

# SURVEY OF INFLAMMATORY BIOMARKERS IN BLOOD AND SPUTUM IN PULMONARY TUBERCULOSIS PATIENTS

**James Perdinan Simanjuntak, Siti Sakdiah, Fardiah Tilawati Sitanggung, Eva Ayu Maharani**

Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Jambi, Jambi

Email: james.p.simanjuntak@poltekkesjambi.ac.id

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**ABSTRACT**

Tuberculosis (TB) is still a global health problem that exists today. Various efforts to reduce the number of TB patients have been carried out ranging from detection efforts to treatment and prevention efforts. Many studies have been conducted previously to look at the detection potential of laboratory diagnoses in finding new cases of tuberculosis. Likewise, efforts to detect prognosis in the laboratory to monitor the development of the condition of TB patients. The development of inflammatory conditions in TB patients is important to accelerate the healing process of these patients. Inflammatory marker tests have been widely performed on several types of laboratory test parameters. CRP and LDH are types of testing that have potential as markers of inflammatory development in TB patients. However, proven tests generally use blood specimens, which are commonly used in laboratory analysis for these parameters. Meanwhile, the current research theme has also been directed at the use of other types of specimens that can show inflammatory conditions experienced by a person. Saliva is known to have potential as another alternative specimen that can be used for such purposes. Saliva is easily obtained and does not require invasive techniques such as needle invocation, so it is considered to have more advantages if it can be proven as a laboratory test specimen that can provide information that is as good and accurate as blood or serum. This study aimed to assess the potential of saliva as an alternative specimen for laboratory analysis with the aim of markers in the assessment of prognosis of TB patients. CRP examination and LDH enzyme activity were chosen in this study of serum and saliva specimens of TB subjects and controls. From this study, it is expected to be shown that saliva can be used as an alternative specimen in laboratory analysis for inflammatory marker tests on both parameters. The results obtained showed that the measurement of LDH activity and and CRP levels in saliva observed in this study proved to have good potential in predicting the presence of inflammation and cell damage experienced by TB subjects. LDH test results have a strong relationship between serum and saliva as laboratory test materials, even salivary LDH levels are higher than serum. While CRP, although saliva showed low levels, the correlation test carried out still showed a strong relationship between the results of the two types of specimens.

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**INTRODUCTION**

Tuberculosis (TB) is one of the major problems for human health worldwide. In addition, it is also known that TB has become a huge economic burden for society [1]. Various examination methods that have been developed today have their own

limitations and advantages in the ability to diagnose tuberculosis [2]. Acid-resistant staining results from sputum specimens are still commonly used in early diagnosis of tuberculosis [3].

However, the diagnosis of tuberculosis is often ruled out due to the low sensitivity of the positive level of acid-resistant bacilli. Although culture of *Mycobacterium tuberculosis* is the gold standard for tuberculosis diagnosis, it cannot be used as an early diagnosis due to its low detection rate and long and complicated bacterial culture time [4,5]. Biomolecular testing that is growing rapidly today has a positive rate of *Mycobacterium tuberculosis* DNA only has a sensitivity of up to 66.67%. Extrapulmonary tuberculosis is often diagnosed by pathology examination, but the histopathological sensitivity of tuberculosis ranges from 40.8% to 56.5% [6].

Laboratory tests that can be used as a marker of the development of inflammation conditions that occur when a person has an infection have been increasingly developed today. C-Reactive Protein (CRP) has been reported in the literature as a predictor of the diagnosis of respiratory diseases, including tuberculosis [7]. Likewise, leukocyte cell count parameters, eosinophil counts and leukocyte ratios such as NLR, MLR and NMLR have also been proven as markers of inflammatory respiratory diseases. Research conducted is generally related to testing inflammatory markers on blood specimens. Likewise, measurements of lactate dehydrogenase (LDH) enzyme activity in chronic pulmonary obstruction patients have been reported to show high significance and potential as inflammatory markers in patients with chronic obstructive pulmonary disease (COPD) [8–12].

However, there have been no reports of observations of these marker tests on salivary specimens from patients with tuberculosis infection. The number of eosinophil cells is closely related to the expression of inflammatory factors in hypersensitive conditions [13]. Meanwhile, Mehmet et al showed that the monocyte-lymphocyte ratio was positively correlated with microalbuminuria, even as an independent factor of diabetic kidney injury by logistic regression analysis [14]. Some literature also suggests that ELR is involved in the inflammatory process, and was significantly higher in the case group than in the control group [15–17]. In the pandemic period of COVID-19, as a marker of hematologic inflammation, eosinophil count was a predictor involving the diagnosis and clinical prognosis of COVID-19 infection [18,19].

The authors hypothesize that eosinophil cell counts are also closely related to inflammation in tuberculosis. CRP is usually highly expressed in inflammatory diseases and is used to assess the degree of infection of the disease. Elevated CRP during treatment indicates severe progression and poor prognosis [20,21]. NLR has been confirmed as a marker of inflammation in tuberculosis, and high levels of NLR are associated with severe tuberculosis [22].

Biomarker testing was carried out by applying laboratory test parameters to blood and saliva specimens from tuberculosis patients in this study. Examination of CRP and LDH enzyme activity was performed on both types of specimens. This study is expected to predict the risk of inflammatory development in the tuberculosis patient population. The prognostic potential of the biomarker assays performed will be analyzed as a determinant variable of the anti-tuberculosis treatment response

given to the observed tuberculosis patients. This study aims to analyze the relationship between CRP test results and LDH enzyme activity as a biomarker of pulmonary tuberculosis.

## **RESEARCH METHODS**

### **Types of Research**

The type of research used was comparative observational with a case-control design.

### **Location and Time of Research**

This research was located at the TB referral health center in Jambi city, namely Simpang Wire Health Center, Pakuan Baru Health Center, Simpang IV Sipin Health Center, Putri Ayu Health Center and Paal X Health Center Jambi City. CRP examination and LDH enzyme activity are carried out in the clinical chemistry laboratory majoring in Medical Laboratory Technology Poltekkes Kemenkes Jambi. The study was conducted from January to August 2023. This research has previously obtained ethical approval from the ethics committee at the Jambi Ministry of Health Poltekkes.

### **Population and Sample**

The case population is patients who seek treatment at the referral health center for TB patients in Jambi city. The sample in this study was TB patients who were over 18 years old and willing to be respondents and sign informed consent. The size of the study sample was calculated using the OpenEpi application for case control with an OR of 4.8 and a confidence level of 95%. After entering the data, a sample number of 68 was obtained, where for case 34 and control 34. The consecutive sampling technique is patients who come to the TB referral health center in Jambi city and meet the criteria taken as samples until they meet the set time target.

### **Data Collection**

The research site consists of specimen collection locations located at the Care Health Center where asthma patients who were observed undergoing previous treatment. If during the study period the patient did not undergo treatment, specimen collection was carried out at the hematology laboratory of the Jambi Ministry of Health Poltekkes or at their respective residences. Sampling is done after the patient expresses his willingness to be a respondent and signs informed consent. Examination of all laboratory parameters observed will be carried out in the immunology laboratory and hematology laboratory of the Department of Technology, Medical Laboratory, Poltekkes, Ministry of Health, Jambi. The study is planned to be carried out from January to July 2023.

### **Data Processing and Analysis**

Research data is analyzed descriptively to see the frequency distribution or percentage of each variable. Comparative analysis to analyze the relationship between CRP test results and LDH enzyme activity using regression test and chi-square test

## **RESULTS AND DISCUSSION**

Data on the number of subjects involved in the study observed by sex showed a percentage that did not differ between men and women. The age of subjects ranged from 18 to 75 years with an average of  $45.8 \pm 16.7$  years and most were in the

productive age range (82.5%). In the male group (44.2 + 15.0 years), the age of the subjects had a lower average than the female group (47.2 + 18.3 years). While observations based on differences in the interpretation of CRP test results, the age of subjects tends to have the same average (46.04 years).

**Table 1. Observed characteristics of TB subjects**

Character	Subject TB	Control
Age group		
- 18 – 65 year	33 (82,5%)	28 (93.3%)
- >65 year	7 (17,5%)	2 (6.7%)
Gender		
- Man	21 (52,5%)	12 (40%)
- Woman	19 (47,5%)	18 (60%)
Smoking Habits		
- Smoke	8 (20%)	5 (16.7%)
- No smoking	32 (80%)	25 (83.3%)
Stage of treatment		
- Intensive	12 (30%)	-
- Advanced	28 (70%)	-

Based on the stage of treatment being undertaken by the subjects of TB patients observed, as many as 30% fall into the category of intensive stage (< 2 months) and 70% are in the advanced stage. Varying average ages were observed in group subjects with intensive treatment stage ( $36.3 \pm 12.6$  years) and advanced stage ( $49.9 \pm 16.8$  years). In addition, in this study it has been observed that smoking habits are still found in 20% of TB patient subjects who are entirely male.

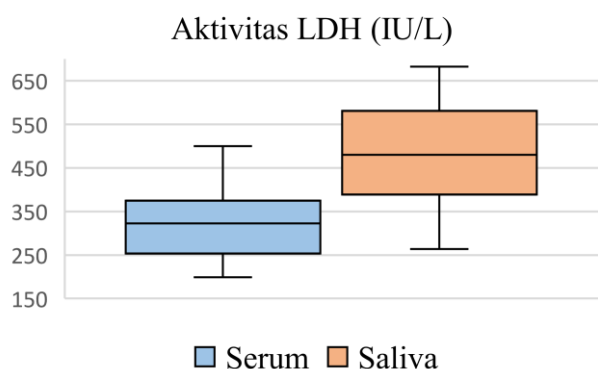


Figure 1. Comparison of LDH activity in serum and saliva

The activity of LDH enzymes observed in this study was obtained from testing of both types of specimens observed, namely serum and saliva. In figure 1, the ratio of the distribution of values of LDH enzyme activity in serum looks lower compared to activity in saliva. The distribution of measurement results from the two specimens was not normally distributed, so descriptive analysis was carried out based on the median value (IQR) and statistical analysis of the Mann-Whitney difference test. Serum showed LDH enzyme activity values of  $329.9 + 96.8$  IU/L, significantly lower

than salivary  $480.5 + 109.0$  IU/L ( $p = 0.000$ ). Saliva as an examination specimen has the advantage of being easy to collect and does not require invasive procedures such as serum. Patients can also do their own specimen collection using clean containers that have been provided by laboratory personnel.

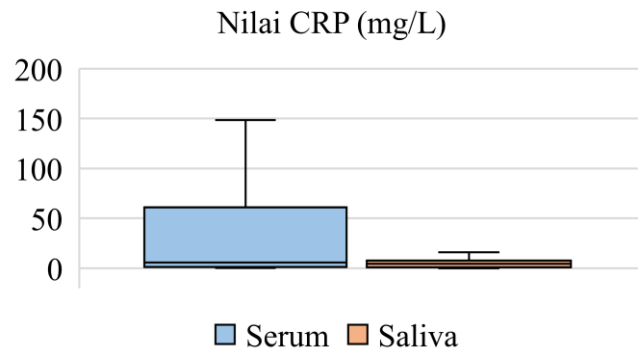


Figure 2. Comparison of CRP values in serum and saliva

CRP levels obtained from both types of specimens show a comparison of the opposite results. Serum gives CRP levels (median = 4.33 mg/L | IQR = 0.97 - 7.74) which was significantly higher compared to saliva (Median = 5.65 mg/L | IQR = 1.5-58.5) ( $p = 0.000$ ). Saliva does not show the potential that can be used in predicting inflammatory events as demonstrated by serum. This is because these proteins are not present in sufficient quantities to be analyzed in saliva.

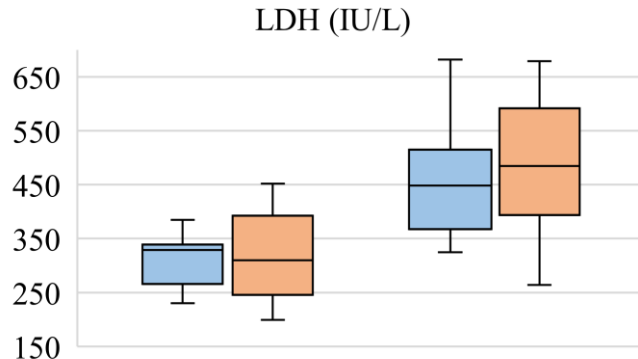


Figure 3. Comparison of serum and salivary LDH based on duration of treatment

Based on the duration of treatment undertaken by TB subjects, the LDH enzyme activity of both specimens gives the same comparative picture. Figure 3 shows each of these enzyme activities at the intensive stage (2-month < treatment) and advanced stage (2-month > treatment). In measuring serum LDH activity, the intensive stage ( $323.6 + 72.4$  IU/L) gave significantly lower results compared to the advanced stage ( $449.1 + 104.7$  IU/L) ( $p = 0.003$ ). Likewise, the results of saliva measurements, at the intensive stage ( $332.6 + 106.6$  IU / L) gave significantly lower results compared to the advanced stage ( $494.0 + 110.0$  IU / L) ( $p = 0.000$ ). There was no difference between the results of measuring LDH activity from each specimen at

both stages of treatment, both intensive stage ( $p = 0.757$ ) and advanced stage ( $p = 0.234$ ).

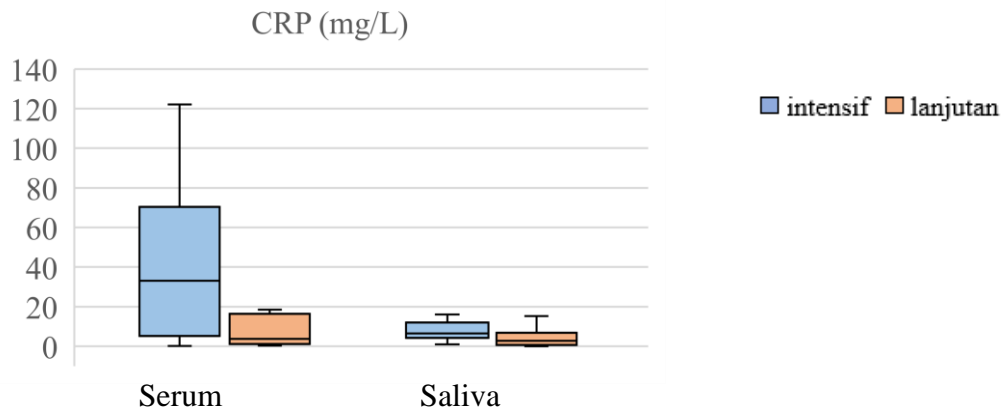


Figure 4. Comparison of serum and salivary CRP based on duration of treatment

In figure 4, the same duration of treatment shows different images of CRP test results in both specimen types. Serum measurement, at intensive stage (Median = 33.15 | IQR = 6.1 - 68) was significantly higher compared to the advanced stage (Median = 3.75 | IQR = 1.15 - 14.3) ( $p = 0.0124$ ). Although saliva showed significantly lower CRP levels than serum, salivary CRP levels were at an intensive stage (Median = 6.46 | IQR = 4.22 - 10.67) still showed significantly higher measurement results compared to the advanced stage (Median = 2.77 | IQR = 0.61 - 6.83) ( $p = 0.046$ ). When observed based on the treatment stage, the difference in CRP levels between the two specimens was only found in the intensive stage ( $p = 0.039$ ), while the advanced stage was no different ( $p = 0.164$ ).

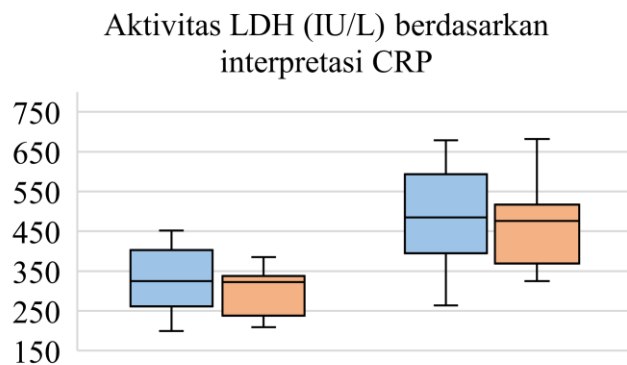


Figure 5. Comparison of serum and salivary LDH based on duration of treatment

LDH activity in TB subjects was also observed based on the interpretation of CRP test results which are considered as markers of inflammation. CRP levels greater than 12 mg/L have been recognized as a marker of inflammation development in a patient. Salivary LDH levels appeared to be higher than in serum, in both the CRP

positive group ( $p = 0.000$ ) and the CRP negative group ( $p = 0.000$ ). However, when viewed based on the type of specimen observed, serum gave LDH levels that did not differ between the CRP negative group ( $327.1 + 74.0$  IU/L) and the CRP positive group ( $294.4 + 53.7$  IU/L) ( $p = 0.126$ ). Likewise, saliva differences were not significant between the CRP negative group ( $494.9 + 113.0$  IU/L) and the CRP positive group ( $456.5 + 101.4$  IU/L) ( $p = 0.274$ )

Explain the meaning revealed by the results (no longer telling the numbers but rather the meaning of the numbers). Explain how its scientific meaning compares to opinions or theories prevailing among fellow scientists (comparison or comparison). If discrepancies are found, it must be explained what causes it to be supported by theories or supported by references, but conjectures (synthesis) based on theories or other related findings (not exactly), this must also be supported by adequate references.

Discussing the results of the research obtained includes interpretation of the research results. Authors can compare with published research results or current information in related fields.

## CONCLUSION

The conclusion must refer to the objectives, therefore see and re-read the objectives of the study and this must be answered expressly in the conclusion.

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