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PERIOPERATIVE ACUTE PHASE RESPONSE AND OUTCOME OF HEPATIC RESECTION, AN EXPERIMENTAL STUDY

By

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Abstract

Identification of patients at risk of postoperative complications could have an impact on the indications for a procedure as well as permitting modifications of treatment to reduce the surgical risk.

This experimental study evaluated the correlation between peri-operative acute phase response and outcome of hepatic resection.

The study was conducted on sixty healthy golden hamsters, which underwent partial hepatectomy. They were arranged in 3 groups (20 per each). One day prior to resection, fracture of the left hind leg was done in group I (G I) & wound infection had been created in group II (GII); while nothing done in group III (GIII) that acted as a control. Blood samples to estimate SGPT and serum albumin (as basic investigations for hamsters liver function) and serum IL–6 and CRP (as acute phase reactants) were taken preoperatively, immediately after resection and for the consecutive 3 days post operatively.

The mean serum level of both acute phase reactants increased in GI and GII preoperatively and continues to rise immediately after resection. Post-operatively; among the three groups, the mean serum level of both reactants was higher in GI than in GII that was in turn higher than in GIII except when the postoperative complications were more severe than the other group, then this relation changed.

Key words: Hamster**,** Partial Hepatectomy, Hepatic Resection, Surgical Risk.

Introduction

The morbidity and outcome in surgical patients are affected to a great extent by postoperative inflammatory res-ponse to infection that may result from preliminary priming of the immune system (Haupt *et al,* 1997). Despite the recognition that hepatic insufficiency may play a major role in the amplification of multiple organ

dysfunctions frequently observed the following trauma and systemic inflammatory response syndrome (Doyle *et al,* 1993; Karcz *et al,* 2012), the diagnostic investigations to evaluate hepatic function properly are not readily available. This results from the lack of a generally accepted, rapid response tool that can provide liver specific physiologically relevant diagnostic information (Panos and Baber, 1996).

Therapeutic intervention with anti-cytokines showed some promise in arresting the massive loss of protein observed after injury, and led to better understanding of the underlying mechanism involved, and so much effort are required to achieve a better understanding of the complex alteration in body chemistry that occurs in the perioperative period (Hill and Hill,2003; Ahn *et al,* 2012).

The aim of this work was to evaluate the correlation between perioperative acute phase response and outcome of hepatic resection in hamsters.

Materials and Methods

All animals were healthy female golden hamsters (*Mesocricetus aurarus*), 2-3 month old and 120-140 gm boy weight. Breeding was done in the Biological Materials Production Centre in Theodor Bilharz Research Institute (TBRI). All the experiments were conducted according to the guide for the care and use of laboratory animals of TBRI. All animals were housed under the ordinary environmental conditions. Ordinary food (rat pellets ad libitum) and tap water were allowed before and after the operation

Experimental design: The study was conducted on sixty hamsters for which partial hepatectomy was performed and they were arranged into 3 groups of 20 hamsters each: GI were subjected to fracture of the Lt. hind leg (bone fracture group), GII were subjected to induction of skin wound (wound infection group) and GIII were neither wound infection nor bone fracture was induced (control group).

The experimentally hamsters were injected with diluted thiopental 25mg/ 100g body weight, IM in the Rt. thigh to induce the general anesthesia maintaining spontaneous breathing.

Preoperative management: One day prior to hepatic resection:

- GI: fracture of the Lt. hind leg was done by applying artery forceps at the junction of the distal one third and the proximal two third of hamster Lt. hind leg then pushing of the distal part of same leg till a click was felt then checking the free mobility of the distal part of the leg to make sure of the completion of fracture.
- GII: skin wound was done on the right side of the hind back longitudinally without shaving or using antiseptic precautions. This was achieved by elevating the skin with a forceps then cutting skin with tissue scissors as one snip followed by increasing the wound to be two cm in length until the back muscles were seen and leaving the wound without stitches or dressing to induce infection.

- GIII: hamsters were similarly anesthetized, just to have the same drug effect on this group without wound infection or bone fracture. In the same day of hepatic resection, blood samples (0.4-0.5 ml) were taken from all hamsters to estimate serum glutamic pyruvic transaminase (SGPT) and serum albumin as basic investigations to confirm the preoperative normal liver function and serum interleukin-6 (IL–6) and C-reactive protein (CRP) as acute phase reactants. Blood samples were taken using non heparinized glass capillary tube that was introduced in the medial or lateral canthus of the hamster eye after making it much protruding. By gentle screw movement of the capillary tube, the blood was dropping through the capillary tube. Blood was collected in a small vial then the capillary tube was removed and minimal compression was applied on hamster closed eye to ensure hemostasis.

All the operations were carried out between 10 am & 4 pm to avoid diurnal variation in plasma hormones levels which may affect the acute phase reactants levels. Hamsters that were died before partial hepatectomy (after making wound infection, bone fracture, anesthesia overdose or much blood sampling) or immediately after the operations were excluded from the study and replaced by others.

Correlations between the results of perioperative acute phase reactants levels and the outcome of the hepatic resection have been studied.

Operative procedures: 2-3 cm midline abdominal incision from xiphoid process downward followed by partial hepatic resection for 2/3 of Lt. lobe (about 2% of liver mass) after ligation just proximal to this part using 2/0 silk tie. Proper haemostasis and mass closure of the abdominal muscles using 2/0 prolene stitches was achieved.

The resected liver part was preserved in 10% formalin to assess the histopathological condition.

Postoperative follow up: Then blood samples were taken 2, 24, 48 & 72 hours postoperatively to measure the same acute phase reactants by the same method of blood sampling mentioned before. The dead hamsters were opened to get biopsies from liver, kidney, heart and lung to help assuming the cause of death.

Serum variables: Blood samples were centrifuged for 3 minutes at 3500 cycles/minute to separate the serum for the estimation of the SGPT, albumin, CRP & IL-6. SGPT and serum albumin were measured by the ordinary automated method while an ELISA method provided by UBI MAGIWEL, CRP Quantitative AD-401, was used for quantitative measurement of C-reactive protein in serum. Sensitivity for CRP was 0.6mg/dl. Besides, the AviBion IL-6 ELISA (ref. IL 06001; AniBiotech Oy. Orgenium Laboratories Business Unit. Tiilitie 3; FIN-07120 Vantaa Finland) was used for the quantitative measurements of IL-6 in serum. Sensitivity for IL6 ELISA was 2 pg /ml.

Histopathological preparation: Specimens were fixed in 10% formalin (routine paraffin processing), then subjected to serial sections at different plane. Hematoxylin and Eosin (H. & E.) staining were supplemented by Masson Trichrome staining technique.

Statistical analysis: The results were expressed as mean ± standard deviation (SD) or number (%). Comparison between the mean values of different parameters in the three groups was performed using one way analysis of variance (ANOVA). If a significant difference was found, post-hoc for the multiple group comparisons was performed using Least Significance Difference test. Comparison relative to the preoperative time in the same group was done using Mann Whitney U test. Categorical data was analyzed by Chi-square test. P value was expressed as significant (≤ 0.05), highly significant (< 0.01) and very highly significant (< 0.001). The statistical analysis was performed with the aid of the SPSS computer program (version 14 windows).

Results

One day prior to surgery: In GI, fracture of the Lt hind leg was successful and easily recognized by clear deformity with 2 mortalities on the same day. Induction of infection was failed in 3 animals. These animals were replaced by others in each group respectively. Serum SGPT and albumin were within normal range in all the animals (Tab. 1).

The mean serum levels of CRP and IL-6 in GIII represented the normal base line values. Serum CRP was significantly higher in GI ($p<0.01$) and GII ($p<0.05$) than in GIII. Serum levels of IL-6 were significantly higher in both GI&GII ($p<0.001$) compared to GIII (Tab. 2).

Two hours after operation: Serum CRP significantly increased ($p<0.05$) in GIII. Its increase in GI & GII was insignificantly compared to preoperative levels but significant ($p<0.05$) was compared to GIII. In the mean time it was significantly higher in GI ($p<0.001$) and GII ($p<0.01$) compared to the preoperative values in GIII. Serum IL-6 significantly increased in GI & GII ($p<0.05$) and GIII ($p<0.001$) compared to preoperative values. This increase was significantly increased ($p<0.001$) in all groups compared to the preoperative values in GIII (Tab. 2).

Five mortalities were recorded, three in GI, one in GII and one in GIII. They were excluded from the study and replaced by other ones (Tab. 3).

In six hamsters (4 in GI& 2 in GII), the levels of both reactants were very high in comparison to their levels in other hamsters. Follow up of these six hamsters revealed that all of them were found dead in the next 24 hours. In the same hamsters, the increase in levels of both reactants were still more in GI hamsters than in GII hamsters that were in turn more than in the control hamsters.

Average weight of the resected liver part was 0.7-1.5gm that equaled to about 20% of average hamster liver weight. Liver biopsies taken from the resected liver parts from all hamsters

were histopathological normal.

Twenty four hours post-operation: Serum CRP continued to increase insignificantly in all groups compared to the day of operation. Its level was significantly higher in GI ($p<0.01$) and in GII ($p<0.05$) compared to GIII at that time. The levels in all groups were significantly higher ($p<0.001$) compared to the preoperative levels in GIII. Serum IL-6 levels significantly increased to their peaks in all groups ($p<0.01$) compared to the day of operation. The increase was significant in GI ($p<0.01$) and GII ($p<0.05$) compared to GIII. In the meantime, the increase was significant ($p<0.001$) in all groups compared normal preoperative base line values in GIII (Tab. 2).

Six mortalities were recorded at that time (Tab. 3). In other six hamsters (3 in GI & 3 in GII), levels of both reactants were higher than their levels in other hamsters. Follow up of these latter hamsters over the next hours revealed the appearance of the postoperative complications in the form of surgical wound infection and decreased physical activities. The increase in the level of serum IL-6 in both morbidity and mortality hamsters was more significant than the increase in the CRP level.

Forty eight hours post-operation: Serum CRP levels in all groups significantly increased ($p<0.05$) compared to previous day to reach their peak. The values in GI was insignificantly higher than GII but both of them were significantly higher than GIII ($p<0.05$).

In the meantime, their values were significantly higher (Tab. 2) than their preoperative values ($p<0.01$) and the normal preoperative base line values in GIII ($p<0.001$).

Serum IL-6 levels started to decrease but their decrease was insignificant compared to the previous day but still significantly higher ($p<0.001$) compared to the preoperative values. The mortality was recorded in six hamsters (3 in GI & 3 in GII), they were the same hamsters in which the levels of both reactants were very high in the first postoperative day compared to their levels in other hamsters.

Five hamsters (3 in GI & 2 in GII) showed higher levels of both acute phase reactants with remarkable decreased physical activities. They were found dead on the next day (Tab. 3).

Seventy two hours post-operation: The mean serum levels of CRP started to decrease compared to the previous day but the difference was statistically insignificant, although it still significantly higher than the preoperative values in GI ($p<0.05$), GII ($p<0.01$) and GIII ($p<0.001$). In the meantime, serum level was insignificantly higher in GI than GII and in turn GIII. The mean serum level of IL-6 significantly decreased in GI and GII ($p<0.01$) but the decrease in GIII was statistically insignificant. The values in GI were significantly higher (Tab. 2) than those in GII and GIII ($p<0.05$).

Mortality was recorded in the previous five ill hamsters (3 in GI & 2 in GII), and showed significant wound infection with skin necrosis (Tab. 3)

In all the animals, postmortem examination showed the signs of peritonitis. Histopathological study of liver, kidney, heart and lung, revealed the probable cause of death (Figs. 1-12). Clinical follow up in the next four days showed no further mortalities.

Table 1: Mean preoperative values of SGPT (U/L) and albumin (g/L).

Parameter	Group I (mean ± SD) *n=20*	Group II (mean ± SD) *n=20*	Group III (mean ± SD) *n= 20*
SGPT	134.50±55.56	112.80±49.69	34.50±12.25
Albumin	3.62±0.57	4.30±0.34	4.11±0.49

Table 2: Mean values of CRP (mg/L) and IL-6 (pg/ml).

Parameter	Group I (mean ± SD) *n=20*		Group II (mean ± SD) *n=20*		Group III (mean ± SD) *n= 20*	
	CRP (mg/L)	IL-6 (pg/ml)	CRP (mg/L)	IL-6 (pg/ml)	CRP (mg/L)	IL-6 (pg/ml)
Preoperative	94.10±22.11	232.48±55.24	56.48±28.22	129.88±48.22	14.22±4.02	5.84±2.84
2h Postoperatively	104.70±32.16	562.82±199.22	81.52±34.72	382.48±210.84	42.62±18.22	294.38±171.24
24h Postoperatively	128.20±20.12	1622.48±1282.82	91.34±34.82	1189.86±822.88	60.22±26.42	518.86±342.64
48h Postoperatively	234.82±29.16	1392.84±922.88	228.62±58.22	842.84±402.44	171.42±29.34	502.92±412.72
72h Postoperatively	198.22±24.50	598.64±389.42	179.84±19.88	372.86±284.86	149.82±20.72	368.82±298.88

Table 3: Postoperative complications

Parameters	Group I (n=20)	Group II (n=20)	Group III (n= 20)
Postoperative 2 h	3 Anaesth overdose, Not recover Replaced	1 Bleeding not recover Replaced	1 Anaesth overdose not recover replaced
24h Postoperatively	4	2	-
48h Postoperatively	3	3	-
72h Postoperatively	3	2	-

Discussion

This work was preceded by a preliminary study on a big number of hamsters representing the 3 groups to determine the pattern of changes in CRP and IL-6 after the partial hepatectomy. Trend of changes showed that the peak of increase occurred within the first two postoperative days then started to decrease progressively. For this reason, this study was limited to the third postoperative day. In the meantime, the study was restricted to two acute phase reactants only due to the small amount of blood that could be sampled daily for five days.

Normal serum levels of the both reactants in the control group in the preoperative period meant that the control hamsters were not having any stress of trauma or infection that may raise these reactants. In the meantime, continuous rise in levels of both reactants 2 hour after partial hepatic resection in all hamsters indicated that both IL-6 and CRP are considered as good indicators for the acute phase response. This agreed with Pepys and Hirschfield (2003) and Sakakeeny *et al.* (2012) who stated that a high level of CRP doubles the possibility of tissue injury or inflammation. But, Carol (2011) argued that the CRP was not

specific and a low CRP level is not indicative for the absence of the inflammation and in addition a positive CRP also can be detected during the last half of pregnancy or with the use of oral contraception.

The rise in mean serum level of both reactants and appearance of the complications in bone fracture and wound infection groups more than in control group indicated that the preliminary priming of the immune system can lead to inappropriate response to injury that can be the cause for morbidity and mortality. This conclusion was given (Haupt *et al,* 1997; Rachman and Rinaldi, 2006; Warschkow *et al,* 2012).

High levels of both reactants in first postoperative day in hamsters that developed complications later in the second and third postoperative days showed that, high levels of these reactants usually precede occurrence of morbidity and mortality. They could be considered as good predictors for the postoperative complications. In the meantime, they are also good negative indicators, so that if both reactant did not rise higher than their usual levels and started to decrease after their usual peak, this indicates starting recovery without significant complications, and if started to decrease after the initial unusual rise this indicates improving and recovering from this complication. This agreed with Hack *et al.* (1989) and Telman *et al.* (1995) who stated that the presence of high postoperative concentration or persistent of high concentrations of interleukin–6 (IL-6) associated with high mortality. Thus, treatment minimizes IL-6 response to major stress (Badia *et al,* 1998). But, Watters *et al*. (1986) stated that; although very high level of 1L-6 correlate with death after trauma, IL-6 may be a marker of severity of injury like CRP rather than a major causal agent.

The peak levels of IL-6 were recorded within 24 hours postoperatively but for CRP in 48 hours postoperatively in non-complicated cases. This meant that IL-6 was an earlier indicator for acute phase response than CRP, while the mean rise in IL-6 level was always more significant than the mean rise in the CRP levels in response to any stress (e.g. skin wound infection, bone fracture, partial hepatic resection or postoperative complication). This agreed with Telman *et al.* (1995) and Shimada *et al*. (1995). Other authors concluded that IL-6 increases before CRP because IL-6 (formerly called the hepatocyte stimulation factor) is involved in the production of acute-phase reactant such as CRP (Mclntosh and Jeffrey, 1992).

Conclusion

The outcome results showed that the IL-6 and CRP are considered as good indicators for acute phase response to tissue injury or infection, and are good predictors for the occurrence of postoperative complications or mortality. In this context, IL-6 proved to be more sensitive predictor than CRP.

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Explanation of Figures

Fig. 1: Control liver showing preserved hepatic lobular architecture with hepatocytes arranged in thin plates around central vein (H. & E. X10)

Fig. 2: Control liver showing preserved hepatic lobular architecture, and intact portal tracts (Masson Trichrome X10).

Fig. 3: Liver tissue showing prominent hydropic degeneration of hepatocytes, intercellular inflammation and areas of necrosis at zone 1. Portal tracts expanded by dilated, congested vascular channels (H. &E. X10), death cause intraperitoneal hemorrhage

Fig.4: Splenic tissue showing massive hemorrhage and expansion of white pulp (H. & E. X10). death cause hypovolemic shock.

Fig. 5: Heart from a control case (H. & E. X5).

Fig.6: Postoperative heart tissue showing interstitial hemorrhage and edema (H. &E. X5), death cause hypovolemic shock.

Fig.7: Normal control lung showing patent alveoli, mild interstitial edema and mild congestion of blood vessels (H. & E. X10).

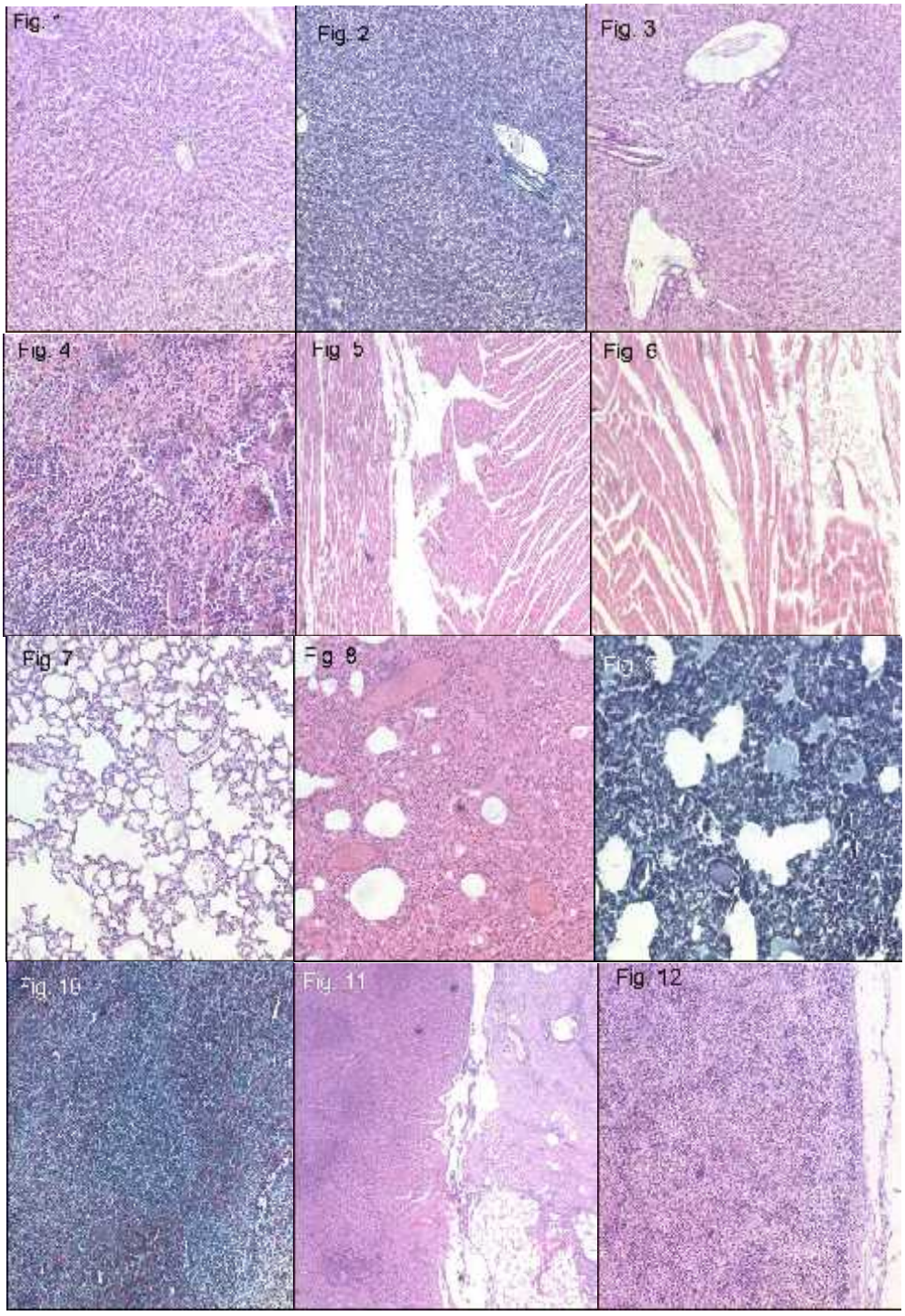
Fig. 8: Postoperative lung tissue from a hamster died from lobar pneumonia showing consolidation of parenchyma by intense acute inflammatory infiltrate, prominent congestion and fibrin deposition (H. & E. X10).

Fig. 9: Same previous case (postoperative lung tissue) (Masson Trichrome X10).

Fig.10: Spleen showing areas of interstitial hemorrhage, focal edema and prominence of lymphoid follicles (red pulp) (Masson Trichrome X10). Cause of death peritonitis.

Fig. 11: Peritoneal tissue from a control case (H. & E. X5).

Fig. 12: Peritoneal tissue from a hamster died from peritonitis showing marked exudation by intense acute inflammatory cells (H. & E. X5).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