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Correlation between C-Reactive Protein Concentrations and Erythrocyte Sedimentation Rate in Patients with Positive Widal Test

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Abstract: Clinical symptoms of typhoid and paratyphoid fever greatly vary, while the gold standard of its laboratory examination is bacterial culture which commonly takes 3 days for the results. Further tests should be performed to support diagnosis of this disease. The tests possibly performed to patients with suspected typhoid are C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR). CRP is an acute-phase protein that is tested to nonspecifically monitor the occurrence of an infection, while ESR is a nonspecific test for inflammation which is clinically functional for screening inflammatory diseases. The aim of this study is to determine the correlation between CRP and ESR in patients with positive Widal test. This study uses correlation analysis to 30 samples which fit inclusion criteria in RS Roemani Muhammadiyah Semarang and are obtained by purposive sampling. The samples were tested the CRP concentration by semi-quantitative latex immunoagglutination method and were tested the ESR by westergren method. The results shows average concentration of CRP was 18.40 mg/L, and average number of ESR was 15.20 mm/h. Statistical analysis uses in this study is pearson correlation test. A strong and significant correlation between CRP and ESR in patients with positive Widal with p -value = 0.000 ($p < 0.05$) and $r = 0.886$ is obtained.

1 INTRODUCTION

Typhoid and paratyphoid fever are two acute systemic diseases of digestive tract that remain global health problems for the world's populations. Approximately 21 million cases and 220,000 deaths were reported per year (WHO, 2014). Typhoid and paratyphoid fever were found to be endemic in Indonesia with the incidence rate of 350-810 per 100,000 population and mortality rate of 0,6-5% (Kepmenkes, 2006). The cases number of the diseases varied in different regions, in Semarang for example, in 2015 there were 9,748 cases reported (Dinkes, 2015). The data above suggested that incidence rate of typhoid and paratyphoid fever are still considered high and an appropriate laboratory test is required.

The laboratory examination commonly performed to diagnose the disease is Widal test. Positive result of Widal test indicates the existence of specific antibody against component of

Salmonella in human blood. It is quickly performed but the specificity is less than 50 %. Gold standard for typhoid and paratyphoid fever is bacterial culture of Salmonella. However, a three days examination and facility of clinical microbiology laboratory has caused the delay of diagnosis (Kepmenkes, 2006), therefore, certain laboratory tests are considered necessary to confirm typhoid and paratyphoid fever in addition to its gold standard as diagnostic examination.

The other laboratory tests possibly performed in suspected infections are C-Reactive Protein and Erythrocyte Sedimentation Rate tests. CRP is acute-phase protein synthesized by liver as an immune response to infection, while ESR is a nonspecific laboratory examination for inflammatory diseases by determining the rate of fall of erythrocytes to form sediment in a certain period (Baratawidjaja, 2006; Bastiansyah, 2008). ESR is much often performed compared to CRP, because ESR is one of the routine laboratory examinations for infections, however,

there have been some studies proving a correlation between the two examinations, one of which was study by Malinda in 2017 showing a strong correlation between ESR values and CRP concentrations in patients with suspected lung tuberculosis with correlation coefficient 0.81 (Rukmana, 2017).

There is an increase of average CRP concentrations of 43 mg/L in children with positive result of *Salmonella typhi* culture, Widal, and typhidot tests (Choo et al, 2001). There is an increase of CRP concentrations and ESR values of 53 mg/L and 30 mm/h in typhoid fever patients, respectively (Idhayu, 2016). There is no report on correlation between CRP concentrations and ESR values in patients with positive Widal test referring to a diagnosis of typhoid or paratyphoid fever.

2 MATERIALS AND METHODS

Materials:

CRP-Latex kit by Glory Diagnostic, Sevilla, Spain, NaCl 0.9 %, Pipette, Micropipette 50 μ l, 200 μ l, 1000 μ l, Westergren pipette, Westergren stand.

Sample Collections:

Venous blood specimens were collected from median cubital vein using syringe, blood was divided into two tubes prior to CRP and ESR examination. Serum was used for CRP test, while plasma was used for ESR test. Samples collections were done in RS. Roemani Muhammadiyah Semarang starting from July until August 2017, totaling 30.

2.1 CRP Test by Semi-quantitative Latex Immunoagglutination

CRP concentrations were measured through several steps including CRP qualitative test and quantitative test using CRP latex kit. Fifty microlitres of positive control, negative control, and patient's blood were added into the well of CRP test-slide. One drop of CRP-latex reagent was added into the slide and then homogenized. Agglutination was examined within 2 minutes. If there is visible agglutination that shows positive result, when the result was positive, further measurement should be done using semi-quantitative test. A five serial dilutions of 50 μ l of NaCl 0.9 % and 50 μ l of patient's serum were done in each well of CRP test-slide until the six circles on slide. One drop of CRP-latex reagent was added, the slide was homogenized, and visible agglutination was

examined within 2 minutes. Dilution factor used was 6 mg/L.

2.2 ESR Test by Westergren method

ESR test used westergren pipette, westergren stand, and NaCl 0.9 % solution. EDTA blood samples were diluted using NaCl 0.9 % solution with ratio of 1:4. The diluted samples were homogenized in a tube and incubated for one hour at room temperature. The result was recorded within one hour. After 1 hour, the result was noted.

The mean and standard deviation of CRP and ESR values were statistically analyzed. Correlation between CRP concentrations and ESR in patients with positive Widal test was done by Pearson test analysis.

3 RESULTS AND DISCUSSION

The average of CRP concentration in 30 patients with positive Widal test was 18.40 mg/l. The lowest CRP concentration was 6 mg/l, and the highest was 48 mg/l. The average of ESR value in 30 patients with positive Widal result was 15.20 mm/h. The lowest ESR value was 3 mm/h, while the highest was 30 mm/h (Table 1).

Table 1: Frequency Distribution of CRP Concentrations and ESR Values in Patients with Positive Widal.

Variable	Sample	Average	SD	Min-Max
CRP	30	18.40	11.245	6-48
ESR	30	15.20	6.289	3-30

SD : Standard Deviation

The comparison between CRP concentrations and ESR values in patients with positive Widal test showed a tendency of ESR values to increase following the increasing of CRP concentration levels (Figure 1).

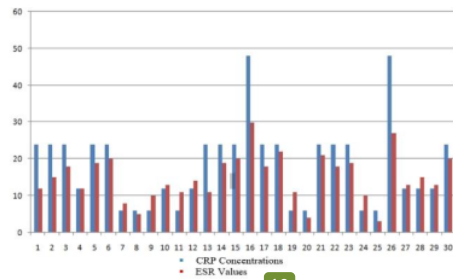


Figure 1: Comparison between CRP Concentrations and ESR Values of Patients with Positive Widal Results.

Relationship between CRP concentrations and ESR values of patients with positive Widal test was shown in a scatter diagram below.

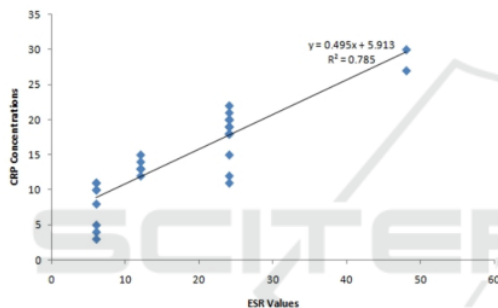


Figure 2: Results of Relationship between CRP Concentrations and ESR Values in Patients with Positive Widal.

Results of Pearson correlation analysis showed that correlation coefficient or $r = 0.886$ suggesting a strong correlation between CRP concentrations and ESR values, whereas value of $p = 0.000$ ($p < 0.005$) showed significant correlation between CRP concentrations and ESR values, suggesting a significant and strong correlation between CRP concentrations and ESR values in patients with positive Widal test. The scatter diagram showed gradient of a straight line going upward, indicated that there was tendency of increasing ESR values following an increase of CRP concentrations.

Pearson correlation analysis showed that correlation value of 0.886 with p value 0.000 was obtained, suggesting a strong and significant correlation between CRP concentrations and ESR values in patients with positive Widal test. The result suggested that a tendency of ESR values to increase following the increasing of CRP concentrations.

The results of this study were relevant with Malinda (2017) on correlation between ESR values and CRP concentrations in patients suspected with lung Tuberculosis, proved a strong correlation between ESR values and CRP concentrations in patients with suspected lung tuberculosis, with $r = 0.81$ (Rukmana, 2017). In addition, this study is also relevant with research by Widarti in 2014 showed a meaningful correlation between ESR values and CRP concentrations in patients suspected with lung tuberculosis with $r = 0.889$ (Widarti, 2014).

During infection, bacterial products such as Lipopolysaccharide (LPS) activated macrophage and other cells to release various cytokines such as Interleukin 1, Interleukin 6, Interleukin 8, And TNF as non-specific immune response to bacterial antigens. These cytokines stimulated liver to synthesize and release some plasma protein called acute-phase protein, such as C-Reactive Protein, Mannan Binding Lectin (MBL), seruloplastin, and fibrinogen (Longo and Fauci, 2013; Baratawidjaja, 2006).

Study by Amal *et al* in 2012 about effect of typhoid fever to cytokines (Interleukin 6 and 8) and C-Reactive Protein showed a significant increase of the average IL-6, IL-8, and CRP as much as 153 pg/ml, 131 pg/ml, and 37.2 mg/l, respectively (Ali *et al* 2012).

The increasing CRP concentrations in blood caused the increase of plasma viscosity. It led to a decrease of potential zeta, a repulsive force among erythrocytes, allowing the formation of rouleaux and faster sedimentation of erythrocytes. The increase of ESR values are not only influenced by the increase of CRP concentrations, but also possibly influenced by erythrocyte factors and other acute-phase proteins like fibrinogen. During infection, the other acute-phase proteins are also increased although it does not occur prior to the increase of CRP concentrations. However, the other acute-phase proteins also caused plasma viscosity and increased the ESR values (Kiswari, 2014).

4 CONCLUSIONS

The conclusion of this study obtains the average CRP concentration from 30 patients with positive Widal test is 18.40 mg/l. The lowest CRP concentration is 6 mg/l, and the highest is 48 mg/l. The average ESR value from 30 patients with positive Widal result is 15.20 mm/h. The lowest ESR value is 3 mm/h, while the highest is 30 mm/h. A strong and significant correlation between CRP

and ESR in patients with positive Widal with p -value $p < 0.05$ and $r = 0.886$ is obtained.

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