymidine kinase activity (P<0.005). In the bilharzial group there was a marked rise in the serum enzyme during the first three days after partial hepatectomy (P<0.005) and still high even after three weeks. In the non-bilharzial group there was a significant rise in the AST observed during the first three days after surgery (P<0.005) and returned to the pre-operative mean value by the second post-operative week. !n the bilharzial group, total proteins showed a significant decrease (P< 0.05) in relation to the pre-operative baseline value, and still after three weeks post-hepatectomy (P<0.05) didn't recover to the pre-operative mean value. In the bilharzial group there was a significant rise in serum total bilirubin during the first three days after partial hepatectomy (P<0.005) which continued up to the third week post hepatectomy. In the non-bilharzial group there was a significant increase observed up to three days after the operation (P < 0.005) and started to drop after the first week posthepatectomy. The bilharzial group showed a significant rise in Serum Alkaline Phosphatase (P< 0.05) during the first three days after the operation and returned to the baseline value after the first week after the operation. The non-bilharzial group didn't show any changes throughout the whole periods after the operation.

Keywords. Liver cell regeneration. Partial hepatectomy

INTRODUCTION

The liver exhibits a remarkable regenerative capacity after major resection provided it is normal. It is commonly assumed that a normal liver tolerates 70-80% resection safely [1]. On the other hand, the capacity for regeneration after partial hepatectomy in chronic liver disease such as produced by schistosomiasis mansoni, is still unknown [2].

After partial hepatectomy in rats, DNA synthesis is activated within 15-18 hours and is maximal after 24 hours[3]. After a temporary decrease a second less intense maximum is reached at 48-56 hours, both peaks are followed by a proportional increase in the mitotic activity[4]. DNA starts primarily in the peri-portal region (zone 1), after 34-40 hours after partial hepatectomy activated DNA synthesis proceeds in zone 2 and 3 (peri-

central) and this preference for the peri-portal regions holds for the second peak in DNA synthesis[5].

Thymidine Kinase is a nucleotide salvage pathway enzyme whose activity is highly dependent on the growth state and cell cycle phase of a cell. Cells in the resting or quiescent (GO) phase express very low levels of thymidine kinase, mRNA and protein. When quiescent cells are stimulated to enter the cell cycle thymidine kinase activity increases coordinately after about 10-15 hours at the beginning of S phase of the cycle [6].

The aim of this work is to assess DNA, thymidine kinase activity and liver function tests after partial hepatectomy in normal hamsters compared to those infested with schistosoma mansoni, this is to show the effect of schistosomal infestation on the regenerative ability of the liver.

MATERIAL AND METHODS

The hamster was chosen for this study because it is an excellent host for schistosoma mansoni. infestation with the parasite is achieved after 40 days in contrast to the mice in which the infestation occurs after 10 weeks [7]. Also, the hamster is a good model to demonstrate the liver regeneration after hepatectomy, being relatively big and easy to handle, so it is a good candidate for surgical experiments.

The hamsters were divided into the following groups: 1- Non-infested group (60 hamsters). Subdivided into hepatectomized group (33% of the liver), 30 hamster and Sham operated, 30 hamster.

2- Infested group (60 hamsters). Subdivided into hepatectomized group (33% of the liver), 30 hamster and Sham operated, 30 hamster.

Animals were sacrified in both groups, and collection of both the remnant part of the liver tissue as well as blood sample, were done at specific intervals: 1, 2, 3 days as well as 1, 2, 3 weeks post hepatectomy (each group 5 hamsters). The excised left lobe was subjected to pathological examination to prove the Bilharzial infestation in the infested group.

The rate of regeneration is studied according to:
- Comparison of the liver weight percentage as well as the liver weight in relation to body weight percentage.

The liver weight percentage = $\frac{\textbf{Weight of the liver remnant}}{\textbf{Calculated Liver weight}}$

The calculated liver weight = $\frac{\textbf{Weight of the excised lobe X100}}{\textbf{33}}$

- Assessment of both thymidine kinase activity [8] and total DNA [9].

- Measurement of liver function tests: Total bilirubin [10], ALT, AST[11], Total proteins [12]and alkaline phosphatase[13].

Statistical methods:

The Data were processed and statistics was carried Out using the ANOVA test, F for multiple means and T test to compare between observed means of a set of couple of pairs.

Taking 95% as the lowest level of significance, the results can be postulated as:

Insignificant,P< 0.5(less than 95 %); Significant,P< 0.05 (95 %) and Highly significant, P< 0.005 (more than 95 %).

1- Mortality rate

The overall mortality in the bilharzial group was 33.3% and that of the non-bilharzial group was 16.7%. The difference in mortality was statistically significant.

2- liver weight gain:

In the non-bilharzial group the remnant part of the liver showed significant increase in size until it reached 94% of the calculated liver weight after 3 weeks post-hepatectomy.

The bilharzial group showed less significant increase in size and it reached 83% of the calculated liver weight 3 weeks after hepatectomy.

3- Total liver DNA:

In the bilharzial group the range of total DNA in the liver was 33.48-54.21 micrograms, the mean was 42.97 micrograms and standard deviation was 15.2, before surgery. In the non-bilharzial group the range of DNA was 36.98-56.12 micrograms, the mean was 46.78 micrograms and standard deviation was 13.6, before surgery. No statistical difference between the two groups.

In the bilharzial groups there was a significant increase in the liver DNA level seen during the first three days after hepatectomy ($P < 0.005$), and still on the third week after the operation the total DNA was high ($p < 0.005$). Comparing the different periods within the group the most significant rise was observed on the second post-operative day.

In the non-bilharzial group there was a highly significant increase in the liver DNA level seen during the first three days after hepatectomy (P < 0.005), and still on the third week after the operation the total DNA was high (P <0.005). Comparing the different periods within the group the most significant increase was observed on the second post-operative day.

Comparing the different periods between the bilharzial and the non-bilharzial groups, it was found that there was a highly significant rise in the total DNA in the non-bilharzial in relation to the bilharzial group seen on the first three days affer partial hepatectomy (P <0.005).

4- Thymidine kinase activity:

In the bilharzial group, the range of thymidine kinase activity was 1.36 - 2.7 units, the mean was 2.12 units and the standard deviation was 21.5, before surgery. ln the non-bilharzial group, the range of thymidine kinase activity was 1.36 - 2.7 units, the mean was 2.27 units and the standard deviation was 28.6, before surgery.

- In the bilharzial groups there was a significant increase in the liver thymidine kinase activity seen during the first three days after hepatectomy (P <0.005), and still on the third week after the operation the thymidine kinase activity was high (P <0.05). Comparing the different periods within the group the most significant rise was observed on the second postoperative day.

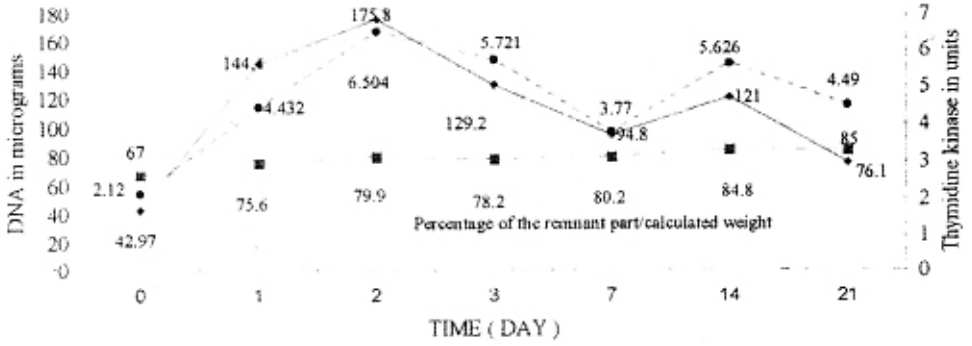
- In the non-bilharzial group there was a highly significant increase in the liver thymidine kinase activity seen during the first three days after hepatectomy (P < 0.005), and still on the third week after the operation the thymidine kinase activity was high but statistically insignificant. Comparing the different periods within the group the most significant increase was observed on the second post-operative day.

Comparing the different periods between the bilharzial and the non-bilharzial groups, it was found that there was a highly significant rise in the thymidine kinase activity seen in the nonbilharzial in relation to the bilharzial group on the first three days(P < 0.005) as well as one week after partial hepatectomy (P <0.05). (Fig. 1 and 2)

5- Liver frinction tests:

• AST:

- In the bilharzial group there was a marked rise in the serum enzyme during the first three days after partial hepatectomy (P < 0.005) and still high even after three weeks but statistically insignificant. Comparing the different periods within the group the most significant increase was observed on the second post-operative day (Fig 3).



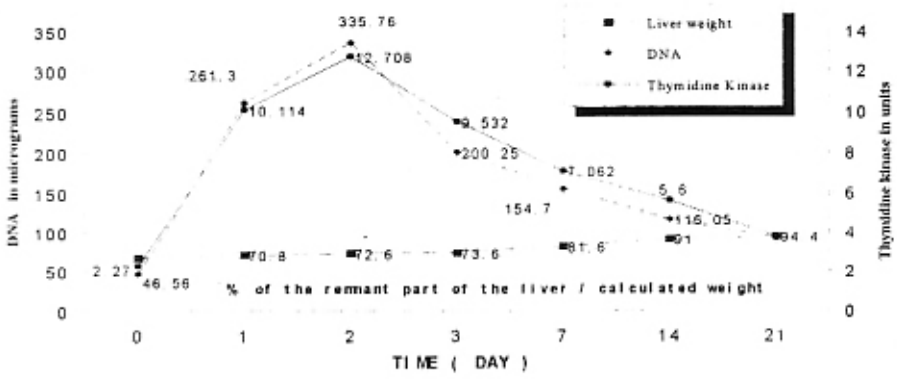


Fig. (1) and (2), demonstrate the relation of DNA, thymidine kinase and the changes in the remnant part of the liver after partial hepatectomy in both groups the bilharzial and non- bilharzial.

In the non-bilharzial group there was a significant rise in the AST observed during the first three days after surgery (P <0.005) and returned to the pre-operative mean value by the second post-operative week. Comparing the different periods within the group the most significant increase was observed on the second post-operative day (Fig. 4).

• ALT:

- In the bilharzial group there was a marked rise in the serum enzyme during the first three days after partial hepatectomy (P <0.005) and still high even after three weeks but statistically insignificant. Comparing the different periods within the group the most significant increase was observed on the second post-operative day (Fig .3).

- In the non-bilharzial group there was a significant rise in the AST observed during the first three days after surgery (P <0.005) and returned to the pre-operative mean value by the second post-operative week. Comparing the different periods within the group the most significant increase was observed on the second post-operative day (Fig. 4).

• Total proteins:

- In the bilharzial group, total proteins showed a significant decrease (P < 0.05) in relation to the pre-operative baseline value, and still after three weeks post-hepatectomy (P <0.05) didn't recover to the pre-operative mean value. In the non-bilharzial group there was a significant drop in serum proteins in relation to the pre-operative baseline value throughout the whole periods. (Fig 5)

• Serum bilirubin:

-In the bilharzial group there was a significant rise in serum total bilirubin during the first three days after partial hepatectomy (P <0.005) which continued up to the third week post hepatectomy, at the same time serum direct bilirubin was raised (P < 0.005) throughout the same periods reflecting the obstructive element of the raised bilirubin.

-In the non-bilharzial group there was a significant increase observed up to three days after the operation (P< 0.005) and started to drop after the first week post-hepatectomy, at the same time serum direct bilirubin was shown to be raised during the first three days after the operation (P < 0.005).

• Serum alkaline phosphatase:

The bilharzial group showed a significant rise (P<0.05) during the first three days after the operation and returned to the baseline value after the first week after the operation. The non-bilharzial group didn't show any changes throughout the whole periods after the operation.

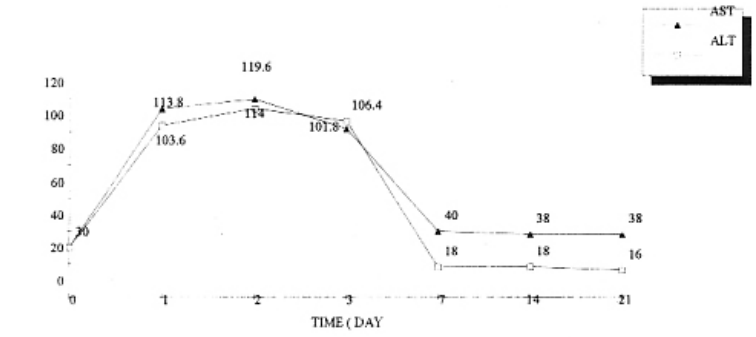


Figure (4)

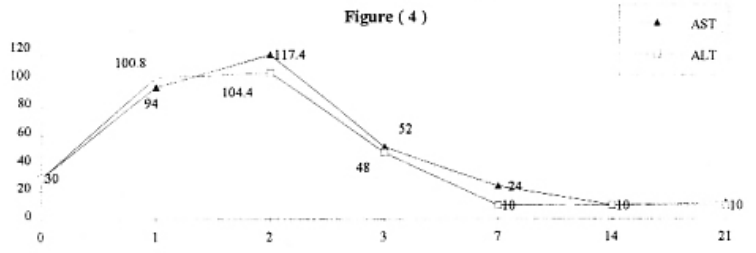


Fig. (3) and (4), show the changes in AST and ALT (units/liter) after partial hepatectomy in the bilharzial and non- bilharzial hamsters respectively

No significant changes were seen in the Sham groups either bilharzial or nonbilharzial, except in the animal weight and serum total proteins, where they showed a drop in relation to the pre-operative baseline values.

DISCUSSION

In a preliminary study preceded this work it was found that the body weight of the bilharzial hamsters was significantly (P<0.05) lower than the non-bilharzial hamsters, liver weight was significantly (P< 0.05) more than the non-bilharzial hamsters and this is attributed to the bilharzial affection itself [(14)]. The remnant part of the liver in the bilharzial group reached 85% of the calculated liver weight after three weeks from partial hepatectomy, but this increase was not statistically significant throughout the whole periods. In the non-bilharzial group the remnant part of theliver reached 94.4% of the calculated liver weight after three weeks from partial hepatectomy, in contrary to the results of Betsy et al., 1994 [(15)] who showed that the remnant part of the liver attained its original mass after 10-12 days after partial hepatectomy. Comparing the weight changes between both groups during the first three days after the operation, it was found that the remnant part of the bilharzial liver was statistically (P <0.05) more than the non-bilharzial group mainly on the second day after the operation, it was explained by the increase in liver congestion rather than in tissue mass, due to the increase in both portal and systemic blood flow that occur during liver regeneration after the operation [(16)] in an organ already suffers congestion from the chronic liver disease. The difference in weight in the remnant part of the liver between both groups after three weeks was statistically significant (P <0.005) and gave and idea that the normal liver is capable of regeneration to replace the excised segment while the bilharzial liver cannot. These results were in agreement with the results of Shimada et al. 1994[(17)], who showed that the regeneration rate in normal liver is significantly higher than those seen in chronic liver diseases such as liver cirrhosis and chronic active hepatitis.

Total DNA in the liver and thymidine kinase activity showed a significant increase (P < 0.005) during the first three days after partial heptectomy, with maximum level observed after 48 hours from partial hepatectomy, in both groups but the peak was more intense in the non-bilharzial group. It was found also, that in the bilharzial group there were two peaks of increase in both parameters, one as mentioned previously after 48 hours and the second peak

which is less intense was observed after 2 weeks from the operation. Betsy et al.[15], observed two peaks in total DNA after partial hepatectomy in normal liver one after 24 hours and the the second peak 24 hours latter, and they explained these two peaks by; the first one is due to parenchymatous cell replication and the second one due to non-parenchymatous cell replication.

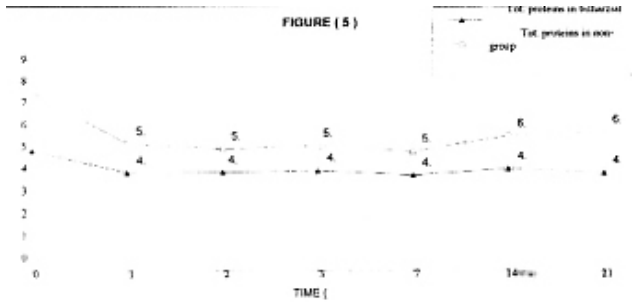


Fig. (5), shows the changes in serum total proteins (gram I deciliter) after partial hepatectomy in the bilharzial and non- bilharzial groups after partial hepatectomy.

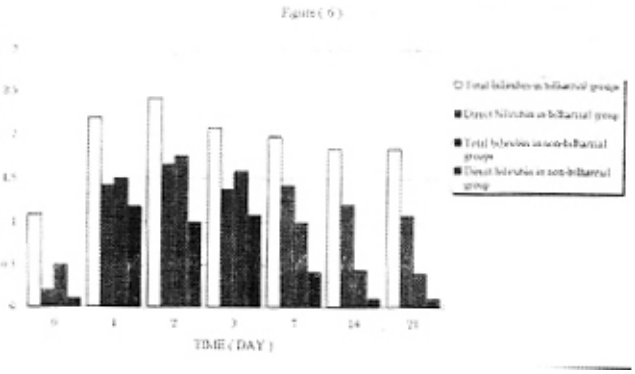


Fig. (6), shows the changes in the total and direct bilirubin (milligram I deciliter) in the bilharzial and non-bilharzial hamsters after partial hepatectomy respectively.

Thymidine kinase activity showed the same peaks and changes that occurred in total DNA contents of the liver in either the bilharzial and non-bilharzial groups, giving an idea that measuring either of them reflects the condition of the other.

These results are in agree with the results of Zucoloto et al., [2] who found that the thymidine kinase activity in the schistosoma-infested mice was lower than that of the control group, and that the thymidine kinase activity in the control group was higher after 48 hours from partial hepatectomy; but, the results are in disagree with those reported by Aguiar et al. [18] and Teixeira and Andrade [7] who found good regeneration in schistosoma-infected mice in comparison to controls.

In Sham operated hamsters there were no changes seen in both groups in thymidine kinase activity after partial hepatectomy. The results of the present study are in agree with the results of Khan et al.,[19] and Zieve et al., [21].But, this is found to be in disagree with the results of Beyer and Zieve,[20] who reported that the activity in the thymidine kinase after Sham operation was lower than normal and they explained this lower level due to decreased food consumption after surgery and that the reduced caloric intake could affect the enzyme activity.

Pre-operative liver function tests in the bilharzial group showed a significant increase in the direct bilirubin (1:1 mg/dl) and alkaline phosphatase (110 u/l), and a significant decrease in total proteins (5.4 gm/dl) compared to the non-bilharzial group where total bilirubin was 0.50

mg/dl, alkaline phosphatase was 95 u/I and total proteins 8.1 gm/dl. Serum direct bilirubin was higher in the bilharzial group but was not statistically significant. This is fount to be in agree with the results of Sadum and Williams [22], concerning the liver functions in bilharzial affection of the liver. Mousa [23], explained the marked reduction in protein levels is attributed to decrease liver synthetic function and the increase in serum bilirubin to both hepatic injury and cholestasis.

AST and ALT in the bilharzial group was significantly higher than the pre-operative value during the first three days after the operation ($P < 0.005$), the enzymes level after three weeks didn't reach the pre-operative level but this high level was statistically insignificant. Comparing the different periods within the same group it was found that the most significant increase was observed on the second post-operative day. AST in the non-bilharzial group was significantly higher than the pre-operative value during the first two days after the operation ($P < 0.005$), the enzyme level reached the pre-operative level on the second week after the operation. Comparing the different periods within the same group it was found that the most significant increase was observed on the second post-operative day. ALT in the non-bilharzial group was significantly higher than the pre-operative value during the first two days after the operation ($P < 0.005$), the enzyme level was within normal range of the pre-operative level on the first week after the operation. Comparing the different periods within the same group it was found that the most significant increase was observed on the second post-operative day. These results in the non- bilharzial group are in agree with the results of Nagata et al. [24], who found that serum AST reached maximum level after 24 hours after hepatectomy and then recovered rapidly to the pre-operative level at 96 hours after the operation and they explained this rise in the AST can be due to the release of tumour necrosis factor (TNF) and interleukin- 1 (IL- 1) from Kupffer cells in the liver as a result of the endotoxaemia occurring after partial hepatectomy. The more marked rise in the enzyme observed in the bilharzial group indicates that the liver infected with schistosomiasis suffers more injury than the normal liver after partial hepatectomy.

Total serum proteins in the bilharzial group showed a significant decrease ($P < 0.05$) in relation to the pre-operative value, and still on the third week after surgery serum total proteins didn't recover completely and showed a significant decrease level compared to the pre-operative value. In the non-bilharzial group the decrease in the total proteins was statistically significant throughout the whole periods ($P < 0.05$). Yamanaka et al. [25], followed the changes in total proteins in normal liver and injured liver (cirrhosis and chronic hepatitis) after partial hepatectomy on humans, they found no significant change in the serum level compared to the serum level before surgery and then increased 4 weeks after surgery, in contrast to the injured liver where serum level dropped below the pre-operative baseline value over the first 1-2 months after surgery.

Up to three days after partial hepatectomy in the bilharzial group there was a highly significant increase in total bilirubin ($P < 0.005$), and this increase was still significant up to three weeks after the operation compared to the pre-operative baseline value ($P < 0.05$) at the same time the serum direct bilirubin was seen significantly ($P < 0.005$) raised throughout the same periods reflecting the obstructive element of the raised bilirubin.

While in the non-bilharzial group there was a highly significant increase observed up to three days after the operation and this rise in the total bilirubin persisted to be significant until the first week after the operation ($P < 0.05$) and at the same time serum direct bilirubin was shown to be significantly increased ($P < 0.005$) during the first three days after the operation.

These changes that occurred after hepatectomy can be due the obstructive effect of oedema and congestion on the remnant part of the liver which took more time to recover in the bilharzial group than the non-bilharzial group, which already suffers from congestion due to the bilharzial affection. The results of this work were in agree with the results of Yamanaka et al. [25] who noticed that the serum level of total bilirubin in human after partial hepatectomy increased sharply after surgery in both normal liver and injured liver (cirrhosis and chronic hepatitis), and the serum level returned to the pre-operative baseline after 2 weeks in normal liver while in chronic hepatitis after 1 month and remained elevated for 4 months in cirrhotic liver.

The serum alkaline phosphatase in the bilharzial group showed a significant increase ($P < 0.005$) in its level compared to the pre-operative mean value, alkaline phosphatase returned to the baseline on the first week after partial hepatectomy. The non-bilharzial hamsters didn't show any significant changes in comparsion to the pre-operative mean value throughout the whole periods after partial hepatectomy and this means that the obstructive effect is more marked in the bilharzil group.

In Sham operated hamsters no changes were seen in liver flinction tests except in serum total proteins where in the bilharzial group there was a significant drop ($P < 0.05$) in the serum level over the first three days after the operation, the non-bilharzial group showed the same results ($P < 0.05$). The drop in the serum level in the Sham group can be due to the metabolic and hormonal changes that occur after surgery with their catabolic effect on proteins.

From the previous data it can be concluded that, there was a significant difference in the rate of regeneration between bilharzial and non-bilharzial groups, indicating that the bilharzial affection of the liver is one of the factors that decreases the response of the liver to regenerate after hepatectomy. Although there was an observed increase in the parameters of the regeneration in the bilharzial liver but significantly less intense than those observed in the normal liver.

It was found also in this study that the bilharzial group showed two peaks of increase in the total DNA content of the liver, one after 48 hours and a second one after two weeks from the operation. While in the non-bilharzial group there was only one peak observed which occurred after 48 hours from partial hepatectomy.

Thymidine kinase activity showed the same peaks and changes that occurred in total DNA contents of the liver in either the bilharzial and non-bilharzial groups, giving an idea that measuring either of them reflects the condition of the other.

Also the bilharzial liver showed marked disturbances in the liver functions after the operation compared to the non-bilharzial liver indicating that the bilharzial liver suffers more after the operation than the normal liver.

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