J Med Sci, Volume 47, No. 4, December: 154-161

Correlation between alkaline phosphatase, γ-glutamyl transpeptidase, and bilirubin with interleukin-1β level in dogs with obstructive jaundice

Nurcahya Setyawan[1*], Vicky S. Budipramana[2]
[1]Digestive Surgery Division, Department of Surgery, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito Hospital, Yogyakarta, [2]Digestive Surgery Division, Department of Surgery, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya

DOI: http://dx.doi.org/10.19106/JMedSci004704201502

ABSTRACT

Surgical management in obstructive jaundice still contributes to significant morbidity and mortality. One of complications following surgery in obstructive jaundice is sepsis. This complication is caused by the toxic effects of bilirubin and bile salts, endotoxins, bacterial translocation, modulation of the immune-inflammatory cascade, decreased cellular immunity and/or nutritional status. Many studies have shown the elevated inflammatory response indicator, interleukin-1 (IL-1β), in patients with obstructive jaundice. However, only a few report described the association between the indicators of obstructive jaundice (alkaline phosphatase [ALP], γ-glutamyl transpeptidase [GGT], and bilirubin) and the indicator of inflammatory response (interleukin-1β [IL-1β]). This study aimed to investigate the association between the indicator of obstructive jaundice (ALP, GGT, and bilirubin) and the level of interleukin-1β (IL-1β) in dogs as the animal model. We performed ligation on distal common bile ducts (CBD) to produce a model of obstructive jaundice. Every three days within a month, the blood samples from ten dogs were extracted to determine the ALP, GGT, direct and total bilirubin, and IL-1β levels. We found a significant correlation between the ALP and GGT with IL-1β level with p-value of 0.036 ($r = 0.626$) and 0.003 ($r = 0.826$). However, there was no association between the increased direct bilirubin with the IL-1β level ($p = 0.068$; $r = 0.537$). Moreover, the increased level of ALP and GGT had a strong correlation with the increased level of direct bilirubin with p-value of 0.004 ($r = 0.810$) and $p = 0.011$ ($r = 0.746$). In conclusion, the increased level of GGT was the strongest indicator for inflammatory response in dogs with obstructive jaundice. Furthermore, the increased levels of GGT and ALP might imply the development of obstructive jaundice in dogs.

ABSTRAK

Pembedahan untuk kasus ikterus obstruksi masih menunjukkan angka morbiditas dan mortalitas yang tinggi. Salah satu risiko terapi terapi pembedahan definitif adalah sepsis. Hal ini disebabkan oleh efek toksik bilirubin dan garam empedu, efek endotoksin, translokasi bakteri, modulasi kaskade proses inflamasi, dan penurunan imunitas seluler serta penurunan status nutrisi pasien. Pada beberapa penelitian menunjukkan bahwa respon inflamasi pada pasien ikterus obstruksi digambarkan dengan peningkatan kadar interleukin-1 beta (IL-1β). Namun, masih sedikit penelitian yang meneliti tentang hubungan antara parameter ikterus

Corresponding author: nurcahya.spb@yahoo.com

obstruksi (Alkaline Phosphatase, Gamma-Glutamyl Transaminase, dan bilirubin) dengan indikator respon inflamasi (IL-1β). Penelitian ini bertujuan untuk menilai hubungan antara indikator ikterus obstruksi (ALP, GGT, dan bilirubin) dengan indikator respon inflamasi (IL-1β) pada hewan coba anjing. Penelitian ini adalah penelitian eksperimental dengan hewan coba anjing. Sepuluh anjing dilakukan ligasi duktus koledokus distal sebagai model ikterus obstruksi. Setiap 3 hari selama 1 bulan, pada 10 hewan coba ini dilakukan pemeriksaan serum darah meliputi ALP, GGT, bilirubin direk dan total, serta kadar IL-1β setiap 3 hari selama 1 bulan. Didapatkan hubungan yang bermakna antara kenaikan kadar ALP dan GGT dengan IL-1β dengan nilai p masing-masing adalah 0,036 (r = 0,626) dan 0,003 (r = 0,826). Namun, tidak terdapat hubungan antara kenaikan kadar bilirubin direk dengan IL-1β (p = 0,068; r = 0,537). Selain itu, kenaikan kadar ALP memiliki korelasi yang sangat kuat dengan kenaikan kadar GGT (r = 0,912; p = 0,000) dan bilirubin direk (r = 0,810, p = 0,004). Demikian juga kenaikan kadar GGT berkorelasi yang sangat kuat dengan kenaikan kadar bilirubin direk (r = 0,746; p = 0,011). Kenaikan kadar GGT merupakan indikator paling kuat untuk menunjukkan respon inflamasi pada ikterus obstruksi pada hewan coba anjing. Selain itu, kenaikan kadar GGT dan ALP merupakan indikator terjadinya ikterus obstruksi pada hewan coba anjing.

Keywords: obstructive jaundice - inflammatory response - animal model - distal common bile ducts

INTRODUCTION

Surgical management of cholestasis or obstructive jaundice, especially in malignancies is still highly correlated with significant morbidity and mortality. Definitive surgical management is needed in cholestasis in accordance with the cause. Some cases required prompt definitive therapy, but the risk of morbidity is still quite high, about 40-60%, such as sepsis, anastomotic failure, bleeding, and failure of wound healing. The occurrence of surgical complication in the definitive surgical management of cholestasis is caused by the elevation of intraductal biliary tract pressure. This effect is caused by the toxic effect of bilirubin and bile salt, endotoxins, bacterial translocation, modulation of the inflammatory immuno-inflammatory cascade, decreased cellular immunity as well as decreased in nutritional status. In some cases, temporary surgical repair is done in a way of preoperative biliary drainage with can lower intraductal pressure thus improving or restoring liver function, which in turn can reduce the risk of complications of liver failure and sepsis.[1-3]

Biochemical markers of cholestasis are the presence of elevated levels of Alkaline Phosphatase (ALP), and Gamma-glutamyl Transpeptidase (GGT). ALP and GGT are the hepatocyte membrane and are released in case of hepatocellular damage. In cholestasis, the synthesis of these enzymes is induced and made soluble. GGT is lifted because the leak out of the bile duct of the increased bile duct pressure. In the next stage of cholestasis; Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and bilirubin will increase because of liver damage as a secondary effect of cholestasis.[4-8]

The exposure of endotoxemia and bacterial translocation due to obstructive jaundice cause uncontrolled induction of the inflammatory cascade. In cholestasis, there is an increase of endotoxin concentration in the portal circulation, as a result of a lack of bile salts in the intestinal lumen, which lead to in an imbalance of microflora and increased the permeability of the intestinal

mucosal barrier, resulting in bacterial translocation. The damage of the hepatocyte and cholangiocyte cause activation and release of proinflammatory cytokines. Tumor Necrosing Factor Alpha (TNFα) and interterleukin-1 (IL-1) and interleukin-6 (IL-6) are produced by macrophages, endothelial cells, and Kupffer cells which are the response of the body immune system. Animal experiments have shown increased concentrations of proinflammatory cytokines such as tumor necrosis factor (TNF), IL-1, IL-6, interleukin-8 (IL-8), and interleukin-10 (IL-10). Cytokine concentration increased contributes to increased risk of complications. This increased levels of cytokine is a manifestation of an inflammatory process, which induces SIRS and sepsis.[3,9-14]

The role of Preoperative Biliary Drainage (PBD) in obstructive jaundice is controversial.[15] Study shows that PBD could increase the surgical outcomes in patients with obstructive jaundice. Several other studies in human have shown no benefit in PBD, so it is still being debated. Nevertheless, some experimental studies have shown PBD beneficial effects, such as reduction of systemic endotoxemia, improvement of liver function and nutritional status; reduction of cytokine release, and as a result, enhance the body's immune status and significantly reduced mortality on animal model.[14]

Various studies have been conducted and reported that the existence of elevated serum level of IL-1, IL-6, TNFα and IL-10 in patients with cholestasis.[12-14] Few research discusses the relationship between indicators of cholestasis (ALP, GGT or bilirubin) on the occurrence of the inflammatory response, and which are the most meaningful and sensitive indicators.[1,2] Based on above description, this study was conducted to analyze the correlation between increased level of ALP, GGT and bilirubin with IL-1β.

MATERIALS AND METHODS

This study was an experimental study using dogs as the animal model. Ligation of distal Common Bile Duct (CBD) was performed on 10 dogs to develop or mimic a model of cholestasis or obstruction. Every three days during the first month, blood serum was examined including Alkaline Phosphatase, Gamma-glutamyl Transpeptidase, direct and total bilirubin, and the level of Interleukin-1 beta. Examination of the inflammatory response (IL-1β) was measured from the first day and repeated every 3 days within a period of 30 days.

The amount of sample was determined by calculating the following formula: Large Sample → $n = 1+2C\ [s/d]^2$ which C: the value selected in accordance with the level (ά) and power (1-ß) is 7.85; S: a mean estimate of surveyed → 0.3; D: represents the expected deviation → 0.4; ά: Confidence level → 12:05. So we calculated the number of research subjects with the formula: $n = 1 + 2x7.85\ [0.3 / 0.4]^2$ is 9.8. We rounded the result so the total number of samples are 10. We determined the inclusion criteria which were the animal subjects that had the same weight, same sex and been given the same treatment. We determined the exclusion criteria which were all animal subjects that were too small or sick. Independent variables in this study were the clinical examination of obstructive jaundice and increased level of ALP, GGT, and bilirubin. Dependent variable in this study were the serum level of proinflammatory cytokine.

We defined jaundice or icterus as a yellowish pigmentation of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes. We defined obstructive jaundice as a particular type of jaundice and occur when the essential flow of bile to the intestine is blocked and remains in the bloodstream

and might be due to blocked bile ducts caused by gallstones, or tumors of the bile duct which can block the area where the bile duct meets the duodenum. We defined the level of proinflammatory cytokine as an examination of serum level of IL-1β, using the ELISA technique.

This study was conducted in Digestive Surgery Department, Airlangga University/ Dr. Soetomo Hospital and in Department of Veterinary Medicine, Airlangga University within the period of April – June 2012. Bivariate correlation analysis was used to analyze the correlation between increased levels of alkaline phosphatase and GGT with the level of IL-1β in cholestasis.

RESULTS

A total of 10 samples (dogs) had an average age of 6 years. One dog year equals about 7 human years. So it was estimated the samples had an average 42 human years. The average weight was 10.8 kilograms consisted of 6 female dogs and 4 male dogs.

This study found a statistically significant correlation between ALP and IL-1β (r = 0.626, p = 0.036), between GGT and IL-1β (r = 0.826, p = 0.003). There were no statistically significant correlation between Bilirubin Direk and IL-1β (p> 0.05). Increased levels of GGT and ALP occurred three days after ligation of the CBD. Increased level of ALP had a strong correlation with increased level of GGT (r = 0.912; p = 0.000). Similarly, increased level of GGT was correlated very strongly with the increased of direct bilirubin levels (r = 0.746; p = 0.011). It was found a very strong positive correlation between ALP and increased level of bilirubin (r = 0.810, p = 0.004).

These results indicated that there was very strong and significant correlation between increased levels of GGT, ALP; and increased levels of IL-1β and direct bilirubin IL-1β, as a manifestation of the inflammatory process as well as damage of liver function. While the increased level of direct bilirubin correlated with IL-1β levels, had a moderate positive correlation but not statistically significant (r = 0.537, p = 0.068).

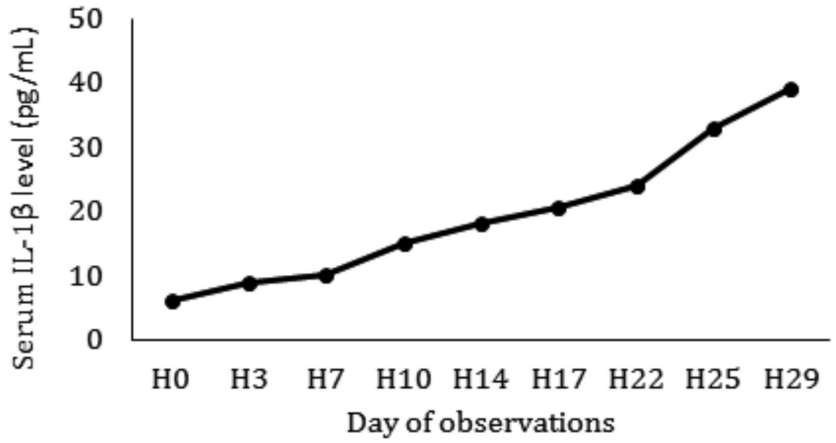

FIGURE 1. The Average Increased Level of IL-1β

The increased level of IL-1β occurred since day 3 and continued to increase until day 29. The increased level of IL-1β between baseline and day 29, had a statistically significant difference (p=0.000).

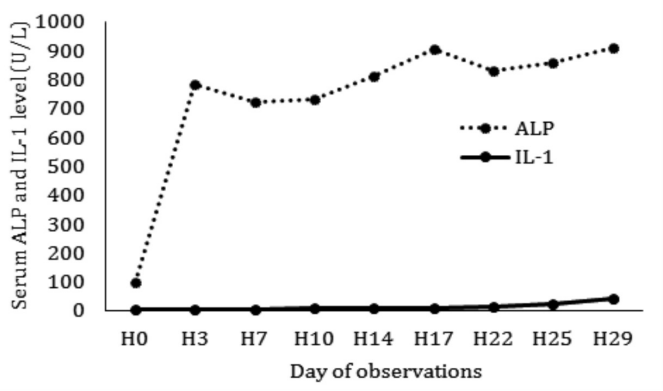

FIGURE 2. The Average Incerased Levels of ALP and IL-1

FIGURE 2 shows the high increased levels of ALP on the third day after ligation of the CBD, then fluctuated until day 29. FIGURE 3 shows a regular increased in GGT levels until day 29.

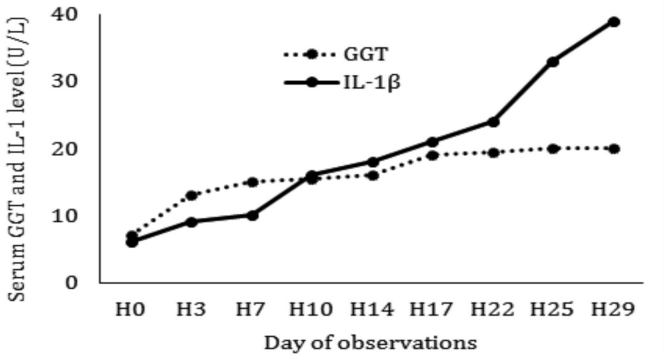

FIGURE 3. The Average Incerased Levels of GGT and IL-1β

Figure 4 shows the increased levels of direct and total bilirubin until the 3rd day, with a reduction on day 14, and rise again until day 29.

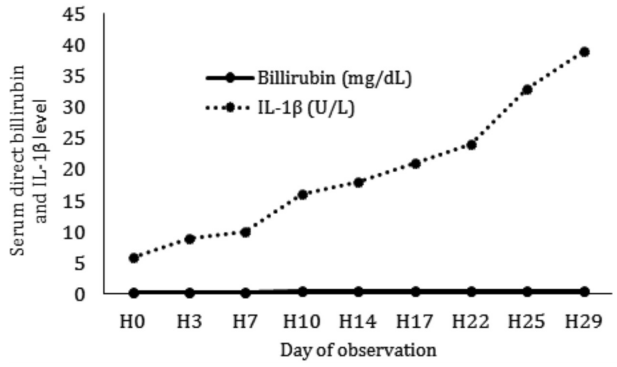

FIGURE 4. The Average Incerased Levels of Direct Billirubin and IL-1β

DISCUSSION

At the initial examination of blood serum, it was found two subjects had an increased in white blood cell count (leukocytosis), but otherwise, they were clinically well. Clinically, we did not find scleral icterus in the subjects. The evaluation began in day 3, we found increased serum level in almost all subjects. 20% of direct bilirubin was not increased, 10% of direct bilirubin had mild increased and 70% of direct bilirubin increased at almost twice the normal value. However, we did not find any signs of scleral icterus in all the subjects. It was found increased levels of alkaline phosphatase, GGT, leukocytes, and IL-1β that were quite high. The increased in leucocytes ranged between 17,100-59,800/uL, with the mean value of 29.620/uL, and 10% of the samples had no leukocytosis. There was a steep increased of the level og IL-1β (range from 0.897pg/dl to 9.274pg/dl). This showed that there were inflammatory or infection.

Biochemical markers of cholestasis are the presence of elevated levels of Alkaline Phosphatase (ALP) and Gamma-Glutamyl Transpeptidase (GGT). ALP and GGT is a sensitive indicator of the occurrence of cholestasis. ALP and GGT are the hepatocyte membrane and released if there is a damage of hepatocellular. In cholestasis, the synthesis of these enzymes is induced and made soluble. GGT is lifted because the leak out of the bile duct of the increased bile duct pressure. In the next stage of cholestasis; AST, ALT, and bilirubin will increase because of liver damage as a secondary effect of cholestasis.[4-8]

A study says that the level of ALP in a dog is increased earlier than GGT, bilirubin, and clinical jaundice.[16] High levels of ALP and GGT show the presence of biliary duct obstruction. High level of ALP (300-500U/L) occurs in chronic liver disease and biliary obstruction. Mild increased (<300U/L) typically occurs in cirrhosis of the liver due to viral, non-alcoholic fatty liver disease, cholestatic liver disease and hepatocellular cancer. The level of ALP increased four times higher than normal value in 1 or 2 days after obstruction. The level of ALP can also be increased in conditions apart from the liver disorder and biliary obstruction; such as malignancies (bronchogenic carcinoma, Hodgkin's lymphoma, renal cell carcinoma), high fat diet, pregnancy, growing child and chronic kidney disease.[17]

Cholestasis is associated with pro-inflammatory status, resulting from the portal and systemic endotoxemia. The concentration of endotoxin in the portal circulation increases, as a result of a lack of bile salts in the intestinal lumen, with resulting in an imbalance of microflora and increased the permeability of the intestinal mucosal barrier, resulting in bacterial translocation. Exposure endotoxemia and bacterial translocation due to obstructive jaundice cause uncontrolled induction of the inflammatory cascade. The damage to the hepatocyte or cholangiocyte causes activation and release of proinflammatory cytokines. The TNFα, IL-1 and IL-6 will be produced by macrophages, endothelial cells, and Kupffer cells are still good that the response of the systems of defense TNFα, IL-1) and IL-6 are produced by macrophages, endothelial cells, and Kupffer cells which are the response of the body immune system. Animal experiments have shown increased concentrations of proinflammatory cytokines such as TNFα, IL-1, IL-6 (IL-8), and IL-10. Cytokine concentration increased contributes to increased risk of complications. This increased levels of cytokine is a manifestation of an inflammatory process, which induces SIRS and sepsis.[3,9-14] A study on the animal model have shown increased concentrations of proinflammatory cytokines such as TNF, IL-1, IL-6, IL-8, and IL-10. The increased concentration of cytokines contributes to

an increased incidence of complications. Increased level of this cytokine is a manifestation of an inflammatory process. The local Increased level of cytokines will cause the cardinal signs of inflammation, such as heat (calor), swelling (tumor), redness (rubor), pain (dolor) and loss of function (functio laesa). While a high level of cytokines contribute to SIRS and sepsis.[3,9-14]

It was found 2 dogs (2%) having anemia, with the hemoglobin values of 5.7g/dL and 3.6 g/dl on the day 7. The cause remained undetermined 100% leukocytosis obtained between 17,100-59,800/uL. There was a sharp increase of IL-1 level. Increased level of AST occurred in 90% of subjects as well as increased level of ALT occurred in 80% of subjects. Increased levels of SGOT and SGPT indicate the beginning of declining of liver function. Increased level of direct bilirubin occurred in 4 (40%) of the subjects. This suggests that an increased level of direct bilirubin in obstruction, can last more than 7 days.[18]

On day 14, after blood sampling, 3 subjects found dead. Most likely it was due to the infection or sepsis. We found the hemoglobin level range from 4.3 to 4.6g/dl, the number of leukocyte 38,100-62,000u/L, ALP 623-1.233 in the dead subjects. Sepsis occurred with the level of SGOT/PT of 123-699U/L. It was found scleral icterus in the 3 dead subjects. There were no signifcant changes in valuation day 17-22. We obtained an increased level of ALP with the lowest level of 432U/L with the highest level of 1.676U/L on day 29. The highest level of direct bilirubin was 1.02mg/dl. Until day 29, we did not find clinically jaundice in the rest of the subjects. All the subjects remain active and energetic without showing signs of sepsis.

The occurrence of a strong and significant correlation between the increase in GGT and ALP with IL-1β, suggesting that the increased levels of Gamma-glutamyl Transpeptidase and Alkaline Phosphatase will be followed by the occurrence of an inflammatory process. While the weak correlation between direct bilirubin and interleukin-1 β (IL-1β), indicating the need of direct bilirubin to increase in order to induce an inflammatory process. The inflammatory process will occur when there is a high enough level of direct bilirubin. In this study, inflammatory process was marked by a sharp elevated in the level of IL-1β and an increased white blood count (leukocytes) on the 3rd day after ligation of the CBD.

CONCLUSIONS

Increased levels of ALP and GGT are sensitive indicators of cholestasis. Increased levels of ALP and GGT had a strong positive and significant correlation to increased level of IL-1β as a pro-inflammatory cytokine. Increased level of GGT had the most powerful and significant positive correlation to increased level of IL-1β, compared to increased level f ALP. an increase ALP levels. Whereas, increased levels of total and direct bilirubin had a low and insignificant correlation to increased level of IL-1β. Inflammatory process occurred on the third day after ligation of the CBD, so that in the case of cholestasis or obstructive jaundice, the risk of infection, SIRS, and sepsis are considered very high, so it should be considered to perform a temporary surgical repair, prior to definitive or curative surgical management.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. dr. H. Abdus Sjukur, SpB-KBD, Prof. dr. P. Soetamto Wibowo, SpB-KBD, dr. Mamiek Dwi Putro, SpB-KBD for their genuine apprehension, encouragement, patient, and guidance and whose expertise and knowledge were generously shared. To the fellow

digestive trainees for sharing their knowledge and idea in helping the researchers in the construction of the research.

REFERENCES

1. Afify M, Samy N, El Maksoud NA, Ragab HM, Yehia A. Biochemical alterations in malignant obstructive jaundice: effect of pre-operative drainage. New York Science Journal 2010; 3(2):80-9.
2. Sewnath ME, van der Poll T, van Noorden CJ, ten Kate FJ, Gouma DJ. Cholestatic interleukin-6–deficient mice succumb to endotoxin-induced liver injury and pulmonary inflammation, Am J Respir Crit Care Med 2004; 169(3):413-20.
3. Arrese M, Trauner M. Molecular aspects of bile formation and cholestasis. Trends Mol Med 2003; 9(12):558-64.
4. Anonim. EASL clinical practice guidelines: management of cholestatic liver diseases, J Hepatol 2009; 51(2):237-67. http://dx.doi.org/10.1016/j.jhep.2009.04.009.
5. Zajic S, Damnjanovic Z, Stojanovic M, Visnjic M, Dencic S, Ilic D, *et al*. Biochemical markers in patients with extrahepatic cholestasis, Acta Medica Medianae 2008; 47(1):5-12.
6. Rege RV. Adverse effects of biliary obstruction: implications for treatment of patients with obstructive jaundice. AJR Am J Roentgenol 1995; 164(2):287-93.
7. Soares AF, Castro e Silva Jr O, Ceneviva R, Roselino JE, Zucoloto S. Biochemical and morphological changes in the liver after hepatic artery ligation in the presence or absence of extrahepatic cholestasis. Int J Exp Path 1993; 74(4):367-70.
8. Kinugasa T, Uchida K, Kadowaki M, Takase H, Nomura Y, Saito Y. Effect of bile duct ligation on bile acid metabolism in rats. J Lipid Res 1981; 22(2):201-7.
9. F Charles Brunicardi, MD, FACS, Schwartz's, Principles of Surgery, Ninth Edition, 9th Eds, 2010.
10. Hirschfield GM, Heathcote EJ, Gershwin ME. Pathogenesis of cholestatic liver disease and therapeutic approaches. Gastroenterology 2010; 139:1481-96. http://dx.doi.org/10.1053/j.gastro.2010.09.004.
11. Chand N, Sanyal AJ. Sepsis-induced cholestasis. Hepatology 2007; 45(1):230-41.
12. Alpini G, Ueno Y, Tadlock L, Glaser SS, LeSage G, Francis H, *et al*. Increased susceptibility of cholangiocytes to tumor necrosis factor-α cytotoxicity after bile duct ligation. Am J Physiol Cell Physiol 2003; 285(1):C183-94.
13. Geier A1, Dietrich CG, Voigt S, Kim SK, Gerloff T, Kullak-Ublick GA, *et al*. Effects of proinflammatory cytokines on ratorganic anion transporters during toxic liver injury and cholestasis. Hepatology 2003; 38(2):345-54.
14. Van der Gaag NA, Kloek JJ, De Castro SM, Busch OR, Van Gulik TM, Gouma DJ. Preoperative biliary drainage in patients with obstructive jaundice: history and current status. J Gastrointest Surg 2009; 13(4):814-20. http://dx.doi.org/ 10.1007/s11605-008-0618-4.
15. Bhandari M, Toouli J. Preoperative biliary drainage (stenting) for treatment of obstructive Jaundice. HPB (Oxford) 2006; 8(5):343-5. http:// 10.1080/13651820600804328.
16. Cornell University; Alkaline phosphatase (AP, ALP, SAP); from:http://ahdc.vet.cornell.edu/clinpath/modules/chem/alkphos.htm
17. Shaffer E. Laboratory test of the liver and gallbladder; The Merck Manual; last revision on Juni 2009.
18. Wikipedia; Alkaline Fosfatase; free encyclopedia: from: http://en.wikipedia.org / wiki/Alkaline_phosphatase