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Composite Bacterial Infection Index and Serum Amyloid A Protein in Pulmonary Tuberculosis Patients and their Household Contacts in Makassar

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Abstract

BACKGROUND: Early diagnosis of tuberculosis (TB) cases in limited resource remains challenging. It is urgent to identify the new diagnostic tools which can control the spread of disease with accurate and rapid test.

AIM: This study aimed to investigate the levels of infection markers: Composite bacterial infection index (CBII) and serum amyloid A (SAA) protein in pulmonary TB (PTB), and their healthy household contacts, as the alternative diagnostic markers for TB.

METHODS: CBII and SAA were measured from 44 new PTB patients, and 31 household contact serum samples. The value of CBII was calculated from neutrophils, lymphocytes, monocytes, erythrocyte sedimentation rate, and high-sensitivity C-reactive protein (hs-CRP) level. hs-CRP and SAA levels were quantified from their serum samples using ELISA. QuantiFERON-TB Gold Plus (interferon gamma release assay [IGRA]) was used to screen latent TB infection among household contacts.

RESULTS: Among 31 household contacts, there were 24 positive IGRA results and the rest (n = 7) had negative results. PTB patients exhibited significantly higher level CBII in the serum specimens, than those in household contact ($p < 0.0001$). There was no significant difference in the SAA level between TB cases and household contacts ($p = 0.679$).

CONCLUSIONS: CBII can be used as one of the biomarkers for the identification of PTB from the serum specimens.

Introduction

Global Tuberculosis (TB) Report 2019 from the World Health Organization reveals that in 2018, there were an estimated 10 million new TB cases around the world [1]. Meanwhile in Indonesia, pulmonary TB (PTB) is known as the third leading cause of death, and one of the top three causes of disability-adjusted life years. Indonesia has one of the highest TB disease burdens in the world, due to a combination of a large population, and a high prevalence rate [2]. TB burden increases with high rates of drug resistance, as in the previous study in Makassar revealed that 4.1% multidrug-resistant TB was found among new TB patients [3].

The severity, the risk of mortality, and transmission of the disease will increase if the procedure for diagnostic is time consuming. Therefore, early diagnosis and therapy are significantly important to controlling the spread of this disease. Clinically, TB detection is still highly dependent on the clinical symptom of TB, microscopic smear examination, sputum culture, and chest radiology. However, the sensitivity of microscopic smear examination among TB cases is still relatively low, which is around 20–60%, and could only be detected if the colony is around 10^5 [4], [5]. *Mycobacterium tuberculosis* culture, which is the gold standard for TB diagnosis, takes 4–8 weeks for bacterial growth. In addition, the signs and symptoms of TB patients with the radiological examination in prodromal stages of infection are not very significant

for diagnostic, and other lung diseases will interfere the final decision. Therefore, it is necessary to develop a fast and accurate alternative method for the diagnostic and effective treatment for the control of TB.

Serum proteins as a biomarker provide important clues in identifying physiological and pathological conditions of the body [6]. Biomarkers can be defined as something that is objectively measured and assessed as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention [7]. Detecting biomarkers in serum can be an effective diagnostic tool for the disease. Biomarkers can be used for early diagnosis, surveillance of the disease, effective treatment, and prognosis of the disease. Biomarker detection is also easy, sensitive, specific, non-invasive, and affordable [6]. The role of several serum proteins has been investigated in various diseases. Several changes in serum protein levels are also detected in TB patients [8]. The infection of *M. tuberculosis* in the body can affect the protein expression and release of TB-related proteins, which can be identified in the blood. Therefore, TB disease can be diagnosed by serum proteins as a biomarker for supporting TB diagnosis [9].

Biomarkers can be a part of a specific host or pathogen that can be used to identify pathogenic processes, including the current state of health and the risk of disease in the future. A specific biomarker is required to distinguish the state of disease, whether having active TB, latent *M. tuberculosis* infection, or no TB infection at 1 time. Biomarkers can also potential in predicting reactivation risk and monitoring the elimination of latent *M. tuberculosis* infection [7]. At present, there are still a few biomarkers that can be applied to identify TB patient and latent TB infection from household contact [10].

In this study, we investigated the levels of infection markers that were measured in TB patients and compared them with their household contacts using the enzyme-linked immunosorbent assay (ELISA) method. Clinical data and infection marker values were analyzed using statistical methods. This research will be a reference in the research of markers in TB patients and their contacts, and may support the early diagnosis of TB patients.

Methods

Seventy-five serum samples were collected consisting of 44 samples of TB patients from Makassar Community Center for Lung Health (BBKPM), Makassar, Indonesia and 31 samples of household contacts. All TB patients were diagnosed according to clinical, radiological finding, smear microscopic, and further confirmed by cultured.

The inclusion criteria for PTB patients in this study included new cases of PTB (had not received anti-tuberculous drugs or TB treatment <2 weeks), age 15 years and over, were willing to participate in this study by giving written consent, and have smear-positive results. The exclusion criteria were positive rapid HIV (SD Bioline). The inclusion criteria for household contact are age 15 years and over, have no clinical symptoms of TB, no previous history of TB or anti-tuberculous drugs, and live in a house with TB patients of at least 6 months, and are willing to involve in the study with written approval. Blood and sputum samples were collected from 44 TB patients and blood samples only from 31 household contacts who fulfilled the criteria. Sputum smear (acid-fast bacilli [AFB]) positive was decontaminated and continued to the culture process at the Hasanuddin University Medical Research Center TB Laboratory, Makassar, Indonesia. The blood samples were centrifuged on 4400 rpm for 10 min at 25°C to separate the sample serum. Serum samples were stored at −20°C before the ELISA examination. Specifically, from contact samples, the interferon gamma release assay (IGRA) was checked with the QuantiFERON TB (QFT) Gold Plus Test (Qiagen, Germany), according to the manufacturer's instruction manual [11].

This research was approved by the Research Ethics Commission of the Medical Faculty of Hasanuddin University Makassar, South Sulawesi, Indonesia (No. 517/H4.8.4.5.31/PP36-KOMETIK/2018 on July 27, 2018), and all research subjects were asked for written approval.

Composite bacterial infection index (CBII)

According to Kossiva *et al*., CBII is calculated by the ratio of leukocyte type counts: The ratio of neutrophil counts to the number of lymphocytes and monocytes multiplied by the levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) as follows: N/(L+M)*CRP*ESR [12]

N: Neutrophils (in percent), L: Lymphocytes (in percent), M: Monocytes (in percent),

CRP: Blood CRP level (mg/dL), ESR: Erythrocyte sedimentation rate level (mm/hour)

The value of neutrophils, lymphocytes, and monocytes was obtained from routine blood tests. ESR was measured by automatic method with a Ves-Matic Easy tool (Diesse Diagnostica Senese, Italy). CRP was measured by automated latex-enhanced turbidimetric immunoassay.

ELISA

To determine the levels of serum amyloid A (SAA) in serum, the ELISA method was used. Human SAA ELISA kits (Cat. No. E1225Hu, Bioassay Laboratory Technology, Shanghai Korain Biotech

Co., Ltd, Shanghai, China) were used to detect concentrations of SAA in serum [13].

Statistical analysis

All experimental data were analyzed using SPSS software (version 21.0, Chicago, IL, USA). $p < 0.05$ was considered statistically significant. Data obtained from the levels value were analyzed and assessed for differences between the three groups, PTB patients, household contacts with positive IGRA, and household contacts with negative IGRA. Data (parametric) will be presented as mean SD (median) and will use statistical analysis (one-way ANOVA test) to distinguish values between the three groups. The non-parametric data were presented as the median (minimum-maximal) and analyzed using the Mann–Whitney U-test for the two groups and the Kruskal–Wallis test for the three groups.

Results

The analysis of results was carried out on 75 samples, consisting of three groups, 44 samples of TB patients, 24 samples of positive IGRAs household contacts, and seven samples of negative IGRAs household contacts. CBII (neutrophils, lymphocytes, monocytes, ESR, and CRP) and SAA were examined from these 75 samples. The clinical characteristics of this study are shown in Table 1. Body mass index was significantly different in the three groups ($p < 0.0001$). There was no association between smoking history and active TB status in this study ($p = 0.389$). AFB from the sputum of 44 TB patients was examined, and there were 28 AFB 1+, 12 AFB 2+, and four AFB 3+.

Before calculating the values of CBII, the components measured in CBII: The leukocytes, ESR, and CRP between TB patients and household contacts were compared. The median of monocyte-to-lymphocyte ratio (MLR) and neutrophil-to-lymphocyte ratio (NLR) was also assessed. The values of these parameters with CBII and SAA in the TB patients group, positive IGRA, and negative IGRA household contacts are shown in Table 2. It is found that the values of leukocytes, MLR, NLR, ESR, CRP, and CBII differed significantly between the three groups ($p < 0.0001$). However, there was no significant difference in those parameters between the positive IGRA contact group and the negative IGRA group. The SAA concentration was not significantly different in the three groups ($p = 0.679$). The median values of ESR, CRP, CBII, and SAA values in the active TB group were higher than the positive IGRA contact group, and the median values of the ESR, CRP, CBII, and SAA values of the positive IGRA contact group were higher than the negative IGRA contact group. In TB patients group, there was no association between the values of ESR, CRP, CBII, and SAA with the degree of smear ($p = 0.096$, $p = 0.066$, $p = 0.200$, and $p = 0.112$, respectively).

Table 1: Characteristics of samples based on gender, age, BMI, and smoking history

Variable	TB patients	Positive IGRAs household contact	Negative IGRAs household contact	p-value
Male	23	8	2	0.222†
Female	21	16	5	
Age				
15–39 years	22	15	4	0.607†
≥40 years	22	9	3	
BMI*	18.262 ± 3.40 (17.48)	21.90 ± 3.64 (22.002)	27.71 ± 6.715 (25.31)	<0.0001‡
AFB 1+	28			
AFB 2+	12			
AFB 3+	4			
Smoking	17	7	1	0.389†
No smoking	27	17	6	

*Data are presented as mean ± SD (median). BMI in kg/m^2, †Chi-square test, ‡One-way ANOVA test. BMI: Body mass index, TB: Tuberculosis, IGRA: Interferon gamma release assay, AFB: Acid-fast bacilli.

Based on the age category, it was found that the values of leukocytes, MLR, NLR, ESR, CRP, and CBII did not different statistically between ages 15–39 and ≥40 years ($p = 0.196$, $p = 0.573$, $p = 0.647$, $p = 0.815$, $p = 0.513$, and $p = 0.551$, respectively). Conversely, SAA was lower significantly in ages 15–39 than ≥40 years, with median (min–max) 2.86 (0.42–64.08) versus 7.81 (0.44–136.42) μg/mL, $p = 0.005$. In addition, when we compared between SAA males and females, it was observed that SAA was higher in males than in females (median [min–max] 11.32 [0.42–136.2] vs. 3.77 [0.46–90.26] μg/mL, $p = 0.001$). CRP, MLR, and NLR values were also different among gender. CRP was found to be higher in males than in females (median [min–max] 5.47 (0.02–16.64) vs. 1.16 [0.01–12.41] μg/mL, $p = 0.017$). Likewise, the MLR and NLR were higher in males than females (median [min–max] 0.27 [0.05–2.55] vs. 0.15 [0.04–0.94] μg/mL, $p = 0.018$, and 3.4 [1.03–21.29] vs. 2.4 [0.99–9.5] μg/mL, $p = 0.021$, respectively). The rest of the parameters were not different significantly between males and females.

The ROC analysis of CBII resulted in cutoff 234.62 to differentiate new PTB diagnoses with non-TB (both positive and negative IGRA household contacts) with sensitivity and specificity values was 84.1% and 100%, respectively. AUC value (± standard error) was 0.968 ± 0.017 (95% CI 0.936–1.000) as presented in Figure 1.

Discussion

Based on the results obtained from the IGRA examination with QFT Gold Plus test on household contact, there were 77.42% of them with positive IGRA results. This indicated the high rate of TB transmission at their house in Makassar. However, the limitations of this study were the number of samples that can be examined. Thus, these results may not describe the prevalence of latent TB in household contacts in the community. Other studies in Busan, South Korea, as many as 45.7% of household contacts had positive IGRA

Table 2: Leucocytes, neutrophils, lymphocytes, monocytes, MLR, NLR, ESR, CRP, CBII, and SAA in the TB patients group, positive IGRAs, and negative IGRAs household contacts

Variable	TB patients	Positive IGRAs household contact	Negative IGRAs household contact	p-value
Leukocytes (/µL)*	10.253 ± 2706.88 (9920)[§]	8120 ± 1543.45 (8065)[¶]	9712 ± 2807.2 (9630)[¶]	0.004[‡]
Neutrophils (%)*	72.46 ± 9.15 (72.9)[§]	57.12 ± 7.58 (58.05)[¶]	59.07 ± 55.3 (9.29)[¶]	<0.0001[‡]
Lymphocytes (%)*	19.32 ± 9.93 (18.49)[§]	34.35 ± 6.47 (34.75)[¶]	32.51 ± 8.27 (34.5)[¶]	<0.0001[‡]
Monocytes (%)*	6.08 ± 2.92 (5.4)[§]	3.52 ± 1.69 (3.05)[¶]	3.47 ± 1.25 (3.4)[¶]	<0.0001[‡]
Monocyte/lymphocyte (MLR)**	0.305 (0.054–2.548)[§]	0.09 (0.04–0.3)[¶]	0.1 (0.05–0.19)[¶]	<0.0001[†]
Neutrophil/lymphocyte (NLR) **	3.897 (1–21.29)[§]	1.66 (0.99–3.22)[¶]	1.58 (1.2–3.79)[¶]	<0.0001[†]
ESR (mm/hour)**	81 (3–111)[§]	36 (1–119)[¶]	15(5–72)[¶]	<0.0001[†]
CRP (mg/dL)**	5.47 (0.05–16.64)[§]	0.082 (0.01–1.26)[¶]	0.051 (0.03–1.55)[¶]	<0.0001[†]
CBII**	1104.645 (3.11–8183.97)[§]	3.352 (0.16–229.41)[¶]	1.519 (0.33–125.87)[¶]	<0.0001[†]
SAA (µg/mL)**	4.72 (0.42–120.14)[§]	3.715 (0.91–136.2)[§]	3.56 (0.75–12.07)[§]	0.679[†]

*Data are presented as median (minimum–maximum), **Data are presented as mean ± SD (median), [§,¶]value in each row which shared similar superscript is not significantly different between two groups by T-test or Mann–Whitney, [†]Kruskal–Wallis, [‡]One-way ANOVA test. MLR: Monocyte-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, CBII: Composite bacterial infection index, SAA: Serum amyloid A, TB: Tuberculosis, IGRAs: Interferon gamma release assays

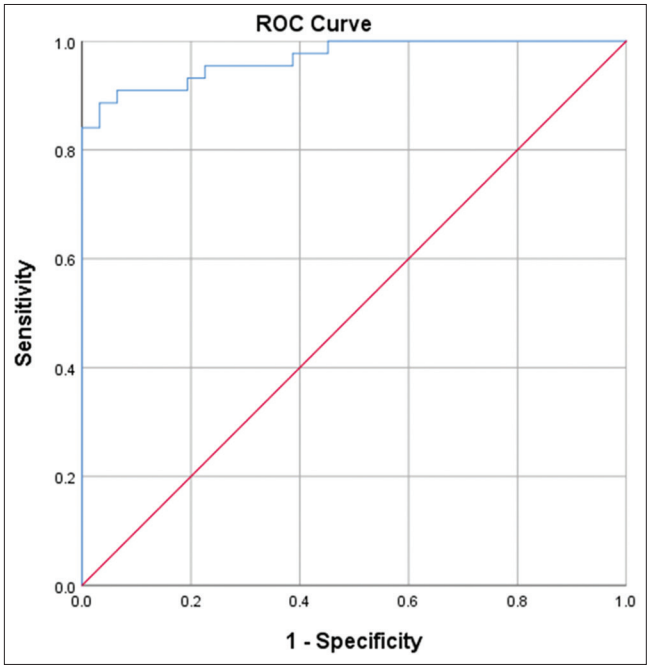

Figure 1: ROC curve analysis of new pulmonary tuberculosis diagnosis

results [14]. Study in Jinju, South Korea, showed 35.2% of household contacts with positive IGRA results [15]. Research in 10 countries in Europe showed 33.9% of household contacts with a positive IGRA [16]. Research in Thailand showed that 21.4% of household contact had positive TST and IGRA results, where those of age ≥20 years and have close relationships with patients (husband/wife/parents) were risk factors for latent TB infection [17]. This study examined only household contacts who have stayed together with the TB patient for 6 months or more, and have a close relationship with patients; that is, spouse, parents, daughter, or son. The high incidence of positive IGRA in household contacts in Makassar can be associated to their low-income status, house conditions with less ventilation or densely populated, and dense residential environment.

CBII is an index calculated by the ratio of the number of neutrophils to the number of lymphocytes and monocytes, multiplied by levels of CRP and ESR [12]. Kossiva *et al*. proposed this formula to determine the presence of infection and to investigate whether the CBII can be used to distinguish fever caused by a bacterial or viral infection. The cutoff value of CBII was 32.45 for the diagnosis of infection and 2044 for the diagnosis of bacterial infection. The median value of CBII in bacterial and viral infections was 4725 and 496, respectively. In this study, the median of CBII in TB patients was 1104.645. This result was significantly lower than Kossiva's study, median result in this study has a high discrepancy with Kossiva's study (5.47 vs. 125.9 mg/dL). It means that TB infection usually has lower CRP value than other bacterial infection, as described in another study [18].

Leukocyte count alone may not be an accurate predictor for bacterial infection because of low sensitivity and specificity [19]. However, in this study, leukocytes in active TB were significantly increased compared to household contacts, both IGRA positive and negative. Neutrophils play a notable part in building innate immunity, possibly through an initial inflammatory response which initiates decrease of mycobacterial load [20]. Another study showed the ratio of monocyte to lymphocyte differ significantly in TB disease, compared to healthy controls and HIV patients without TB infection. The lymphocyte count was lower in active PTB patients than other patients without HIV, and the monocyte count was higher in active PTB patients and other patients without HIV [21]. In this study, it was found that the ratio median of monocyte to lymphocyte was 0.31, and this result was higher and differed significantly ($p < 0.0001$) compared to household contact. This result was similar to Wang *et al*. study. They found that the median ratio of monocytes to lymphocytes was 0.36 in new TB patients, and the ratio of monocytes to lymphocytes <9% or >25% was significant predictors for active TB [22].

The NLR can also be used as predictors to the presence of bacterial infection [23]. It was discovered that the ratio NLR was significantly different in TB patient and household contact ($p < 0.001$), with the median value of NLR in TB patients was 3.897 (1–21.29). In another study, the mean value of NLR in TB patients was 5.59 ± 3.3. The most appropriate cutoff value of NLR to distinguish TB from the control group was 2.16 [24]. NLR values in an adult, non-geriatric, population in good health were between 0.78 and 3.53 [25]. NLR may also be useful in distinguishing infections due to TB or other bacterial pneumonia, particularly in limited resource settings.

The study by Furuhashi *et al*. revealed that ESR, CRP, and SAA concentrations in PTB patients have significant correlations, with a strong correlation between CRP and SAA concentrations [26]. Furuhashi *et al*. reported that the median value of CRP in PTB was 3.3 mg/dL [26], while Mendelson *et al*. obtained 14.1 mg/dL [18]. Meanwhile, in this study, the median value of CRP in PTB was 5.47 mg/dL, which was higher than positive and negative household contact (0.082 and 0.051 mg/dL, respectively). Another study by Almani *et al*. found that the mean of high sensitivity in smear-positive drug-naïve PTB was 0.63 mg/dL and that was higher than in control (0.18 mg/dL) [27]. CRP is an acute inflammatory marker protein that can increase up to 1000 times in conditions of infection or inflammation. CRP is produced by hepatocytes and can also be produced by smooth muscle cells, macrophages, endothelial cells, and adipocytes. CRP includes acute phase reactants that are produced due to increased IL-6 produced by macrophages and adipocytes in acute and chronic inflammatory conditions, such as infections caused by bacteria, viruses, or fungi; inflammatory diseases, tissue injuries, and malignancies. CRP plays a role in innate immunity as the initial defense system against infection. According to the reference for a healthy human, the value of CRP is <1 mg/dL [28].

SAA protein is a major acute-phase protein. It is a specific marker for granulomatous inflammation in sarcoidosis. It regulates *M. tuberculosis* catalase-peroxidase antigen-driven granulomatous lung inflammation in an experimental model of sarcoidosis. Furuhashi *et al*. showed that SAA values in TB patients increased significantly (108 μg/mL), which then decreased after therapy (12 μg/mL) [26]. In another study, the serum level of SAA was significantly higher in TB than healthy controls [29]. Those are different from the result that this study obtained. The SAA concentrations in this study were not significantly different between the three groups. This result may be due to the fact that this study only compared SAA to household contacts, most of whom had positive IGRA results. However, when compared to healthy people, it might get significantly different results, referring to the previous studies where there were different SAA values in PTB patients and healthy controls. In Wang study, SAA was significantly increased in smear-positive (median: 2.21 μg/mL) compared to smear-negative TB (median: 0.93 μg/mL) ($p = 0.02$) [29]. While in this study, it did not involve smear-negative TB patients, but compared the SAA concentration with the degree of AFB smear 1+, 2+, and 3+. There was no association between the values of SAA with the degree of smear ($p = 0.112$). However, there was a significant difference between SAA values in males and females, and between ages 15 and 39 and ≥ 40 years, suggesting that age and separate gender reference range may be used in future analysis. Contradict to the result of this study, Liu *et al*. reported that the SAA levels were not notably different between gender and age [30]. This difference may have occurred because Liu only examined healthy populations.

Conclusions

The accurate diagnostic of TB will not be adequate if using only individual biomarker. Thus, it is recommended to using a combination of some biomarkers to increase the accuracy of diagnostic tools. In this study, one potential diagnostic marker (CBII) for TB was acquired. NLR and MLR can be used as an alternative marker in the diagnosis of TB compared to the household contact. SAA level did not differ significantly between TB patients compared to both positive and negative IGRA household contacts. Thus, SAA in this study may not significant for diagnostic purposes. The result of the present study may facilitate to establish a method for supporting the diagnosis of TB.

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