Changes in Tumor Necrosis Factor Alpha and Interleukin 6 Levels in Patients with Obstructive Jaundice due to Pancreatobiliary Cancer Who Underwent Biliary Drainage

Adang Sabarudin, Rino Alvani Gani**, Murdani Abdullah***, C Martin Rumende*****

* Department of Internal Medicine, Mitra Keluarga Bekasi Timur Hospital, Bekasi
** Division of Hepatobiliary, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta
***Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta
**** Division of Pulmonology, Department of Internal Medicine,Faculty of Medicine Universitas Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta

Corresponding author:
Rino Alvani Gani. Division of Hepatobiliary, Department of Internal Medicine, Dr. Cipto Mangunkusumo General National Hospital. Jl. Diponegoro No.71 Jakarta Indonesia. Phone: +62-21-31900924; Facsimile: +62-21-3918842. E-mail: personaly@yahoo.com

ABSTRACT

Background: *Obstructive jaundice represents the most common complication of biliary tract malignancy. Obstructive jaundice causes releases of proinflammatory cytokine. There has been controversy about effect of biliary drainage on the change in proinflammatory cytokine level in pancreatobiliary cancer patients. The present study was designed to determine levels of tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6) in preprocedure of either endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic biliary drainage (PTBD) and postprocedure of them in obstructive jaundice patient caused by pancreatobiliary cancer.*

Method: *The study method was before-and-after case study design with consecutive sampling. Blood was collected five days prior to either endoscopic retrograde cholangiopancreatography (ERCP) procedure or percutaneus transhepatic biliary drainage (PTBD) procedure and five days after either of them. Enzyme linked immunosorbed assay (ELISA) was used to determine TNF-alpha and IL-6.*

Results: *Forty subjects were included in this study which consisted of 22 men and 18 women. The age was 55.3 (SD 13.7) years old. According to the results of imaging and endoscopy procedure, twenty-two people were diagnosed cholangicarcinoma, ten people were diagnosed ampulla vateri and eigth people were diagnosed pancreatic tumor. In preprocedure, the TNF-alpha concentration was 4.81 (SD 2.91) pg/mL, the IL-6 concentration was 7.79 (SD 1.57) pg/mL and the bilirubin concentration was 15.5 (SD 6,9) mg%. In postprocedure, the TNF-alpha concentration was 8.05 (SD 6.7) pg/mL, there was a significant increase in TNF-alpha concentration ($p = 0.02$). However, IL-6 concentration was 7.75 (SD 1.76) pg/mL, there was not any significant chance in IL-6 concentration ($p = 0.52$). The bilirubin concentration was 11.3 (SD 6,5) mg%.*

Conclusion: *There was a significant increase in mean concentration value of TNF-alpha after biliary drainage procedure. On the other hand there was not any significant decrease in the mean concentration value of IL-6 after biliary drainage procedure.*

Keywords: *obstructive jaundice, pancreaticobilliary cancer, tumor necrosis factor alpha (TNF-alpha), interleukin 6 (IL-6), endoscopic retrograde cholangiopancreatography (ERCP), percutaneus transhepatic biliary drainage (PTBD)*

ABSTRAK

Latar belakang: *Ikterus obstruktif merupakan salah satu komplikasi tersering keganasan sistem bilier. Keadaan ini akan memicu pelepasan sitokin proinflamasi. Terdapat kontroversi mengenai pengaruh drainase bilier terhadap perubahan kadar sitokin proinflamasi pada penderita kanker pankreatobilier. Penelitian ini bertujuan untuk mengetahui kadar tumor necrosis faktor alfa (TNF-alfa) dan interleukin 6 (IL-6) sebelum dan sesudah endoscopic retrograde cholangiopancreatography (ERCP) atau percutaneus transhepatic biliary drainage (PTBD) pada penderita ikterus obstruksi etiologi kanker pankreatobilier.*

Metode: *Desain penelitian adalah penelitian satu kelompok sebelum dan setelah dengan pemilihan sampel secara konsekutif. Sampel darah diambil sebelum dan lima hari sesudah ERCP atau PTBD. Pengukuran kadar TNF-alfa dan IL-6 dengan cara enzyme linked immunosorbed assay (ELISA).*

Hasil: *Terdapat 40 orang responden yang diikutsertakan dalam penelitian ini, 22 laki laki dan 18 perempuan dengan usia rata rata 55,3 (SD 13.7) tahun. Berdasarkan imaging dan endoskopi, ditegakkan diagnosis kolangiokarsinoma sebanyak 22 orang, tumor ampulla vateri 10 orang, dan tumor pankreas 8 orang. Kadar rata-rata TNF- alfa sebelum tindakan 4,81 (SD 2,91) pg/mL dan sesudah tindakan 8,05 (SD 6,7) pg/mL, terdapat peningkatan yang bermakna setelah tindakan drainase bilier (p = 0,02). Kadar rata-rata IL-6 sebelum tindakan 7,79 (SD 1,57) pg/mL dan sesudah tindakan 7,75 (SD 1,76) pg/mL, tidak terdapat perbedaan yang bermakna setelah tindakan drainase bilier (p = 0.52). Kadar rata-rata bilirubin sebelum tindakan 15,5 (SD 6,9) mg% dan sesudah tindakan 11,3 (SD 6,5) mg%.*

Simpulan: *Terjadi peningkatan kadar rata-rata TNF-alfa secara bermakna setelah drainase. Tidak ada penurunan yang bermakna kadar rata-rata IL-6.*

Kata kunci: *ikterus obstruksi, kanker pankreatobilier, tumor necrosis factor alpha (TNF-alfa), interleukin 6 (IL-6), endoscopic retrograde cholangiopancreatography (ERCP), percutaneus transhepatic biliary drainage (PTBD)*

INTRODUCTION

Every year, it is predicted that there are 600,000 of new pancreatobiliary cancer cases with mortality rate of 500,000.[1,2] One of the complications of pancreatobiliary cancer is obstructive jaundice, which is an obstruction in the biliary flow from liver to duodenum.[3] Kurniawan et al found that 55% causes of obstructive jaundice was malignancy in patients hospitalized in Cipto Mangunkusumo Hospital in 2009-2014.[4] Meanwhile, Sjamsuhidayat et al, reported that 70% cases of extrahepatic tumour causing obstructive jaundice were malignancy and carcinoma of the head of pancreas which were the most common cause of malignant obstructive jaundice (> 55%).[5] Biliary tract obstruction causes decrease of bile level in duodenum and bile accumulation in the biliary tract, thus alterations in intestinal normal flora, intestinal mucosa epithelial barrier impairment, and intestinal bacteria translocation occur.[3] Bile accumulation causes impairment of Kuppfer cells and hepatocytes which stimulate the release of proinflammatory cytokines, including interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-alpha).[6]

In patients suffering from obstructive jaundice due to unresectable pancreatobiliary cancer, drainage procedure was an optional palliative therapy; however the survival rate of malignant obstructive jaundice who underwent biliary drainage was not significant.[4,7] Biliary drainage procedure will decrease bilirubin level in the blood and is expected to decrease hepatocytes impairment, decrease stimuli to inflammatory cells and Kuppfer cells; hence, the inflammation process is decreased. However, the drainage procedure alone possesses the risk of trauma and infection after the procedure which can stimulate proinflammatory cytokines. TNF-alpha and IL-6 were the main proinflammatory cytokines. The main sources of TNF-alpha and IL-6 are macrophages and more than 60% of macrophages in the body are located in the liver as Kuppfer cells.[8] Some experts stated that biliary drainage in patients suffering from obstructive jaundice with malignancy aetiology would decrease TNF-alpha

and IL-6 levels.[9] Meanwhile, some others stated that biliary drainage did not decrease TNF-alpha and IL-6 levels.[10]

This study was aimed to understand the pathogenesis of inflammation which occur after biliary drainage in patients with obstructive jaundice due to pancreatobiliary cancer by measuring alteration in TNF-alpha and IL-6 levels before and after biliary drainage; thus, it was expected to provide better understanding regarding this matter.

METHOD

This study was a prospective longitudinal study, one-group before-after study. This study was performed in Internal Medicine Inpatient Ward Cipto Mangunkusumo Hospital in October 2015 to March 2016. Samples were collected using consecutive sampling method. Samples were patients suffering from obstructive jaundice due to pancreatobiliary cancer in Internal Medicine Inpatient Ward Cipto Mangunkusumo Hospital during October 2015 to March 2016 period, who fulfilled study criteria. Inclusion criteria included patients aged ≥18 years old with diagnosis of obstructive jaundice due to pancreatobiliary cancer. Exclusion criteria in this study were patients who had undergone PTBD or ERCP previously, and patients who refused to participate in the study.

Independent variable in this study was biliary drainage (ERCP or PTBD), while dependent variable in this study were TNF-alpha and IL-6 levels. Sample examination was performed in Hepatobiliary Laboratory Faculty of Medicine Universitas Indonesia/ Cipto mangunkusumo Hospital. Reagent kit being used were Quantukune ELISA Human IL-6 and Human TNF-alpha products from R&D System, Inc, Mineapolis, USA. Collected data were primary data, which included age, sex, antibiotics use, non-steroidal anti-inflammatory drugs (NSAIDS) use, comorbid score, bilirubin level, TNF-alpha levels, and IL-6 levels. Data processing and analysis was performed using SPSS 22.0 software. This study had received ethical approval from Faculty of Medicine Universitas Indonesia and had obtained ethical approval from Cipto Mangunkusumo Hospital Jakarta with letter number LB.02.01/X.2/40/2016.

RESULTS

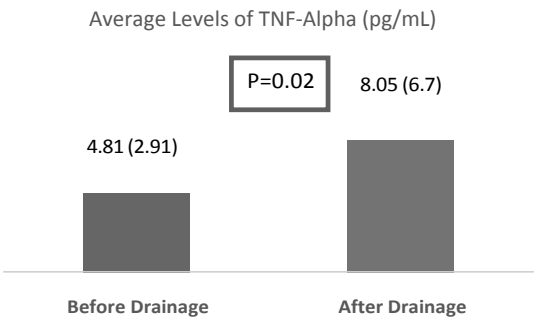
Basic characteristics of 40 research participants were presented in Table 1. This study included 40 obstructive jaundice patients due to pancreatobiliary cancer, 22 males, 18 females with average age of 55.3 (SD 13.7) years old. Cholangiocarcinoma was the most common aetiology found in 22 individuals, followed by ampulla vateri tumour found in 10 individuals, and pancreatic tumour found in 8 individuals. ERCP procedure was performed in 34 cases and PTBD in 6 cases.

Table 1. Patients basic characteristics of study (n = 40)

Data Demography Variable	n (%)
Male	22 (55)
Female	18 (45)
Mean age (years old)	55.3 (SD 13.7)*
Diagnosis	
Cholangiocarcinoma	22 (55)
Ampulla vateri tumour	10 (25)
Pancreatic tumour	8 (20)
ERCP Procedure	34 (85)
PTBD Procedure	6 (15)

*Mean; SD

Figure 1 showed results of TNF-alpha levels before and after biliary drainage (n = 40).



Figurer 1. TNF-alpha levels before and after drainage

Figure 2 presented the results of IL-6 levels before and after biliary drainage procedure (n = 40).

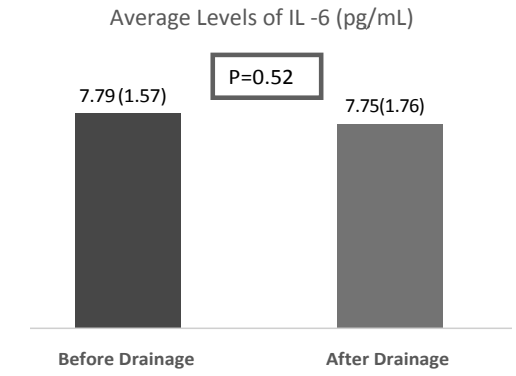


Figure 2. IL-6 Levels before and after drainage

TNF-alpha levels before and after biliary drainage differed significantly with p = 0.02, 4.81 pg/mL (SD

2.91) vs. 8.05 pg/mL (SD 6.7), while IL-6 levels before and after the procedure did not differ significantly with p = 0.52 (7.79 pg/mL (SD 1.57) vs. 7.75 pg/mL (SD 1.76). Table 2 showed results of bilirubin level and leucocyte count before and after biliary drainage.

Table 2. Results of laboratory tests (n = 40)

Parameter	Before procedure	After procedure
Bilirubin (mg%)	15.5 (SD 6.9)	11.3 (SD 6.5)
Leucocyte count/mL	11.570 (SD 6100)	11.700 (SD 5800)

There was a decrease in the average of bilirubin levels before procedure from 15.5 (SD 6.9) mg% to 11.3 (SD 6.5) mg% after drainage procedure. Meanwhile, leucocyte count before and after procedure increased slightly 11570/mL (SD 6100) vs. 11700/mL (SD 5800).

Table 3 presented results of systemic inflammatory response syndrome (SIRS), comorbid score, use of antibiotic and use of NSAIDs before and after ERCP or PTBD.

Table 3. Evaluation on the presence of SIRS, comorbid score, antibiotic use, and NSAIDs use

Parameter	Before procedure	After procedure
Presence of SIRS	13 (32.5%)	15 (37.5%)
Comorbid score 2	28 (70%)	28 (70%)
Comorbid score 3	12 (30%)	12 (30%)
Antibiotic use	40 (100%)	40 (100%)
NSAIDs use	40 (100%)	40 (100%)

SIRS: systemic inflammatory response syndrome; NSAIDs: non-steroidal anti-inflammatory drugs

There was an increased number of SIRS patients as much as 2 patients after undergoing drainage procedure. There was no change in comorbid score, NSAIDs use, and antibiotic use before and after drainage.

There were 2 patients (6.5%) suffering from pancreatitis 24 hours after ERCP procedure. Pancreatitis diagnosis was established based on increased results of amylase and lipase enzyme examination more than 3x of upper normal limits. Three days after the procedure, the results of those enzymes decreased to less than 3x upper normal limits.

There was an increased level of TNF-alpha before and after ERCP (4.84 pg/mL vs. 8.93 pg/mL), while after PTBD was similar (5.48 pg/mL vs. 5.10 pg/mL). IL-6 levels before and after ERCP or PTBD did not differ significantly.

Highest TNF-alpha levels before drainage procedure was found in patients with ampulla vateri tumour, which showed 6.13 pg/mL (SD 2.61), followed by cholangiocarcinoma with 4.80 pg/mL (SD 1.9), and pancreatic tumour with 3.28 pg/mL (SD 2.1). After biliary drainage all increased to 12.41 pg/mL (SD 4.6), 7.64 pg/L (SD 3.2) and 5.11 pg/mL (SD 3.8), respectively. Meanwhile, IL-6 levels before and after procedure were similar in all types of tumour.

DISCUSSION

Levels of TNF-alpha and IL-6 vary in healthy population. TNF-alpha and IL-6 levels are very possible to be influenced by environmental factor pollution), habits (smoking), and vitamin D levels in the blood.[11-13] Experts agree that in the presence of biliary obstruction, the level of proinflammatory cytokines will increase, including TNF-alpha and IL-6. In this study, we found 40 patients with obstructive jaundice with malignancy aetiology with the average age of 55.3 (SD 13.7) years old with 55% of them were males. This result was similar to the previous study with average age of 53 years old and males were 58%.[4] This showed that during 2010-2015 period, demographical data did not change considerably. Besides, we obtained that the most common cause of obstruction was pancreatic tumour, followed by cholangiocarcinoma, and ampulla vateri tumour. This difference was thought to be caused by different sampling method.

The role of NSAIDs and antibiotic in this study could not be evaluated because all patients received NSAIDs, paracetamol, and antibiotics before and after the procedure. In this study, we found an

Table 4. TNFα and IL-6 levels before and after biliary drainage based on type of procedure

Type of biliary	TNFα (pg/mL)		IL-6 (pg/mL)	
procedure (n=40)	Before biliary drainage	After biliary drainage	Before biliary drainage	After biliary drainage
PTBD (n= 6)	5.48	5.10	7.39	7.97
ERCP (n = 34)	4.87	8.93	8.49	8.02

PTBD: percutaneus transhepatic biliary drainage; ERCP: endoscopic retrograde cholangiopancreatography; TNF-α: tumor necrosis factor alpha; IL-6: interleukin 6

Table 5. TNFα and IL-6 levels before and after biliary drainage based on types of tumour

Type of tumour (n = 40)	n (%)	TNFα (pg/mL)		IL-6 (pg/mL)	
		Before biliary drainage	After biliary drainage	Before biliary drainage	After biliary drainage
Cholangiocarcinoma	22 (55)	4.80	7.64	7.86	7.71
Ampulla vateri tumour	10 (25)	6.13	12.41	7.86	7.71
Pancreatic tumour	8 (20)	3.28	5.11	7.56	7.59

TNF-α: tumor necrosis factor alpha; IL-6: interleukin 6

increased average of TNF-alpha level accompanied with the tendency of increased leucocytes; therefore, the presence of infection after drainage needs to be considered. There were 2 individuals suffering from SIRS after procedure, 1 of them suffered from pancreatitis post ERCP, with increased levels of TNF-alpha and IL-6. We did not observe the other patient for the cause of SIRS because this study was not designed for that purpose. Evaluation on the presence of SIRS was very possible to be influenced by the administration of paracetamol and antibiotics.

Biliary drainage procedure succeeds to decrease bilirubin level with average of 4 mg%. There is no specific criteria regarding the success rate of biliary drainage; but, Kurniawan et al in their preliminary study stated that a biliary drainage is considered successful if there is a decreased level of bilirubin more than 2 mg% in 5 days. Qui et al in their analysis suggested that biliary drainage was said to be successful if after drainage was performed, bilirubin level decreased to more than 20% or with imaging evaluation the width of biliary tract was back to the normal size.[14] In this study we did not evaluate the width of biliary tract.

The success rate of biliary drainage in this study (average level of bilirubin decreased from 15.5 mg% to 11.3 mg%) was not followed with significant decrease of IL-6. Padillo et al obtained significant decrease of IL-6 in obstructive jaundice with benign or malignant aetiology who underwent ERCP.[15] This difference is possibly caused by the bilirubin level which continued to stay high after drainage, i.e. 11.3 mg% in this study. High level of bilirubin will lead to hepatocyte impairment and will stimulate Kuppfer cells to release proinflammatory cytokines. Meanwhile, Gwangdae et al did not found significant changes of IL-6 in obstructive jaundice patients with cholangiocarcinoma aetiology (16 individuals) and pancreatic tumour (5 individuals) who underwent ERCP or PTBD.[13] This study also obtained no changes in IL-6 level and cholangiocarcinoma as the most common aetiology. This is in accordance with the theory that cholangiocarcinoma is closely related to chronic inflammation process and microenvironment.[15]

Other factors which can be considered to influence IL-6 level are time of sample collection after procedure, drainage technique, different type of tumour. A study conducted by Griseldis et al in rats which created full thickness injury of 6 mm showed increased levels of IL-6 and TNF-alpha from hour 6 to 15-72 hours after injury.[16] From day-5 of injury, TNF-alpha started to decrease and reached the baseline level in day-14 when complete healing was achieved. Gokhan et al in their study attained that IL-6 and TNF-alpha levels increased significantly 24 hours after ERCP; this supported that proinflammatory cytokines after biliary drainage was influenced by injury during procedure.[17]

Qiu et al in their meta-analysis study concluded that the inflammation cascade which happen after ERCP procedure had not been fully understood; nonetheless, the technique of procedure was believed to have great influence (physical trauma, chemical, thermal, and duration of procedure).[14] This study encountered difficulty in uniforming the procedure being performed.

In this study, we attained 21 (52.5%) cholangiocarcinoma cases. Currently, it is thought that chronic inflammation process in the biliary tract plays role in the presence of cholangiocarcinoma. Prolonged high level of proinflammatory cytokines will induce cell transformation and inhibit antitumour receptor. Furthermore, tumour micro-environment will produce proinflammatory cytokines, including TNF-alpha and IL-6 in a high level, thus causing prolonged inflammation process.[15]

In this study, we found significant increase in the average of TNF-alpha level after biliary drainage. This was thought to be caused by trauma during procedure, infection, use of urographin 76% in ERCP, use of OAINS, and complications after ERCP. ERCP or PTBD procedure causes trauma in the tissue which will cause increased levels of TNF-alpha and IL-6. Grisledis et al concluded that TNF-alpha levels would increase 6 hours after tissue injury and would reach its peak at 72 hours, which later decreased gradually in day-5.[16] PTBD procedure in 6 patients did not show increased levels of TNF-alpha, this was possibly due to the liver tissue had very good regeneration characteristic.[3] Meanwhile, ERCP procedure in 34 patients increased TNF-alpha level; this was thought to be caused by trauma which happened during ERCP due to sphingterotomy and electric cauterisation. Biliary drainage procedure is very difficult to simplify, because of the size of injury due to sphingterotomy in PTBD and liver impairment due to needle insertion in PTBD was not equal in all patients. This might influence the TNF-alpha level.

TNF-alpha is a proinflammatory cytokine which has main role in the response of acute inflammation to gram negative bacteria. It is found that there are approximately 1.5% infection cases post ERCP with *Escherichia coli* as the main aetiology.[18] In this study, we observed an increase in the average of leucocytes

count, increased number of patients suffering from SIRS (2 individuals), 2 individuals suffered from pancreatitis accompanied with increased average of TNF-alpha level, thus the possibility of infection needs to be considered. Abeed et al through an in vitro study found that lipopolysaccharide-stimulated monocyte cells from patients with obstructive jaundice with malignancy aetiology secrete lesser TNF-alpha compared to normal individual, therefore it could be concluded that they are more prone to be infected by bacteria.[19]

In this study, we used urographin 76% during ERCP. Urographin is an ionized iodine contrast which has high osmolality and has cytotoxic effect upon mucous cells of the biliary tract. Michele and Quintavalle stated that iodine contrast medium may increase apoptosis of endothelial cells and kidney tubular cells which may cause acute kidney injury, therefore the possibility of apoptosis in endothelial cells of biliary tract and hepatocytes could be assumed.[20,21] Excessive apoptosis may stimulate the release of proinflammatory cytokines. Other factor which was thought to play role in the increase of TNF-alpha was NSAIDs use. In this study, all patients before and after procedure received NSAIDs (ketorolac or ketoprofen). It was found that single dose administration of NSAIDs (celecoxib and ketorolac), in healthy individuals will decrease the level of PG E2 and increase the level of TNF-alpha. TNF-alpha level will return to normal after administration of PgE2 intravenously.[22]

In this study, we found 2 individuals (5%) suffered from pancreatitis which was manifested as complaint of stomachache and increased amylase serum to more than 3 times of upper normal limit. ERCP procedure may cause local oedema, thus, causing obstruction to the pancreatic duct and lead to pancreatic enzyme reflux. Incidence of pancreatitis post ERCP in this study was similar to other studies.[18] In this study, biliary drainage procedure is beneficial to improve liver function which is signified by decreased average rate of bilirubin as much as 4.2 mg%. However, we should remain cautious as this procedure causes increased level of TNF-alpha which indicated a progressing inflammation.

Selecting patient before biliary drainage procedure, the use of NSAIDs, paracetamol, and antibiotics before and after biliary drainage procedure will influence the evaluation on the presence or absence of SIRS. Details of procedure are not possible to generalized, including the duration of procedure, trauma, use of contrast; all of these factors possibly influence IL-6 and TNF-alpha values.

CONCLUSION

In this study, we found a significant increase in the average level of TNF-alpha after biliary drainage, but there was no significant decrease in the average level of IL-6 after biliary drainage procedure.

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