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Nutritional Status of Male Albino Rats undergo Obstructive Jaundice experimentally

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***Abstract**: Effect of Synthetically obstructive jaundice, and biliary drainage on the nutritional status was studied in male albino rats and compared with control and sham operated animals. During the first week after operation, food intake was reduced in bile duct ligated (BDL) group ($P < 0.05$), resulting in weight loss when compared to control and sham operated animals. Weight gain and nitrogen balance were improved after biliary drainage with a significant difference between internal and external methods ($P<0.05$). Serum albumin decreased firstly in all groups except control one, returned to normal in sham operated group, and persisted low in BDL group at the end of the fourth week ($P<0.01$). Serum albumin rose to normal levels after internal and external biliary drainage at the end of the experimental period. Obstructive jaundice was associated with anemia (hemoglobin and hematocrite value decreased significantly at the end of fourth week in BDL group. Biliary drainage improved the hemoglobin and hematocrite values especially in the internally drained group. All the parameters used for nutritional assessment (food intake, body weight, and nitrogen balance) were improved after internal biliary drainage.*

In the present work, changes occurred in serum albumin, hemoglobin and hematocrite values showed that they are not good indicators for nutritional assessment of this animal model. Internal biliary drainage may be of value in decreasing morbidity and mortality after surgery for patients with obstructive jaundice.

1. Introduction

Altered nutritional status is one of the problems encountered in both malignant and non-malignant obstructive jaundice. Malnutrition and alternation in reticulo-endothelial function correlate with increased mortality and morbidity after surgery in patients with obstructive jaundice (Nehez & Anderss, 2002 and Pauli-Magnus & Meier, 2005). Infection of bile and septicemia are well known complications after surgical procedures and after bile duct ligation in experimental animals (Wadei et al., 2006). Previous studies have suggested that hyperbilirubinemia may be responsible for the poor prognosis in cases of obstructive jaundice (Papadopoulos et al., 2007). Preoperative biliary drainage either externally or internally has been suggested to reduce morbidity and mortality of surgical treatment in obstructive jaundice (Fang et al., 2013). Others found that there was no statistically significant difference in morbidity and mortality for those patients who had not undergone drainage prior to surgery (Sewnath et al., 2002). The effect of biliary drainage in reversing nutritional abnormalities has not been studied in detail.

The aim of this study is to evaluate malnutrition in rats after bile duct ligation and to study if external or internal drainage has any effect on their nutritional status.

2. Materials and Methods:

Thirty five male albino rats weighing approximately 250 grams were maintained at room temperature (25°C) and subjected to 12 hours artificial light cycle. They were obtained from the animal house of the National Research Center, Cairo, Egypt. All rats were individually housed in metabolic cages with mesh wire bottoms. The basal diet was formulated to meet the rats' nutrient requirements as mentioned by **Osfor (2000)**. Basal diet and their contents are shown in table (1). Water and Food were available all the time during the experimental period.

All surgical procedures were carried out under Di-ethyl ether anaesthia. The animals were equally divided into five groups:

Group-1: Non-operated rats and served as control group

Group-2: Sham ligated group

Group-3: Common bile duct ligated group for 28 days

Group-4: Common bile duct ligated group for 14 days followed by internal drainage and observed for 14 days.

Group-5: Common bile duct ligated group for 14 days, followed by external drainage and observed for 14 days.

Table (1): Composition of the basal Diet:

Ingredient	Percentage (%)
Wheat flour	**77.0**
Meat meal	**06.0**
Animal fat	**03.5**
Alfalfa	**10.0**
Di-Calcium phosphate	**01.0**
Lysine	**00.6**
Methionine	**00.4**
NaCl	**00.5**
Mineral & Vitamin Mix*	**01.0**
Calculated Nutrient Composition:	**14.01**
Crude Protein	**3296.5**
Energy (ME / kg)	**03.83**
Crude fiber	**05.27**
Ether Extract	**03.06**
Ash	**01.09**
Methionine	**00.61**
Lysine	**00.91**
Calcium	**00.64**
Phosphorus	

***The mixture of Mineral and Vitamins per kg feed:**

Calcium phosphate (200 mg); Ferrous sulfate (225 mg); Copper sulfate (6 mg); Manganese sulfate (40 mg); Zinc (30 mg); Iodine (0.55 mg); Selenium (0.1 mg). Vitamin A (5000 IU); Vit. D (1500 IU); Vit. E (45 mg); Vit. K_3 (1.5 mg); Vit C (75 mg); Vit B_1 (2 mg); Vit. B_2 (45 mg); B_6 (3 mg); Vit B_{12} (0.015 mg); Biotin (0.04 mg); Ca-pantothinate (18 mg); Nicotinic acid (39 mg); and Folic acid (1 mg).

3. Surgical procedures:

In group (2), the rats underwent laparatomy with sham ligation of their bile ducts. In bile duct ligated rats (group 3, 4 and 5), 20 cm laparatomy was performed after skin preparation. The common bile duct was ligated and divided, as described by **Lee (1982)**. Internal and external biliary drainage (group 4& 5) were done two weeks after ligation and division of bile duct, the abdomen was re-opened through the previous incision and the liver was mobilized to the anterior abdominal wall. The dilated bile duct was dissected and a silastic tube (0.085 inch in its outer diameter) with a silastic cuff was inserted into the bile duct and secured by two purse string 6-0 silk suture. The distal end of the silastic tube (1.5 cm in length), was inserted into the first part of the duodenum for internal drainage **(Ryan et al., 1977)**, or a longer tube (2.5 cm) was inserted into the urinary bladder for external drainage and both secured in place by double purse string 6-0 silk suture **(Diamond and Rowlands, 1990)**.

Hematology and biochemical analysis:

Blood sample were collected weekly from the retrobulber venous plexus for biochemical and hematological studies. Serum total protein and albumin were determined according to the method of **Ratliff and Hall (1973)**. Nitrogen balance was estimated by subtracting the total urinary nitrogen from total nitrogen intake. The total nitrogen intake was calculated from the amount of food consumed. Serum bilirubin was estimated by using commercial diagnostic kits, Bio-Merieux, France. Hemoglobin concentration and hematocrite percentage were determined after **Schalm et al (1975)**.

Statistics:

Unpaired data are reported as mean ± standard deviation and analyzed using "Students" t test. The level of significance was $P<0.05$ **(Snedecor and Cochrun 1975)**.

4. Results:

One mortality occurred in BDL group, and one animal in internally drained group was excluded because of biliary peritonitis. One of the animals in the externally drained group died, five days following the drainage procedure, probably due to cholangitis. The remaining animals were healthy throughout the study.

Serum bilirubin elevated markedly in the BDL group (group-3) and decreased after external or internal drainage, while in group 1 and 2, it remained normal throughout the study (Table-4).

Body weight changes are demonstrated in table-1. Significant loss of the body weight occurred after BDL during the first week, compared to the control and sham operated animals (P<0.05). The weight gain was improved after the drainage procedure. The improvement was more evident in internally drained than externally drained group. The daily food intake increased in both BDL and sham operated animals after surgery. After seven days, food intake was significantly less in BDL group compared to the sham animals and control ($P<0.05$). It improved in group 4 and 5 after drainage with no statistically significant difference between them (Table-1).

Levels of serum albumin significantly decreased after BDL, when compared to control and sham operated groups ($P<0.01$), and remained so throughout the experimental period in the ligated group. Following the drainage procedure, serum albumin returned to the normal level in group 4 and 5 (Table-3). In contrast to serum albumin, which decreased after BDL, levels of total protein increased significantly compared to control and Sham operated rats ($P<0.05$), (Table-3). Mean of nitrogen balance was negative in BDL groups in the first seven days after bile duct ligation and then became positive after drainage (Table-3).

Hemoglobin significantly decreased after BDL ($P<0.05$), and returned to normal values after drainage. The same changes occurred in hematocrite values (Table-4).

5. Discussion:

Sepsis, renal failure, upper gastrointestinal hemorrhage, and impaired wound healing are well known complications of obstructive jaundice (**Kozarek, 2013 and Moole et al., 2015**). Eight risk factors had been reported to be associated with poor prognosis after surgery for obstructive jaundice, namely: old aged patients over 60 years, patients with malignant disease, leucocytosis (over 10.000 cell / ml), low hematocrite (<30%), high serum creatinine (>1.3 %), high serum alkaline phosphatase (>100 IU), high serum bilirubin (<3g %) (**Moole et al., 2016**). Nutritional status of the body is important for surgery as malnutrition has been associated with a high postoperative complication rate (**Moole et al., 2015**).

Among the previous eight factors serum albumin correlates highly with mortality in patient with obstructive jaundice (**Yuosef et al., 2012 and Shin et al., 2012**). **Armstrong and his team (1984)** showed that reduced wound healing in patients with obstructive jaundice was due to malignancy and associated poor nutritional status rather than to hyperbilirubinaemia. In malignant patients studied by **Armstrong et al., (1984)**, nutritional status was affected by both malignancy and obstruction of the biliary passages. Nutritional status in non-malignant obstructive jaundice has not been studied in detail and the available previous studied showed contradictory results. **Arnaud and his scientific team (1981)** showed a normal weight in rats after bile duct ligation while significant weight loss was reported by others (**Aljiffry et al., 2009**).

In our study, weight loss in rats was significantly higher in the BDL group than in control animals after the first week. During the second postoperative week, the jaundiced rats gained weight comparable to the control rats. At the third and fourth weeks, BDL group failed to gain weight compared to control and to biliary drained groups (Figure-2). Decreased food intake was only observed in the early stage after BDL. This transient depression in appetite is more evident in BDL animals than in sham operated rats (Figure-3), so operative trauma is not the only factor responsible. Recovery of normal intake of the BDL animals was obvious after the drainage procedure. These results showed that anorexia is correlated with hyper-bilrubinaemia.

Serum albumin decreased significantly after BDL (Figure-4); which may be due to reduced hepatic synthesis associated with bile duct obstruction. After biliary drainage, serum albumin regained its normal level (Figure-4). This showed that changes in serum albumin followed the changes in bilirubin and not the changes in the nutritional status. Serum albumin correlates highly with postoperative mortality in obstructive jaundice patients (**Yuosef et al., 2012**), but it is not a good indicator of nutritional status in the jaundiced rats in our study. Serum albumin responds poorly to short term changes that occur in protein and energy intake and it can be used only as a gross indicator of visceral protein status in chronic caloric and protein malnutrition (**Valle et al., 2010**). In contrast to serum albumin, total serum protein significantly increased after BDL (Figure-5). This may be due to increase in synthesis of globulins by the liver as an acute phase response to the operative trauma (**Valle et al., 2009**), or due to regurgitation of IgA, which is normally excreted in the bile, into the serum (**Ramirez-Merino et al., 2013**). Systemic endotoxaemia, which frequently followed BDL, may explain the significant increase of globulin in the BDL group than in other groups, and it could lead to neutrophilic response and increase in the circulating visceral proteins (**Okusaka et al., 2010**). Very rare studies with N-labeled amino acids indicate an increase in protein turnover in addition to the increase in protein synthesis. This reflects an increase in muscle proteolysis associating the increase in hepatic phase protein synthesis (**Steel et al., 2011**). There is still controversy about the nature of the mediators involved in the alternations of protein metabolism. Recently a positive correlation between interleukin-6 (IL-6) plasma levels and protein turnover and IL-G has been demonstrated to be the main factor of hepatic acute phase protein synthesis (**Figueroa-Barojas et al., 2013**).

Hemoglobin and hematocrite were decreased after bile duct ligation and returned to normal levels after biliary drainage (Figures 7&8). The results were independent of changes of nutritional status of the experimental animals **(Dhir et al., 2012)**. This rapid retairn to normal level may show that low hemoglobin and hematocrite appears to be related to increased hemolysis rather that an effect of malnutrition **(Dhir et al., 2012)**. **Zoepf and his team (2005)** showed that preoperative external biliary decompression had no therapeutic benefit when compared (non-randomized) with those patients without alleviation of jaundice prior to surgical intervention. Another study reported a reduction in morbidity and mortality after preoperative external drainage **(Park et al., 2010)**. **Armstrong et al., (1984)** suggested a limited role for this drainage as it improves only one of the adverse factors, namely hyperbilirubinaemia; Internal biliary drainage does not carry the risks of external trans-hepatic biliary drainage in human patients, namely liver puncture that carries the risk of bleeding and leakage of bile from the trans-hepatic track **(Park et al., 2010)**. Although in our study, the external biliary drainage is not trans-hepatically, intr=ernal drainage of the eight risk factors related to increased morbidity and mortality in obstructive jaundice **(Nguyen et al., 2010)**. The return of bile to its normal pathway by internal drainage may be important in prevention of bacterial translocation, which is promoted in experimental animals by the absence of intestinal bile **(El-Kousy et al., 1994)**.

In conclusion, it was found that improvement of the nutritional indices, as food intake, body weight, nitrogen balance, serum albumin and hemoglobin, occurred in our experiment after biliary drainage. Also, we found a statistically significant difference between internal and external drainage, so internal drainage in obstructive jaundice can improve postoperative morbidity and mortality. Further studies are needed in human patients as external drainage is different in our study than that used in humans. Moreover, the role of nutritional supportive therapy preoperatively needs further study.

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