SERUM p53 PROTEIN AND TOTAL LYMPHOCYTE PROFILE IN SEPSIS PATIENTS TREATED WITH CIPROFLOXACIN AND CEFOTAXIME

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ABSTRACT

Sepsis is still the main cause of death in the hospital. The frequency of cefotaxime resistant bacteria is increasing. On the other hand, cefotaxime has a potency to stimulate a high LPS release. Ciprofloxacin is an alternative antibiotic agent against sepsis with low LPS release characteristics. Higher LPS release is associated with the increase of lymphocyte apoptosis. The mechanism of cell death (apoptosis), especially lymphocytes, is through the expression of serum p53 protein. The objective of this study was to determine serum p53 protein and total lymphocyte profile of patients with sepsis treated with ciprofloxacin and cefotaxime. This research was an observational study. The effect of ciprofloxacine versus cefotaxime on concentration of p53 protein and total lymphocyte in the sera of patients with sepsis was evaluated. This study included 50 patients suffering sepsis caused by bacteria, treated with either ciprofloxacine or cefotaxime after thorough clinical and microbial evaluation and followed up for sera of p53 protein and total lymphocyte. Serum p53 protein and total lymphocyte from the ciprofloxacin group and cefotaxime were analyzed on the first, sixth, and ninth days of treatment. Data were presented descriptively to determine the profile of serum p53 protein and total lymphocyte. From all of the patients, 40 (80%) cured and 10 (20%) died. In cefotaxime group, 19 (28%) cured and 6 (12%) died. In ciprofloxacin group, 21 (42%) cured and 4 (8%) died. The average serum p53 protein in cured patients showed a decrease as compared to normal value (<0.2 U/ml) either in ciprofloxacine or cefotaxime group. The average serum p53 protein of ciprofloxacin in the cured group were 2.23 U/ml, 1.36 U/ml, 0.75 U/ml on the first, sixth, and ninth day. The average serum p53 protein in the cured cefotaxime group on the first, sixth and ninth day were 6.14 U/ml, 4.09 U/ml, and 3.08 U/ml respectively. Average total lymphocyte in cured patients showed alteration. The average total lymphocyte in cured patients showed an increase as compared to normal limit (2000/UI) in both groups. The average total lymphocyte in cured ciprofloxacin group on the first, sixth, and ninth day were 15243/uL, 2007.8/uL, and 2309.3/uL respectively, and that in cured cefotaxime group were 1287.4/uL, 2006/uL, and 2095.6/uL. The average total lymphocyte in cured patients during treatment increased both in the ciprofloxacin (1524.3/uL) and the cefotaxime group (1287.4/uL). In conclusion, there was a tendency of decreased serum p53 protein and increased total lymphocytes in sepsis patients who was treated either with ciprofloxacin or cefotaxime. Based on this study, serum p53 protein and total lymphocyte may have potential indicators for prognosis in sepsis patients.

***Keywords**: serum p53 protein, total lymphocyte, sepsis*

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INTRODUCTION

Sepsis and its complications remains a major cause of death among hospitalized patients. Although it has been overcome by the advance of medical technology, death rate of sepsis remains high, ranging between 40 and 90%. Forty percent of death in intensive care unit in the United States is caused by gram positive and 90% by gram negative bacteria (Llewelyn 2001; Suharto 2001). However, cumulative data on death rate of sepsis in Indonesia have not been reported. Sepsis complication that often leads the patient to death is septic shock. Septic shock occurs due to endothelial damage resulting from the effect of cytokines triggered by the presence of antigen and bacterial toxin (Mattot 2001). Most of the germs that cause neonatal sepsis in developing countries are gram negative germs (Bochud 2001). Since most of the sepsis-causing germs are those of gram negative and the ever increasing resistance against third generation cephalosporin antibiotics, to overcome the infection, antibiotic administration has an important role and should be given cautiously. Previously, antibiotics were regarded reliable to overcome various bacterial infections. Recently, their capacity has been questioned, as there as been increasing resistance against certain antibiotics, such as cefotaxime, which has long been

used to treat sepsis, whose resistance pattern has long not been evaluated systematically (Kuntaman 2002). Along with the advance in pharmacy, fluoroquinolone has become popular as alternative antibiotic, having a high effectiveness, particularly in overcoming gram negative infection and lower capacity of LPS release as compared to cefotaxime.

Another benefit of fluoroquinolone is its spheroplasting effect (reducing LPS release) and reducing proinflammatory mediators (IL-12 and TNF-α) as well as increasing anti-inflammatory mediators (IL-10) (Khan 2000). Cefotaxime has a potentiality of LPS release higher than cyprofloxacin, so that it releases much proinflammatory mediators, which, finally, through the expression of p53 protein, multiplies lymphocyte apoptosis. Unfortunately, the expression of p53 was investigated in rats (Bochud 2001; Perez 2001; as quoted by Primal 2005). This study would reveal the profile of serum p53 protein level and total lymphocyte of septic patients receiving cyprofloxacin and cefotaxime.

MATERIALS AND METHODS

This was an observational study using quasi experimental control group pre- and posttest design. The population in this study was septic patients at the Division of Tropic Infection Disease, Department of Internal Medicine, Dr Soetomo Hospital, Surabaya. Samples were taken using random sampling with Federer's formula (Hanafiah 2002). Since there was a possibility of drop out, for prevention we added the total sample to reach the number of 25 persons in cefotaxime group and 25 in cyprofloxacin group. Total number of sepsis patients was 50 persons. Cyprofloxacin group received intravenous cyprofloxacin 2 x 400 mg for 5 days, and cephalosporin group received intravenous cefotaxime 3 x 1 gram for five days. The dose was given to reach the bactericidal effect as maximum as possible (Lipman 1998).

The inclusion criteria were aged 12-80 years, and SIRS patients with infection. SIRS was marked by 2 or more markers (Bone 1992; Stephen 2005), the temperature was more than 38 degree C or less than 36 degree rectal temperature, heart beat > 90x/min, respiration > 20x/min or PaCO2 < 32 mmHg (<4.3 Pa), white blood cells < 4000/mm3 > 12.000/mm3; or > 10% immature/band. The exclusion criteria were pregnant women, breastfeeding women, and patients who needed other antibiotics for other infections; patients were found to be hypersensitive against quionolone or cephalosporin; patients were unable to follow the research procedure; the patients was not being received immunosuppresive therapy or following the research of other drugs; the patients had no severe disease, such as hepatic cirrhosis with liver encephalopathy, chronic renal disease degree 4 and 5, heart failure, and diabetes mellitus with acute complication (Burke 2002; Gogos 2004; Labbok 2000; Princeton 2005). The drop out criteria were as follows: the patients refused to join the study after being randomized/receiving treatment and, improvement after 3 clinical days and had to change to antibiotic that meet the germ's pattern (Gardjito 2002; Lim 2005), the patients withdraw the treatment with the reason that it has no benefit/the disease worsened; and the patient made a forceful discharge.

p53 protein level was examined in appointed laboratory, the Laboratorium Klinika, Surabaya, using ELISA–BMS256 method with sensitivity of 21% and specificity of 80%. Total lymphocyte was counted using SYSMEX KX21 flow cytometry with sensitivity of 33% and specificity 98%. The study was carried out in the wards of Tropical and Infectious Disease Subdivision, Department of Internal Medicine, Dr Soetomo Hospital, for 6 months, since April to August 2002. The effectiveness was evaluated according to daily clinical changes during treatment, laboratory changes on day 6 and 9, while the evaluation of safety was observed based on side effects, starting every day since the first day administration, during the whole time of study, until the patient was discharged. Data were processed descriptively to provide the picture of p53 protein level and total lymphocyte count on the first, sixth, and ninth day of treatment after being treated with cefotaxime and cyrpofloxacin. However, statistical analysis could not be carried out since there had been no similar study. Data were processed using SPSS 1.0 for Windows.

RESULTS

In this study, 50 patients, comprising 25 females and 25 males. The age of septic patients in this study ranged between 12-80 years with the mean of 45.42 year. The distribution was mostly in age group of 41-50 year (20%) and the least was in age group of 71-80 year (8%).

From 50 septic patients, 12 (24%) were immunocompromized and 38 (76%) were non-immunocompromized. The immunocompromized patients were mostly found in cefotaxime group, comprising 9 persons (18%), and the non-immunocompromized was mostly found in cypro-floxacin group, consisting of 22 persons (44%).

From 50 septic patients, 10 died (20%) and 40 recovered (80%). Cured patients were mostly found in

cyprofloxacin group, 21 persons (24%), and those who died were mostly from cefotaxime group, consisting of 6 persons (12%).

Table 1. The distribution of septic patients according to age and sex

Age (years)	Male		Female		Total	
	n	%	n	%	N	%
12 - 20	4	16	5	20	9	18
21 - 30	4	16	2	8	6	12
31 - 40	5	20	1	4	6	12
41 - 50	4	16	6	24	10	20
51 - 60	2	8	4	16	6	12
61 - 70	4	16	5	20	9	18
71 - 80	2	8	2	8	4	8
Total	25	100	25	100	50	100

Table 2. The distribution of septic patients according to immunocompromized, non-immunocompromized and antibiotics

	Immuno-compromized		Non-immuno-compromized	
	N	%	N	%
Cyprofloxacin	3	6	22	44
Cefotaxime	9	18	16	32
Total	12	24	38	76

Table 3. Distribution of septic patients according to cured, died, and antibiotics.

	Cured		Died		Total	
	n	%	n	%	N	%
Cyprofloxacin	21	42	4	8	25	50
Cefotaxime	19	38	6	12	25	50
Total	40	80	10	20	50	100

Among 50 septic patients, those who had one underlying disease were 32 (64%), two underlying diseases were 13 (26%), and three underlying diseases 5 (10%). Patients with one underlying disease was mostly found in cyprofloxacin group, comprising 18 persons (36%). Patients with two underlying diseases were mostly found in cefotaxime group, 8 persons (16%), and those with three underlying diseases were mostly in cefotaxime group, 3 persons (6%).

Table 4. Distribution of septic patients according to underlying disease and antibiotics

	One Disease		Two Diseases		Three Diseases		Total	
	n	%	n	%	n	%	N	%
Cyprofloxacim	18	36	5	1	2	4	25	50
Cefotaxime	14	28	8	16	3	6	25	50
Total	32	64	13	26	5	10	50	100

From 50 died septic patients, those with one underlying disease were 7 (14%), and two underlying diseases 2 (4%). Patients with one underlying disease were mostly found in cyprofloxacin group, comprising 4 persons (8%), and those with two underlying diseases were mostly found in cefotaxime group, 2 persons (45).

Table 5. Distribution of died septic patients according to underlying disease and antibiotics

	One Disease		Two Diseases		Three Diseases		Total	
	n	%	n	%	n	%	N	%
Cyprofloxacim	4	8	0	0	0	0	4	8
Cefotaxime	3	6	2	4	0	0	5	10
Total	7	14	2	4	0	0	9	18

From all cured septic patients, the mean of total lymphocyte on the first day was higher than that in cyprofloxacin group (1524.3/uL). The mean of total lymphocyte on day 6 was also higher in cyprofloxacin group (2007,8/uL). The mean of total lymphocyte group on day 9 was found to be higher in cyprofloxacin group (1524.3/uL). The mean of total lympchocyte in septic patients during treatment was also higher in cyprofloxacin group (1947.13/uL), while the mean of total lymphocyte in septic patients during treatment was lower in cefotaxime group (1796.33/uL).

Table 6. Mean distribution of total lymphocyte in cured septic patients in cyprofloxacin and cefotaxime groups

	Mean of total lymphocyte (Unit/uL)		
	Day 1	Day 3	Day 6
Cyprofloxacin	1524.3	2007.8	2309.3
Cefotaxime	1287.4	2006	2095.6

In this study, septic patients died before the sixth day of treatment, therefore, the data of total lymphocyte on day 6 dan 9 were not found. The mean of total lymphocyte on day 1 was found to be higher in cefotaxime group (1172/uL). The mean of total lymphocyte on day 1 was found to be lower than in cyprofloxacim group (925/uL).

Table 7. Mean of total lymphocyte in died septic patients in cyprofloxacin and cefotaxime group

	Mean of total lymphocyte (Unit/uL)		
	Day 1	Day 3	Day 6
Cyprofloxacin	925	-	-
Cefotaxime	1172	-	-

From all cured septic patients, it was found that the mean of p53 protein level on the first day was higher in cefotaxime group (6.14 U/mL). The mean of p53 protein level on day 6 was higher in cefotaxime group (4.09 U/mL). The mean of p53 protein level on day 9 was found to be higher in cefotaxime group (4.09 U/mL). The mean of p53 protein level on day 9 was higher in cefotaxime group (3.8 U/mL). The mean of p53 protein level in septic patients during treatment was found to be higher in cefotaxime group (4.67 U/mL). The mean of p53 protein level in septic patients during treatment was lower in ciprofloxacim group (1.44 U/mL).

Table 8. Distribution of p53 protein level in cured septic patients in cyprofloxacin and cefotaxime groups.

	Mean of p53 protein level (Unit/uL)		
	Day 1	Day 3	Day 6
Cyprofloxacin	2.23	1.36	0.75
Cefotaxime	6.14	4.09	3.8

In this study, the death of septic patients occurred before the sixth day of treatment, so that the data on p53 protein level on day 6 and 9 could not be obtained. The mean of p53 protein level on day 1 was higher in cyprofloxacin group (42.45 U/mL). The mean of p53 protein level on the first day was found to be lower in cefotaxime group (18.36 U/mL).

Table 9. Mean of p53 protein level in died septic patients in cyprofloxacim and cefotaxime group

	Mean of p53 protein level (Unit/uL)		
	Day 1	Day 3	Day 6
Cyprofloxacin	42.45	-	-
Cefotaxime	18.36	-	-

DISCUSSION

In this study, the result of therapy in 50 patients revealed that 40 persons (80%) left the hospital cured and 10 (20%) died during their treatment in the hospital. The death rate of septic patients in this study was apparently lower than that found by Liewlyin's study, which were 40 (90%). Another researcher, Young et al., who studied 41 septic patients due to gram negative bacteria, found that antibiotic administration was able to reduce death rate from 51% to 28% (Young 1995). In the United States, 40% of the death in intensive care unit was caused by gram positive germs, while that caused by gram negative ones was 60% (quoted by Suharto 2000). Other studies found that death resulting from sepsis was 20% (Gogos 2004). In group receiving cyprofloxacin, 21 persons (42%) left the hospital cured, while Gogos et al. found 26 individuals (44.8%) cured. In cyprofloxacin group, 4 persons (8%) died during treatment, while in the study of Gogos et al. 4 persons (8%) died. Death rate in patients receiving cyprofloxacin was apparently lower than those receiving cefotaxime, while Gogos et al. found that death rate in both groups was similar. In this study, all died patients were caused by septic shock. This was in line with the study from Young et al. (1995) and Gogos et al. (2004). Septic shock occurred due to high predominance of pro-inflammatory cytokines in septic patients and triggered tissue or cellular ischemia, which resulted in abundant production of free radicals. In further development, these free radicals may trigger the apoptotic process of endothelial cells or other cells.

The principle in septic patient therapy is to eliminate the source of infection and supportive encouragement. This study has proved that cyprofloxacin was able to eliminate the source of infection, as could be seen from the lower death rate of cyprofloxacin group than cefotaxime group, because cyprofloxacin has capability to overcome gram negative microorganism, which was the major cause of sepsis (90%) (Llewelyn 2001; Kuntaman 2002). Gogos et al. (2004) also found that sepsis-causing microorganisms were mostly gram negative (67%). Cyprofloxacin is also able to alleviate the negative effect of ROS, which can be observed by the ever reducing p53 level and increasing lymphocyte count during the treatment. Cyprofloxacin also has immunomodulatory effect by reducing the concentration of proinflammatory mediators TNF-α and IL-12 and increasing the concentration of anti-inflammatory mediators IL-10 (Khan 2000).

The mean of total lymphocyte in cured patients, particularly in cyprofloxacin group showed a trend toward increasing total lymphocyte count from time to time during the treatment, i.e. 1524,3/uL on the first day, 2007.8/uL in sixth day and 2309.3/uL on the ninth day, while in cefotaxime group 1287.4/uL on the first day, 2006/uL on the sixth day and 2095.6/uL on the ninth day. The trend toward the increase of total lymphocyte was caused by the effect of antibiotics that is able to eliminate the cause of infection and along with the improvement of cellular immune system in the body of the patient. In this study, total lymphocyte might be potential as a prognosis indicator, although further studies are needed for confirmation. The mean of total lymphocyte in died patients, however, was lower, 925/uL in cyprofloxacin group and 1172/uL in cefotaxime group. The lower total lymphocyte level in

those died patients resulted from, among others, apoptosis in lymphocyte cell during the sepsis, although this statement still needs confirmation. Hotchkiss et al. (2005) suggested that there was an increasing number of apoptotic lymphocyte in septic patients, either in mitochondrial mediated pathway or receptor mediated pathway. However, the lymphocyte count evaluated on the first day was not evaluated in sixth and ninth day.

In this study, we found that the mean decrease of p53 protein level in cyprofloxacin group was 2.23 U/mL on the first day, 1.36 U/mL on the sixth day and 0.75 u/mL on the ninth day, while in cefotaxime group the reduction was 6.14 U/mL on the first day, 4,09U/mL on the sixth day, and 3.8 U/mL on the ninth day (the normal value of p53 protein is < 0.2 U/mL). The reduction of p53 protein level was expressed by the effect of ROS whose level is increasing in septic patients and triggers the process of apoptosis in various cells, including the lymphocyte, which, finally, the apoptotic process was inhibited indirectly by the administration of antibiotics. Hotchkiss et al. (2000) suggested that there was the increase of p53 protein level in sepsis, either in mitochondrial mediated pathway or receptor mediated pathway. However, the p53 protein level evaluated on the first day was not evaluated on the sixth and ninth day, and also not in human but in rats. Therefore, this study suggests that p53 protein level may have potential as prognosis indicator of septic patients, although this suggestion remains to be investigated further. More severe condition of septic patients was an advanced result of suppressed lymphocytes due to apoptosis, which result in the suppression of immune system, allowing a chance for microorganisms to grow and develop uncontrollably. This condition was also worsened by the effect of excessive proinflammatory mediator, including IL-1, occurring in septic patients.

In this study, several factors affecting the apoptotic process, such as virus, genetic abnormalities, endocrine abnormalities, BCL-2, CD 95 ligand, growth factor, autoimmune disease, apoptotic pathway, such as Receptor Mediated Pathway, were not investigated, so that it was possible that other apoptotic pathways that involved in lymphocyte apoptotic process could not be evaluated. The inclusion criteria still used those of Bone, whose sensitivity and specificity is low, but it is still used by WHO (Bone 1992; Gogos 2004; Stephen 2005). Studies similar to this one remain unexist, so that the author found difficulties in analyzing. However, there were almost similar studies, although limited only to in the observation of proinflammatory mediators, such as TNF-α, IL-1β, IL-8 and IL-6, which are released by macrophage and lymphocyte due to the administration of cyprofloxacin and ceftazidim antibiotics in gram negative bacteria (Gogos 2004). Therefore, this study used extrapolation approach, in which the occurrence of lymphocyte apoptosis was regarded as the result of p53 protein expression through mitochondrial mediated pathway. Another shortcoming is the lower accuracy of p53 level examination using ELISA, in which the sensitivity was 21% and specificity 80%. Nevertheless, the literature mentioned that examination with ELISA is the best method compared to others (Yu Guo Qiang 1998). The other shortcoming is also the lower accuracy of total lymphocyte count measurement, which was carried out using Sysmex KX-21, which provided a discourse that the process of intracellular apoptosis can be detected using p53 protein level and total lymphocyte in blood, although previous study showed that p53 protein level was not specific for apoptosis in total lymphocyte (Aaron 2001). Although both p53 protein level and total lymphocyte are simultaneously potential as an indicator of prognosis, it seems that the p53 protein is more reliable to be the prognostic indicator of septic patients. This statement, however, still needs further corroboration.

CONCLUSIONS

Ciprofloxacin can be considered as alternative antibiotics for the treatment of septic patients. Although both p53 protein level in the serum and total lymphocyte have potentials as prognostic indicator in septic patients, the result of this study shows that p53 protein serum can be more reliable as the prognostic indicator of sepsis. Since there are various apoptosis pathway mechanism and in order to limit the bias, further studies should be limited on apoptotic mechanism in septic patients caused by the expression of p53 protein through mitochondrial mediated pathway. If the objective of the study is to obtain the clinical outcome, further studies should use true experimental randomized control group pre- and post-test design with stricter inclusion and exclusion criteria. These studies should prove the possibility of p53 protein as an alternative parameter for prognosis indicator in septic patients.

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