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SHORT COMMUNICATION

Blood Chemistry Profile of Long-tailed Macaque (*Macaca fascicularis*) Infected by *Klebsiella pneumoniae*

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Abstract
Klebsiella pneumoniae is a nosocomial bacterium that can infect animals and humans. This study used *Macaca fascicularis* because of its potential as a vector for zoonotic diseases. This research aimed to study the health of the kidneys and liver of *Macaca fascicularis* infected with Klebsiella pneumoniae. The method used in this study was blood chemistry examination of Blood Urea Nitrogen (BUN), creatinine, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, *alanine aminotransferase* (ALT), and *alkaline Phosphatase* (ALP) compared to the normal value of blood chemistry of *M. fascicularis*. *K. Pneumoniae* infection causes an increase in blood chemistry values, namely BUN and albumin/globulin ratio, and decreases in creatinine, globulin, and total protein values. This shows that these macaques have kidney problems caused by *K. Pneumoniae* infection. Blood chemistry values that were within the normal range were albumin, total bilirubin, ALT, and ALP, which indicated that *M. fascicularis* had no liver function disorder

Keywords: organ health status, Southeast Asia Macaques, Zoonosis

1. Introduction

Non-human primates are widely distributed in Southeast Asia, especially in Indonesia. Indonesia is home to extensive natural habitats that support numerous non-human primate species, including the long-tailed macaque (*Macaca fascicularis*). *M. fascicularis* has been widely used over the years for scientific purposes, particularly in the development of biomedical sciences such as drug efficacy trials intended for human application (Ling *et al*. 2018).

M. fascicularis has the potential to become a medium for disease transmission due to its wide roaming range and interactions with humans, animals, and the environment. At least 61% of human diseases can spread from humans to animals or vice versa (zoonosis) (Taylor *et al*. 2001). Although not yet listed by the WHO as a zoonotic disease, *Klebsiella pneumoniae* has been reported as zoonotic in several publications (Hu *et al*. 2020). *K. pneumoniae* can be transmitted from humans to *M. fascicularis* through direct contact, such as during feeding activities (Kasuya *et al*. 2017).

M. fascicularis housed in the same cage as an individual infected with *K. pneumoniae* has a high potential to contract the disease. To determine the health status of other monkeys within a specific population, various health examinations are conducted, including blood chemistry tests of various organs. Blood chemistry tests are expected to serve as early detection of the populations health status before conducting molecular PCR testing to confirm *K. pneumoniae* infection (Bao *et al*. 2021). Specific blood chemistry values can serve as indicators of organ function. Reference values for the blood chemistry of clinically healthy *M. fascicularis* have been widely published, but blood chemistry values for *M. fascicularis* infected with *K. pneumoniae* remain limited.

It is important to assess the health status of organs such as the liver and kidneys. Blood chemistry profiles can serve as indicators of organ function with specific results. Kidney function can be accurately assessed by measuring the waste products excreted by the kidneys, namely, Blood Urea Nitrogen (BUN) and creatinine. Liver function can be assessed through total bilirubin, *alkaline phosphatase* (ALP), and *alanine aminotransferase* (ALT). *Aminotransferase enzymes*, or *serum glutamate pyruvate transaminase* (SGPT) tests, provide a more accurate picture of liver function because they are primarily derived from hepatocytes (Basten 2019).

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2. Materials and Methods

2.1 Materials

The equipment used in this study included 1 ml EDTA tubes, test tubes, micropipettes, microtubes, a BKC-TL4C centrifuge (Biobase®), and a 5010 V5+ photometer (Riele®). Reagents for the examination of BUN, creatinine, albumin, globulin, albumin/globulin ratio, total protein, total bilirubin, ALT/GPT, ALP (Greiner®) and distilled water (aquadest), The materials used included 15 blood samples *M. fascicularis* that had been confirmed positive for *K. pneumoniae* infection based on PCR testing, from Primate Research Center (PRC), IPB University.

2.2 Methods

The blood chemistry examination began with the collection of 15 blood samples from juvenile *M. fascicularis* aged 1.5-4 years that were PCR-positive for *K. pneumoniae* infection. The PCR test results for *M. fascicularis* at the PRC-IPB University, showed that 11 males and four females were naturally infected with *K. pneumoniae*. Blood sampling was carried out by a licensed veterinarian at the PRC-IPB University. The blood samples were centrifuged for 5 minutes at a speed of 5000 rpm using a BKC-TL4C centrifuge (Biobase®) to obtain blood serum. The samples were then analyzed for BUN, creatinine, albumin, globulin, albumin/globulin ratio, total protein, total bilirubin, ALT, and ALP levels using a 5010 V5+ photometer (Riele®). Reagents and samples were allowed to reach room temperature, and the photometer was calibrated to zero absorbance using distilled water (AquaDest).

2.2.1 Biochemical Parameters

2.2.1.1 Blood Urea Nitrogen (BUN)

A total of 1 ml of reagent and 5 µl of sample were pipetted into a test tube and incubated for 1 minute at 25°C. The test tube was then placed in the cell holder, and absorbance was measured at 1 and 2 minutes. The BUN concentration was calculated using the following formula:

$$\Delta A \times \text{Factor } 952$$

Notes:
- ΔA: Absorbance at the second minute minus the absorbance at the first minute (mg/dl)
- Factor 952: Absorbance factor at a wavelength of 340 nm

2.2.1.2 Creatinine

A total of 1 ml of reagent and 50 µl of sample were pipetted into a test tube and incubated for 1 minute at 25°C. The test tube was then placed in the cell holder, and absorbance was measured at 1 and 2 minutes. The creatine concentration was calculated using the same formula as BUN above.

2.2.1.3 Total Protein

A total of 1 ml of reagent and 20 µl of sample are pipetted into a test tube and incubated for 1 minute at 25^0C. The test tube is then placed in the cell holder, and absorbance readings are taken at 1 and 2 minutes. The concentration is calculated as follows:

$$\Delta A \times \text{Factor } 4$$

Notes:
- ΔA: Absorbance at the second minute minus the first minute (mg/dl)
- Factor 4: Absorbance factor at a wavelength of 546 nm.

2.2.1.4 Albumin

A total of 1 ml of reagent and 20 µl of sample are pipetted into a test tube and incubated for 1 minute at 25^0 C. The test tube is then placed in the cell holder, and absorbance readings are taken at 1 and 2 minutes. The Albumin concentration was calculated using the same formula as total protein above.

2.2.1.5 Globulin

Globulin levels are not directly measured; they are calculated by subtracting albumin levels from the total protein levels in the blood. The formula is:

$$\text{Globulin} = \text{Total Protein} - \text{Albumin}$$

2.2.1.6 Albumin/ Globulin Ratio

The Albumin/Globulin ratio (A/G ratio) is calculated using the formula:

$$\text{A/G ratio} = \text{Albumin} / \text{Globulin}$$

2.2.1.7 Total Bilirubin

A total of 1 ml of reagent and 100 µl of sample are pipetted into a test tube and incubated for 1 minute at 37^0C. The test tube is then placed in the cell holder, and absorbance readings are taken at 1 and 2 minutes. The total bilirubin was calculated using the same formula as total protein.

2.2.1.8 Alanine Aminotransferase (ALT)

A total of 1 ml of reagent and 100 µl of sample are pipetted into a test tube and incubated for 1 minute at 37^0C. The test tube is then placed in the cell holder, and absorbance readings are taken at 1 and 2 minutes. The concentration is calculated as follows:

$$\Delta A \times \text{Factor } 3333$$

Notes:
- ΔA: Absorbance reading at the 2nd minute minus the 1st minute (IU/L)
-Factor 3333: Absorbance factor at a wavelength of 492 nm.

2.2.1.9 Alkaline Phosphatase

A total of 1 ml of reagent and 10 µl of sample are pipetted into a test tube and incubated for 1 minute at 37^0C. The test tube is then placed in the cell holder, and absorbance readings are taken at 1 and 2 minutes. The concentration is calculated as follows:

$$\Delta A \times \text{Factor } 2757$$

Notes:
- ΔA: Absorbance reading at the 2nd minute minus the 1st minute (IU/L)
-Factor: 2757 Absorbance factor at a wavelength of 405 nm.

2.3 Data Analysis

The blood chemistry measurement data were processed in SPSS version 26 to obtain the mean, standard deviation, minimum, and maximum values, and then analyzed descriptively and compared with the normal blood chemistry ranges for *M. fascicularis* reported by Park *et al*. (2016).

Table 1. Normal Blood Chemistry Values of *Macaca fascicularis* (Park *et al*. 2016)

Blood Chemistry Parameter	Unit	Normal Range
Blood Urea Nitrogen (BUN)	mg/dl	15.5-36.80
Creatinin	mg/dl	0.69-1.37
Albumin	g/dl	3.79-5.05
Globulin	g/dl	2.93-5.34
Albumin/Globulin ratio	-	0.84-1.57
Total protein	g/dl	7.20-9.83
Total bilirubin	mg/dl	0.07-0.66
Alanin aminotransferase(ALT)	IU/L	19.7-283.7
Alkaline phosphatase (ALP)	IU/L	562.0-2633.2

Notes: mg/dl milligrams per deciliter
IU/L internatioal units/liter

3. Results

BUN and creatinine are indicators used to evaluate kidney function. The results of BUN and creatinine tests are presented in Table 2.

Table 2. Serum BUN and creatinine measurements in a Population of *M. fascicularis* Infected with *K. pneumoniae*

Population (individuals)	Mean BUN (mg/dl)	Mean Creatinine (mg/dl)
Male (11)	48.10 ± 17.33	0.66 ± 0.08
Female (4)	63.55 ± 62.00	0.64 ± 0.06

Note: mg/dl milligrams per deciliter

Table 3. Total protein serum, albumin, globulin, and A/G ratio measurements in a Population of *M. fascicularis* Infected with *K. pneumoniae*

population (indviduals)	Mean Albumin (g/dl)	Mean Globulin (g/dl)	Mean Albumin/ Globulin ratio (g/dl)	Mean Total Protein (g/dl)
Male (11)	3.90 ± 0.43	2.20 ± 0.52	1.86 ± 0.46	6.11 ± 0.34
Female (4)	3.85 ± 0.45	2.57 ± 0.65	1.57 ± 0.53	6.42 ± 0.49

Notes: g/dl grams per deciliter

Total protein, albumin, globulin, and the A/G ratio are indicators used to evaluate liver function. Albumin is produced in the liver to maintain intravascular osmotic pressure. The albumin/globulin ratio is used to determine the cause of abnormal total protein values. The results of total protein, albumin, globulin, and A/G ratio are presented in Table 3.

Total bilirubin, ALT, and ALP are used as liver condition checks. Bilirubin is produced in the macrophage-monocyte system by breaking down hemoglobin into bilirubin. ALT is found in serum and liver tissue. ALP will increase during bone growth in young animals. The results of total protein, albumin, globulin, and A/G ratio are presented in Table 4.

Table 4. Serum total bilirubin, ALT, and ALP measurements in a Population of *M. fascicularis* Infected with *K. pneumonia*

Population (individuals)	Mean Total Bilirubin (mg/dl)	Mean ALT (IU/L)	Mean ALP (IU/L)
Male (11)	0.21 ± 0.15	46.99 ± 7.54	1025.10 ± 143.56
Female (4)	0.17 ± 0.22	71.85 ± 40.94	916.75 ± 158.44

Notes: mg/dl milligrams per deciliter
IU/L internatioal units/liter

4. Discussions

The result examination of the BUN in *M. fascicularis* showed an increase compared to the normal range of 15.5–36.80 mg/dl (Park *et al*. 2016). According to Latimer and Duncan (2011), elevated BUN levels can be caused by a high-protein diet, infection, fever, starvation, bleeding in the small intestine, and corticosteroid use. The high standard deviation in the female *M. fascicularis* population is suspected to be due to one individual with a BUN level approximately twice as high as the population average. This condition suggests the possibility of kidney dysfunction in that individual. Kidney disorders, particularly in end-stage renal disease (ESRD), impair the kidneys' ability to maintain metabolic and fluid-electrolyte homeostasis, with kidney function reduced to 10% (Baradero *et al*. 2009). Diseases typically indicated by elevated BUN levels include azotemia and acute kidney failure (Basten 2019).

The normal creatinine value for *M. fascicularis*, according to Park *et al*. (2016), is 0.69–1.37 mg/dl. Creatinine test results in the *M. fascicularis* population were slightly below the reference range. Ostermann *et al*. (2016) stated that decreased creatinine levels may be caused by reduced muscle mass, liver disease, and kidney disease. Low creatinine levels may indicate acute kidney injury caused by prerenal azotemia (Suganya *et al*. 2016). Prerenal azotemia results from reduced renal blood flow due to conditions such as dehydration, bleeding, overdiuresis, and decreased intravascular volume, which can be caused by low blood pressure (Tyagi and Aeddula 2022).

Creatinine levels are generally higher in male animals (Latimer and Duncan 2011). According to Wu *et al*. (2014), a decrease in creatinine levels may result from gradual loss of muscle mass, reduced renal blood flow, and decreased glomerular filtration rate with aging. Creatinine levels are not influenced by factors such as diet and dehydration, making creatinine a more accurate indicator of kidney disease than BUN.

The normal albumin range for *M. fascicularis* is 3.79–5.05 g/dl (Park *et al*. 2016). Despite being infected with *K. pneumoniae*, the macaque population showed albumin levels within the normal range. A decrease in albumin levels occurs in conditions involving inflammation, liver disorders, kidney disease, intestinal malabsorption syndromes, and protein deficiency (Parker and Parker 2003). K. pneumoniae infection is suspected not to have spread to the liver, thus not causing a decrease in albumin levels (Soeters *et al*. 2019).

The globulin levels in the macaque population were below the normal range of 2.93–5.34 g/dl (Park *et al*. 2016). According to Zhang (2021), protein loss through the kidneys can lead to immune deficiency, resulting in decreased globulin levels. A reduction in globulin concentration may be caused by liver disease, kidney disorders, and hormonal imbalances, particularly involving adrenal steroid hormones (Bolton 2015). Globulin levels can also be influenced by age, growth, hormonal changes, sex, pregnancy, lactation, nutritional deficiencies, stress, and fluid loss (Dhasia and Alfarisa 2015).

The normal albumin/globulin (A/G) ratio for *M. fascicularis* is 0.84–1.57 (Park *et al*. 2016). Male *M. fascicularis* showed an increased A/G ratio. A decrease in globulin levels caused this increase. According to Wu et *al*. (2014), an elevated A/G ratio may indicate liver, kidney, or intestinal disease. A/G ratio results can also serve as biomarkers of cancer (Zhang *et al*. 2021).

The normal total protein level for *M. fascicularis* is 7.20–9.83 g/dl (Park *et al*. 2016). Total protein levels in the study's monkey population were below normal, attributable to low globulin concentrations. Bacterial infections can reduce protein production, leading to decreased total protein levels (Freeman and Klenner 2015). Low total protein levels may indicate kidney or liver disease (Washington and Hoosier 2012).

The normal total bilirubin level for *M. fascicularis* is 0.07–0.66 mg/dl (Park *et al*. 2016). The bilirubin levels in all *M. fascicularis* individuals in this study were within the normal range. This indicates that there was no disruption in bilirubin metabolism. According to Kanel (2017), elevated bilirubin levels indicate liver disease in animals.

ALT levels in *M. fascicularis* fall within the normal range of 19.7–283.7 IU/L (Park *et al*. 2016). Tests conducted during the early stages of liver disease generally show ALT levels within the normal range (Kanel 2017). According to Han *et al*. (2016), *K. pneumoniae* infection can cause hepatocellular dysfunction through inflammation and sepsis, leading to increased ALT levels.

The normal ALP level in *M. fascicularis* is 562–2633.2 IU/L (Park *et al*. 2016). ALP testing is used as an assay for liver diseases caused by bile duct disorders. The ALP levels in this study were within the normal range. ALP concentrations vary depending on the species, but levels can increase due to cholestasis, digestive tract disorders, or damage to the intestines or bile ducts. ALP levels in male *M. fascicularis* are generally higher compared to females (Kasuya *et al*. 2017). Total bilirubin, ALT, and ALP levels in the monkey population studied were within normal ranges, leading to the conclusion that there were no liver abnormalities, despite the animals testing positive by PCR for *K. pneumoniae* infection.

Based on blood chemistry examination of kidney function in *M. fascicularis* that tested positive by PCR for *K. pneumoniae* infection, increased BUN levels above the normal range and creatinine levels below the normal range were found. This indicates that *M. fascicularis* experienced kidney dysfunction. The liver of *M. fascicularis* showed no abnormalities, as liver function test results remained within normal ranges except for globulin levels.

Kidney disease can be detected when tissue damage reaches 75%, and glomerular filtration rate (GFR) is reduced by 25% (Rahmawati 2018). Nephron damage causes proteins to leak into the urine, resulting in decreased serum globulin levels (Brown *et a*l. 2013). In the early stage of kidney disease (less than 3 months), BUN and creatinine levels typically remain normal (Rahmawati 2018). This corresponds to the condition of *M. fascicularis* in this study, which did not yet show clinical symptoms (asymptomatic).

K. pneumoniae can be detected in the environment, particularly in animals, and can serve as a reservoir for human transmission. If this bacterium is found in a population within an environment, it can be predicted that the number of hypervirulent *Klebsiella* infections will increase in the coming years. *K. pneumoniae* infection is a zoonosis that can cause septicemia, pneumonia, and urinary tract infections in humans. This invasive bacterial strain has also been reported to cause liver abscesses, which can be complicated by bacteremia, meningitis, and endophthalmitis in animals (Kasuya *et al*. 2017). Although *K. pneumoniae* infections may be mild in some cases, further research is needed to fully understand its zoonotic potential in humans and animals, thereby enabling effective prevention and treatment strategies.

In conclusion, *M. fascicularis* infected with *K. pneumoniae* is likely to develop kidney dysfunction, as evidenced by increased BUN and decreased creatinine levels, and by decreased immune function, as indicated by hypoglobulinemia. Liver function test results remained within the normal range, indicating no abnormalities in the liver of the study animals.

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