Gmail **Resti Yudhawati <restiyudhawati@gmail.com>**

Fwd: Submission Confirmation for AMSU-D-21-00908R1

Resti Yudhawati <resti.yudhawati2021@gmail.com> Wed, Feb 2, 2022 at 9:28 PM
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Ms. Ref. No.: AMSU-D-21-00908R1
Title: Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-Sectional Study
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Fwd: Editor handles AMSU-D-21-00908R1

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Ms. Ref. No.: AMSU-D-21-00908R1
Title: Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-Sectional Study
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

Your submission "Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-Sectional Study" will be handled by Editor in Chief Riaz Agha.

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Fwd: Your Submission

Resti Yudhawati <resti.yudhawati2021@gmail.com> Wed, Feb 2, 2022 at 9:29 PM
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Title: Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

The reviewers have commented on your above paper. They indicated that it is not acceptable for publication in its present form.

However, if you feel that you can suitably address the managing editor and reviewer comments (included below), I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

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AND/OR

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Yours sincerely,

Dr Riaz Agha

Editor-in-Chief
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Comments

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Please can you make the following changes/checks:

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5. Please add the following statement above references:

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Reviewer 4: -As authors know, sputum taking procedure needs to perform in right time -the better is morning- and by skilled person in this regard. Explain more about that how sputum samples were taken.
-Authors included too many variables for analysis. Cigarette smoking itself could change pattern of the disease and plasma factors. Considering various variables in such study with this small sample size may make deep bias in analysis.
-English writing needs grammatical revision.

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Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients

--Manuscript Draft--

Manuscript Number:	
Article Type:	Cross-sectional Study
Keywords:	positivity of acid-fast bacilli; pulmonary tuberculosis; serum PGE2 levels
Corresponding Author:	Resti Yudhawati INDONESIA
First Author:	Herley Windo Setiawan
Order of Authors:	Herley Windo Setiawan
	Resti Yudhawati
	Irmi Syafaah
Abstract:	Background : Mycobacterium tuberculosis that infected apoptotic macrophages is triggered by PGE 2 . Apoptosis suppresses the growth of Mycobacterium tuberculosis bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria. Objective : Analyzing the association between serum PGE 2 levels and the positivity of AFB in the sputum of tuberculosis patients. Methods : A cross-sectional study was carried out from August 2019 – July 2020. Serum PGE 2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with p <0.05. Results : The average participants' serum PGE 2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE 2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE 2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p -value = 0.780. Conclusion : There is a weak negative association between serum PGE 2 levels and the degree of AFB positivity in sputum but not statistically significant.
Suggested Reviewers:	Chin-Chung Shu ccshu139@ntu.edu.tw
	Ming-Fang Wu Wu wmf680102@gmail.com
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Annals of Medicine and Surgery

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Please state any conflicts of interest
All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

The authors declare that they have no conflict of interest.

Please state any sources of funding for your research
All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

None.

Ethical Approval

Research studies involving patients require ethical approval. Please state whether approval has been given, name the relevant ethics committee and the state the reference number for their judgement.

We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Consent

Studies on patients or volunteers require ethics committee approval and fully informed written consent which should be documented in the paper.

Authors must obtain written and signed consent to publish a case report from the patient (or, where applicable, the patient's guardian or next of kin) prior to submission. We ask Authors to confirm as part of the submission process that such consent has been obtained, and the manuscript must include a statement to this effect in a consent section at the end of the manuscript, as follows: "Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request".

Patients have a right to privacy. Patients' and volunteers' names, initials, or hospital numbers should not be used. Images of patients or volunteers should not be used unless the information is essential for scientific purposes and explicit permission has been given as part of the consent. If such consent is made subject to any conditions, the Editor in Chief must be made aware of all such conditions.

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Written informed consent was obtained from the patient.

Author contribution

Please specify the contribution of each author to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways should be listed as contributors.

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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In accordance with the Declaration of Helsinki 2013, all research involving human participants has to be registered in a publicly accessible database. Please enter the name of the registry and the unique identifying number (UIN) of your study.

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1. Name of the registry: Health Research Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

2. Unique Identifying number or registration ID: 1355/KEKP/VII/2019

3. Hyperlink to your specific registration (must be publicly accessible and will be checked): -

Guarantor

The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish

Resti Yudhawati is the person in charge for the publication of our manuscript.

To,

Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled "Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients" in your esteemed journal as an Original Article.

On behalf of all the contributors, I will act and guarantor and will correspond with the journal from this point onward.

In this paper, I/we report the association between PGE2 and the degree of positivity of acid-fast bacilli (AFB) as a reflection of innate immunity and bacteria to count. This is significant because it would help the clinician in predicting the positivity of AFB sputum in patients with specific chest x-ray imaging but have a difficulty in expectorating sputum. The paper should be of interest to readers in the areas of pulmonology, especially Tuberculosis.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

All authors have approved the manuscript and agree with its submission to the Annals of Medicine and Surgery.

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Thanking you,

Yours' sincerely,

Resti Yudhawati
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Highlight

1. Serum PGE_2 levels of tuberculosis patients tend to be normal even though Acid-Fast bacilli (AFB) values are high.
2. Most of the new and recurrent cases of pulmonary tuberculosis patients had normal PGE_2 levels.
3. Serum PGE_2 levels have a negative association with AFB value.

Association between Serum PGE_2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients

Running head: Serum PGE_2 levels and acid-fast bacilli

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Association between Serum PGE_2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients

Abstract

Background: *Mycobacterium tuberculosis* that infected apoptotic macrophages is triggered by PGE_2. Apoptosis suppresses the growth of *Mycobacterium tuberculosis* bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria. **Objective**: Analyzing the association between serum PGE_2 levels and the positivity of AFB in the sputum of tuberculosis patients. **Methods**: A cross-sectional study was carried out from August 2019 – July 2020. Serum PGE_2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with $p < 0.05$. **Results**: The average participants' serum PGE_2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE_2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780. **Conclusion**: There is a weak negative association between serum PGE_2 levels and the degree of AFB positivity in sputum but not statistically significant.

Keywords: positivity of acid-fast bacilli, pulmonary tuberculosis, serum PGE_2 levels

Introduction

Tuberculosis (TB) is still a global health problem [1]. The increase in TB cases is accompanied by an increase in drug-resistant TB (DR TB) cases. In the Global Tuberculosis Report, WHO reported that 10 million people were suffering from TB, both new and relapsed

cases, with 558,000 of whom had DR TB [2]. Indonesia ranks third in the country with the highest TB incidence globally, both new and relapse cases. The number of new and relapsed TB cases in Indonesia in 2017 was 442,172, and 54% of them were confirmed bacteriologically by either acid-fast bacilli (AFB) sputum staining or sputum culture [3].

The pathogenesis of TB is an interaction between *Mycobacterium tuberculosis* and the host [4]. The process begins with alveolar macrophages and dendritic cells as the first cells facing *Mycobacterium tuberculosis* bacteria. Macrophages' response as the mainline in dealing with *Mycobacterium tuberculosis* infection is influenced by various inflammatory mediators [5]. The failure of macrophages to control the number of *Mycobacterium tuberculosis* will result in the significant growth of bacteria [6, 7].

This condition emphasizes the important role of the host immune system in determining the susceptibility of TB to relapse. Several studies pointed out that Prostaglandin E_2 (PGE_2) affects macrophages as the main cells in the innate immune system. PGE_2 induces apoptosis and inhibits necrosis of macrophages infected with *Mycobacterium tuberculosis* [5, 8, 9]. Macrophage apoptosis is reported to reduce the growth rate of *Mycobacterium tuberculosis,* which is very important in the elimination mechanism of bacteria that infects the lungs, whereas necrosis plays the opposite role [5, 8, 10]. When the growth of *Mycobacterium tuberculosis* cannot be inhibited, the number of bacteria will increase. The high number of bacterias is reflected in the degree of phlegm AFB positivity. The higher the value of positivity for AFB in sputum, the greater the number of *Mycobacterium tuberculosis* bacteria contained in each ml of sputum [11]. The higher the number of bacterias, the easier it is can transmit, broader lung damage, and an increased risk of resistance [12, 13].

Based on the facts above, this study further revealed the relationship between PGE_2, which represents the innate immune system, and the degree of phlegm AFB positivity, which

represents the number of bacterias. This research is important because no similar study was conducted in humans, so it is hoped that this research could provide further research.

Methods

Participants

Participants in this study were both new and relapsed patients with pulmonary tuberculosis. The inclusion criteria were patients diagnosed with pulmonary tuberculosis [3, 14], positive sputum examination results for AFB, aged 21-65 years, who cooperated during the research procedure. Meanwhile, the exclusion criteria included patients with risk factors for immunocompromised (AIDS, malignancy, and systemic lupus erythematosus), patients having received anti-tuberculosis drug therapy for their current illness, patients taking non-steroidal anti-inflammatory drugs and/or corticosteroids in the past one week.

Ethical Clearance

Participants and their families filled out the consent form before the study. Participants filled out the consent form consciously and without coercion. This study received ethical approval based on the Declaration of Helsinki and obtained the registry of research (1355/KEKP/VII/2019) at the Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Study Design

A cross-sectional study was carried out from August 2019 – July 2020. The number of participants in this study was 62 patients that were obtained using Ronald Fisher's classic z transformation formula. The sample collection used a consecutive sampling technique (Figure 1). Serum PGE_2 levels and levels of AFB in sputum were taken from the participants.

This study report is by the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15].

Measurement of Serum PGE_2 Level

Serum PGE_2 level is the total concentration of PGE_2 in the blood of pulmonary tuberculosis patients. This examination was carried out by taking 3-5 ml of the patient's venous blood and analyzed using the Elisa Kit PGE2¬ (pg/ml). Serum PGE_2 level is categorized into high if the value is more than 400 pg/ml, normal if the value is 200-400 pg/ml, and low if the value is less than 200 pg/ml [16].

Acid-Fast Bacilli Test

Sputum culture was conducted to determine the degree of the participant's AFB positivity. The examination of AFB in the participant's sputum used the acid-fast staining method (Ziehl Nielssen) or the rapid molecular test of sputum with the GeneXpert machine [17]. The degree of phlegm AFB positivity was assessed based on the International Union Against Tuberculosis Lung Disease (IUATLD) standards which were categorized into 2: low (1+ and scanty) and high (2+ and 3+) [17, 18].

Statistical Analysis

The analysis in this study used descriptive analysis and bivariate analysis. Descriptive analysis included the presentation of the results descriptively using the distribution table, mean, median, standard deviation, maximum value, and minimum value. Meanwhile, bivariate analysis was used to assess the association between two variables. The association between variables was analyzed using the Chi-Square test and assessed the association strength using the Spearman correlation test. The analysis was declared significant if $p < 0.05$.

The analysis was assisted by IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Characteristic of Participant

Most participants were male who was 43.37 ± 12.58 years old. Meanwhile, the median of participants' age was 44.5 years, with the lowest age being 21 years and the highest being 64 years. Some patients had a smoking habit (56.5%) and comorbidity of diabetes mellitus (32.3%). A total of 37 participants were new tuberculosis patients and the rest were relapsed, tuberculosis patients. Most participants had a body mass index (BMI) in the skinny category as much as 53.2% (table 1). The average BMI value was 19.46 ± 4.05 kg/m^2, with a value range of 14.20 – 38.28 kg/m^2.

Distribution of Serum PGE_2 Levels in Tuberculosis Patients

Most participants had normal serum PGE_2 levels (62.9%; Table 1). The average participants had serum PGE_2 levels of 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. The lowest and highest value of the participants' serum PGE_2 levels were 191.00 pg/ml and 2,374.00 pg/ml, respectively. The serum PGE_2 levels of smoking and non-smoking participants was 228.80 (191.0 – 2,3374.0) pg/ml and 214.40 (198.3 – 1,724.0) pg/ml, respectively. Most serum PGE_2 levels of smoking participants were normal (50%), while the serum PGE_2 levels of non-smoking participants were mostly normal (78%; p = 0.053). The median value of serum PGE_2 levels for participants with and without diabetes mellitus was 217.30 (191.0 – 1,986.0) pg/ml and 216.80 (193.0 – 2,374.0) pg/ml, respectively. The value of serum PGE_2 levels of participants with and without diabetes mellitus were 45% and 71%, respectively, indicating that most participants had normal values (p = 0.118; Table 2).

Most of the participants' serum PGE_2 levels were normal in both groups of participants with a new diagnosis of pulmonary tuberculosis (62%) and relapsed (64%; $p = 0.292$). The median value of serum PGE_2 levels for participants diagnosed with new pulmonary tuberculosis was 215.70 (191.0 – 1,724.0) pg/ml and participants diagnosed with relapsed pulmonary tuberculosis was 224.40 (193.2 – 2,374.0) pg/ml. Participants' serum PGE_2 levels that were categorized by BMI were mostly normal, with 73% of skinny participants, 50% of normal participants, and 60% of fat participants ($p = 0.058$; Table 3). The median value of serum PGE_2 levels of participants with BMI in the skinny category was 222.60 (194.3 – 1,986.0) pg/ml, normal was 210.30 (191.0 – 2,374.0) pg/ml, and fatwas 216.40 (199.0 – 1,497.0) pg/ml.

Distribution of Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

Most participants had a high degree of AFB positivity in sputum as much as 58.1% (Table 1). Most participants who were diagnosed with new cases of pulmonary tuberculosis had a high degree of AFB positivity (68%). Meanwhile, most participants diagnosed with relapsed pulmonary tuberculosis had a low positivity degree (56%; $p = 0.065$). Some participants had a high degree of AFB positivity in participants with and without a history of diabetes mellitus of 65% and 55%, respectively ($p = 0.455$). Participants' BMI was categorized into 3, namely skinny, normal, and fast, in which some participants had a high degree of AFB positivity ($p = 0.561$). Most smoking (56%) and non-smoking (61%) participants had high positivity of AFB ($p = 0.798$; Table 3).

Association between Serum PGE_2 Levels and Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

The results showed that most participants with low (89%) and high (71%) serum PGE_2 levels had a high positivity of AFB in sputum as much as 89%. Meanwhile, participants with normal serum PGE_2 levels had a low positivity degree of AFB in sputum as much as 54% (p = 0.036). The strength of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780 (Table 4).

Discussion

PGE_2 is a derivative of arachidonic acid produced by various inflammatory cells, especially macrophages. PGE_2, as an inflammatory mediator, plays a role in regulating various cell functions, namely macrophages, T cells, etc. In addition, PGE_2 plays a role in various body functions such as blood pressure regulation, temperature regulation, gastric protection, and childbirth [19]. Under various conditions such as changes in environmental temperature, hunger conditions, stress, PGE_2 will be produced so that levels in the body will rise and fall in various ways [20].

Schoenberger et al reported an increase in serum PGE_2 levels in patients with diabetic retinopathy [21]. A study conducted by Lo et al. showed that the increase in serum PGE_2 levels was due to the upregulation of the cyclooxygenase-2 (COX_2) enzyme in patients with diabetes mellitus [22]. Kumar et al. reported differences in plasma PGE_2 levels in TB patients compared to TB-DM [16]. These results are inconsistent with various studies that reported increased levels of PGE_2 in smokers. Amadio et al. reported an increase in PGE_2 production in smokers due to the modulation of expression of tissue factors exposed to cigarette smoke [23]. Chen et al. in their study also reported the role of cigarette smoke in increasing PGE_2 production [24].

The condition obtained in this study seemed to occur because of the patient's experience factor. In patients with relapse cases, the experience of suffering from TB in the

past will make the patient who has a cough immediately come to the health facility. Meanwhile, new case-patients ignore the cough complaint that leads to accompanying complaints such as weight loss, hemoptysis, or fever. When these accompanying complaints occur, the course of TB disease would be long enough to increase the number of bacterias [1].

The profile of serum PGE_2 levels showed that the average participants had 446.23 pg/ml, with a standard deviation of 510.27 pg/ml. According to some literature, normal serum PGE_2 levels range from 200 – 400 pg/ml [16]. PGE_2 is a derivative of arachidonic acid produced mainly by inflammatory cells to face invading pathogens from outside. The effect of PGE_2 will trigger apoptosis of macrophages infected with *Mycobacterium tuberculosis* [4]. Macrophage apoptosis will have an elimination effect because *Mycobacterium tuberculosis* bacteria can be destroyed. PGE_2 also suppresses macrophage necrosis which can lead to bacterial dissemination. Increased levels of PGE_2 are associated with a decrease in the number of bacteria in the lung [7].

The negative association between serum PGE_2 levels and the degree of phlegm AFB positivity is by a study conducted by Dietzold and Amaral. Dietzold et al reported that high levels of PGE_2 and low levels of LXA_4 suppress the growth of *Mycobacterium tuberculosis* [7]. Amaral et al. also reported that PGE_2 is associated with macrophage apoptosis in vitro. Apoptotic macrophages infected with *Mycobacterium tuberculosis* will increase the elimination of these bacterias [4]. The two studies above reported a significant association between PGE_2 and the growth of *Mycobacterium tuberculosis*. The statistical analysis results of this study showed that the association between serum PGE_2 levels and the degree of AFB positivity was not statistically significant. The main difference between this study and the two studies above is that both were carried out on mice and in vitro, whereas this study was conducted on pulmonary TB patients with various complications and uncontrollable comorbidities.

The results of this study can be used as consideration for conducting further research on the predictor factors for positivity of AFB in pulmonary TB patients. The use of PGE_2 together with LXA_4 is expected to be able to assist clinicians in predicting the level of AFB positivity in pulmonary TB patients with specific chest X-ray images but difficulty in expectorating phlegm.

Nevertheless, this study has several limitations. First, extreme serum PGE2 levels were found in some research subjects. This can be caused by various factors that can increase PGE_2 levels that cannot be controlled. Second, this study only examined PGE2 levels in TB patients without comparing them with PGE_2 levels in healthy persons, so it cannot be used as a predictor factor for the degree of positivity of AFB with sputum.

Conclusion

The average age of new and relapsed pulmonary TB patients is 43.37 years, mostly male, have a high school education, have a smoking habit, have a low BMI, and have no history of DM. The median serum PGE_2 level of new and relapsed pulmonary TB patients was 216.95 pg/ml. The majority of new pulmonary TB patients have a high degree of positivity for AFB in sputum, but relapsed pulmonary TB patients have a low degree of positivity for AFB. This study finds a weak negative association between serum PGE_2 levels and the degree of phlegm AFB positivity but not statistically significant.

Acknowledgment

We would like to thank the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia for providing support for our study. We would like to thank Fis Citra Ariyanto as our manuscript editor.

Funding

Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Figure Legend

Figure 1. Participant requitement process

Table and Legend

Table 1. Characteristic of participant

Variable	n (%)
Sex	
Male	36 (58.1)
Female	26 (41.9)
Education	
Elementary School	8 (12.9)
Junior High School	12 (19.4)
Senior High School	34 (54.8)
College	7 (11.3)
Not attending school	1 (1.6)
History of Diabetes Mellitus	
Yes	20 (32.3)
No	42 (67.7)
History of Tuberculosis Treatment	
New case	37 (59.7)
Relapse	25 (40.3)
Smoking Habit	
Smoking	35 (56.5)
No smoking	27 (43.5)
Degree of Acid-Fast Bacilli Positivity	
Low	26 (41.9)
High	36 (58.1)
Serum PGE_2 Level	
Low	9 (14.5)
Normal	39 (62.9)
High	14 (22.6)
Body Mass Index	
Skinny (<18.5 kg/m^2)	33 (53.2)
Normal (18.5 – 25.0 kg/m^2)	24 (38.7)
Fat (>25.0 kg/m^2)	5 (8.1)

Table 2. Distribution of Serum PGE_2 Levels in Tuberculosis Patients

Variable	Serum PGE_2 Levels			*p*
	Low	Normal	High	
Pulmonary				
Tuberculosis	6 (16)	23 (62)	8 (22)	0.292
New case	3 (12)	16 (64)	6 (24)	
Relapse case				
Diabetes mellitus				
Yes	4 (20)	9 (45)	7 (35)	0.118
No	5 (12)	30 (71)	7 (17)	
BMI				
Skinny	1 (3)	24 (73)	8 (24)	0.058
Normal	7 (29)	12 (50)	5 (21)	
Fat	1 (20)	3 (60)	1 (20)	
Smoking				

Yes	6 (18)	17 (50)	11 (32)	0.053
No	3 (11)	22 (78)	3 (11)	

Abbreviation: BMI = body mass index

Table 3. Distribution of Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

Variable	Degree of Acid-Fast Bacilli Positivity		*p*
	Low (%)	High	
Pulmonary Tuberculosis			
New case	12 (32)	25 (68)	0.065
Relapse case	14 (56)	11 (44)	
Diabetes Mellitus			
Yes	7 (35)	13 (65)	0.455
No	19 (45)	23 (55)	
BMI			
Skinny	15 (45)	18 (55)	0.561
Normal	10 (42)	14 (58)	
Fat	1 (20)	4 (80)	
Smoking			
Yes	15 (45)	19 (56)	0.798
No	11 (39)	17 (61)	

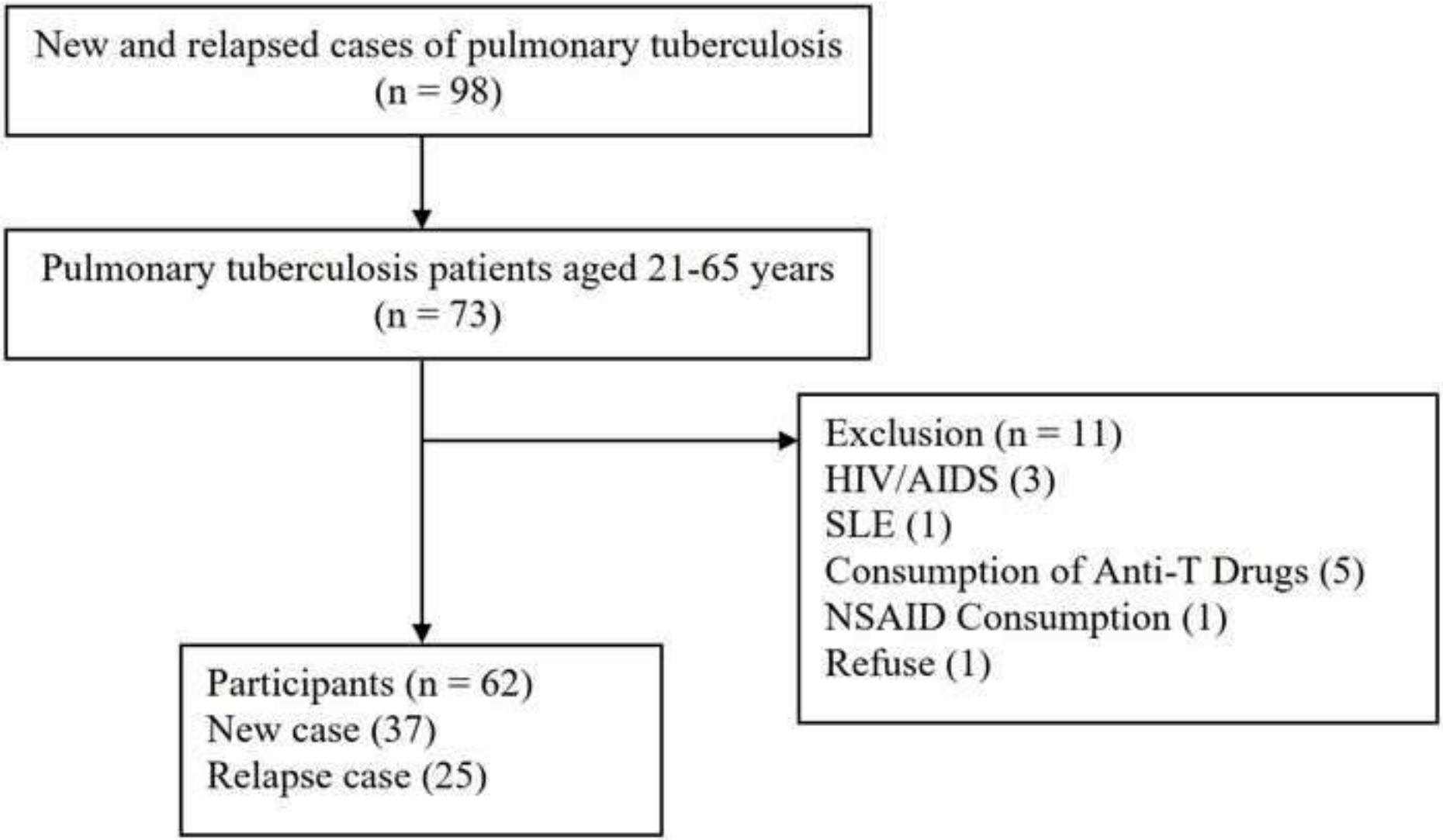
Abbreviation: BMI = body mass index

Table 4. Association between PGE_2 Levels and Positivity of Acid-Fast Bacilli in the Sputum of Tuberculosis Patients

Variable	Tuberculosis Positivity		p^a	*r*	p^b
	Low	High			
PGE_2 Levels					
Low	1 (11)	8 (89)	0.036	-0.036	0.780
Normal	21 (54)	18 (46)			
High	4 (29)	10 (71)			

Note: p^a = Chi-square test; p^b = Spearman's correlation test.

Figure 1



The STROCSS 2019 Guideline		
Item no.	**Item description**	**Page**
TITLE		
1	Title: - The word cohort or cross-sectional or case-controlled is included - The area of focus is described (e.g. disease, exposure/intervention, outcome) - Key elements of study design are stated (e.g. retrospective or prospective)	1
ABSTRACT		
2a	Introduction: the following points are briefly described - Background - Scientific Rationale for this study	1
2b	Methods: the following areas are briefly described - Study design (cohort, retro-/prospective, single/multi-centred) - Patient populations and/or groups, including control group, if applicable - Interventions (type, operators, recipients, timeframes) - Outcome measures	1
2c	Results: the following areas are briefly described - Summary data (with statistical relevance) with qualitative descriptions, where appropriate	1
2d	Conclusion: the following areas are briefly described - Key conclusions - Implications to practice - Direction of and need for future research	1
3	Introduction: the following areas are described in full - Relevant background and scientific rationale - Aims and objectives - Research question and hypotheses, where appropriate	1-2
4a	Registration and ethics - Research Registry number is stated, in accordance with the declaration of Helsinki* - All studies (including retrospective) should be registered before submission *"*Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject*" (this can be obtained from: ResearchRegistry.com or ClinicalTrials.gov or ISRCTN)	9
4b	Ethical Approval: the following areas are described in full - Necessity for ethical approval - Ethical approval, with relevant judgement reference from ethics committees - Where ethics was unnecessary, reasons are provided	9
4c	Protocol: the following areas are described comprehensively - Protocol (*a priori* or otherwise) details, with access directions - If published, journal mentioned with the reference provided	3

4d	Patient Involvement in Research - Describe how, if at all, patients were involved in study design e.g. were they involved on the study steering committee, did they provide input on outcome selection, etc.	3
5a	Study Design: the following areas are described comprehensively - 'Cohort' study is mentioned - Design (e.g. retro-/prospective, single/multi-centred)	3-4
5b	Setting: the following areas are described comprehensively - Geographical location - Nature of institution (e.g. academic/community, public/private) - Dates (recruitment, exposure, follow-up, data collection)	3-4
5c	Cohort Groups: the following areas are described in full - Number of groups - Division of intervention between groups	3
5d	Subgroup Analysis: the following areas are described comprehensively - Planned subgroup analyses - Methods used to examine subgroups and their interactions	3
6a	Participants: the following areas are described comprehensively - Eligibility criteria - Recruitment sources - Length and methods of follow-up	3
6b	Recruitment: the following areas are described comprehensively - Methods of recruitment to each patient group - Period of recruitment	3
6c	Sample Size: the following areas are described comprehensively - Margin of error calculation - Analysis to determine study population - Power calculations, where appropriate	3
7a	Pre-intervention Considerations: the following areas are described comprehensively - Patient optimisation (pre-surgical measures) - Pre-intervention treatment (hypothermia/-volaemia/-tension; ICU care; bleeding problems; medications)	4
7b	Intervention: the following areas are described comprehensively - Type of intervention and reasoning (e.g. pharmacological, surgical, physiotherapy, psychological) - Aim of intervention (preventative/therapeutic) - Concurrent treatments (antibiotics, analgaesia, anti-emetics, NBM, VTE prophylaxis) - Manufacturer and model details where applicable	4
7c	Intra-Intervention Considerations: the following areas are described comprehensively - Administration of intervention (location, surgical details, anaesthetic, positioning, equipment needed, preparation, devices, sutures, operative time) - Pharmacological therapies include formulation, dosages, routes and durations - Figures and other media are used to illustrate	4

7d	Operator Details: the following areas are described comprehensively - Training needed - Learning curve for technique - Specialisation and relevant training	4
7e	Quality Control: the following areas are described comprehensively - Measures taken to reduce variation - Measures taken to ensure quality and consistency in intervention delivery	4
7f	Post-Intervention Considerations: the following areas are described comprehensively - Post-operative instructions and care - Follow-up measures - Future surveillance requirements (e.g. imaging, blood tests)	4
8	Outcomes: the following areas are described comprehensively - Primary outcomes, including validation, where applicable - Definitions of outcomes - Secondary outcomes, where appropriate - Follow-up period for outcome assessment, divided by group	4
9	Statistics: the following areas are described comprehensively - Statistical tests, packages/software used, and interpretation of significance - Confounders and their control, if known - Analysis approach (e.g. intention to treat/per protocol) - Sub-group analysis, if any	4
10a	Participants: the following areas are described comprehensively - Flow of participants (recruitment, non-participation, cross-over and withdrawal, with reasons) - Population demographics (prognostic features, relevant socioeconomic features, and significant numerical differences)	5
10b	Participant Comparison: the following areas are described comprehensively - Table comparing demographics included - Differences, with statistical relevance - Any group matching, with methods	5
10c	Intervention: the following areas are described comprehensively - Changes to interventions, with rationale and diagram, if appropriate - Learning required for interventions - Degree of novelty for intervention	5
11a	Outcomes: the following areas are described comprehensively - Clinician-assessed and patient-reported outcomes for each group - Relevant photographs and imaging are desirable - Confounders to outcomes and which are adjusted	5
11b	Tolerance: the following areas are described comprehensively - Assessment of tolerance - Loss to follow up, with reasons (percentage and fraction) - Cross-over with explanation	5
11c	Complications: the following areas are described comprehensively - Adverse events described - Classified according to Clavien-Dindo classification* - Mitigation for adverse events (blood loss, wound care, revision surgery	5

	should be specified) *Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. A New Proposal with Evaluation in a Cohort of 6336 Patients and Results of a Survey. Ann Surg. 2004; 240(2): 205-213	
12	Key Results: the following areas are described comprehensively - Key results, including relevant raw data - Statistical analyses with significance	5
13	Discussion: the following areas are described comprehensively - Conclusions and rationale - Reference to relevant literature - Implications to clinical practice - Comparison to current gold standard of care - Relevant hypothesis generation	6-8
14	Strengths and Limitations: the following areas are described comprehensively - Strengths of the study - Limitations and potential impact on results - Assessment of bias and management	8
15	Implications and Relevance: the following areas are described comprehensively - Relevance of findings and potential implications to clinical practice are detailed - Future research that is needed is described, with study designs detailed	8
16	Conclusions: - Key conclusions are summarised - Key directions for future research are summarised	8
17a	Conflicts of interest - Conflicts of interest, if any, are described	9
17b	Funding - Sources of funding (e.g. grant details), if any, are clearly stated	9

Annals of Medicine and Surgery

Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-Sectional Study

--Manuscript Draft--

Manuscript Number:	AMSU-D-21-00908R1
Article Type:	Cross-sectional Study
Keywords:	positivity of acid-fast bacilli; Pulmonary tuberculosis; serum PGE2 levels
Corresponding Author:	Resti Yudhawati Universitas Airlangga Fakultas Kedokteran Surabaya, East Java INDONESIA
First Author:	Herley Windo Setiawan
Order of Authors:	Herley Windo Setiawan
	Resti Yudhawati
	Irmi Syafaah
Abstract:	Background : Mycobacterium tuberculosis that infected apoptotic macrophages is triggered by PGE 2 . Apoptosis suppresses the growth of Mycobacterium tuberculosis bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria. Objective : Analyzing the association between serum PGE 2 levels and the positivity of AFB in the sputum of tuberculosis patients. Methods : A cross-sectional study was carried out from August 2019 – July 2020. Serum PGE 2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with p <0.05. Results : The average participants' serum PGE 2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE 2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE 2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p -value = 0.780. Conclusion : There is a weak negative association between serum PGE 2 levels and the degree of AFB positivity in sputum but not statistically significant.
Suggested Reviewers:	Chin-Chung Shu ccshu139@ntu.edu.tw
	Ming-Fang Wu Wu wmf680102@gmail.com
	Chia-Lin Hsu clhsu7@ntu.edu.tw
Response to Reviewers:	we have revised the manuscript according to the reviewer's suggestion

Annals of Medicine and Surgery

The following information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned. If you have nothing to declare in any of these categories then this should be stated.

Please state any conflicts of interest
All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

The authors declare that they have no conflict of interest.

Please state any sources of funding for your research
All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

None.

Ethical Approval

Research studies involving patients require ethical approval. Please state whether approval has been given, name the relevant ethics committee and the state the reference number for their judgement.

We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Consent

Studies on patients or volunteers require ethics committee approval and fully informed written consent which should be documented in the paper.

Authors must obtain written and signed consent to publish a case report from the patient (or, where applicable, the patient's guardian or next of kin) prior to submission. We ask Authors to confirm as part of the submission process that such consent has been obtained, and the manuscript must include a statement to this effect in a consent section at the end of the manuscript, as follows: "Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request".

Patients have a right to privacy. Patients' and volunteers' names, initials, or hospital numbers should not be used. Images of patients or volunteers should not be used unless the information is essential for scientific purposes and explicit permission has been given as part of the consent. If such consent is made subject to any conditions, the Editor in Chief must be made aware of all such conditions.

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Written informed consent was obtained from the patient.

Author contribution

Please specify the contribution of each author to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways should be listed as contributors.

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Registration of Research Studies

In accordance with the Declaration of Helsinki 2013, all research involving human participants has to be registered in a publicly accessible database. Please enter the name of the registry and the unique identifying number (UIN) of your study.

You can register any type of research at http://www.researchregistry.com to obtain your UIN if you have not already registered. This is mandatory for human studies only. Trials and certain observational research can also be registered elsewhere such as: ClinicalTrials.gov or ISRCTN or numerous other registries.

1. Name of the registry: Health Research Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

2. Unique Identifying number or registration ID: 1355/KEKP/VII/2019

3. Hyperlink to your specific registration (must be publicly accessible and will be checked): -

Guarantor

The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish

Resti Yudhawati

To,

Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled "Association between Serum PGE2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-sectional Study" in your esteemed journal as an Original Article.

On behalf of all the contributors, I will act and guarantor and will correspond with the journal from this point onward.

In this paper, I/we report the association between PGE2 and the degree of positivity of acid-fast bacilli (AFB) as a reflection of innate immunity and bacteria to count. This is significant because it would help the clinician in predicting the positivity of AFB sputum in patients with specific chest x-ray imaging but have a difficulty in expectorating sputum. The paper should be of interest to readers in the areas of pulmonology, especially Tuberculosis.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

All authors have approved the manuscript and agree with its submission to the Annals of Medicine and Surgery.

We hereby transfer, assign, or otherwise convey all copyright ownership, including all rights incidental thereto, exclusively to the journal, if such work is published by the journal.

Thanking you,

Yours' sincerely,

Resti Yudhawati
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Padjadjaran – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
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Response to Reviewer

Managing Editor

Please can you make the following changes/checks:

1. Ensure your work is fully compliant with the STROCSS criteria www.strocssguideline.com, which should be cited within the methods section of your article and please submit a completed STROCSS checklist stating the page numbers where you completed each item (your work will be returned if this is not done).

   Please also ensure your methods section states that the work has been reported in line with the STROCSS criteria and cite the paper as follows:

   Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C and Mathew G, for the STROCSS Group. The STROCSS 2019 Guideline: Strengthening the Reporting of Cohort Studies in Surgery. International Journal of Surgery 2019;72:156-165.

   Author response: we have added to the method.

2. Please ensure you submit your work with a Research Registry UIN: e.g. from www.researchregistry.com – it can't progress without being registered – even it its retrospective research. Please ensure you also state your registration unique identifying number (UIN) in your methods section and reference it including a hyperlink to it.

   Author response: we have conducted a research register based on the Declaration of Helsinki at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia with certificate number "1444/KEPK/VIII/2019" and we have added it to the title page.

3. Please go through your paper and proofread it to correct spelling, grammar and syntax errors. If you need our author support services, you can access them here: https://www.ijspg.com/services/author-support

Author response: we have used a professional translator and double-check combines Grammarly.

4. If you haven't already, please include your "highlights" which are 3-5 bullet points summarising the novel aspects and/or learning points (maximum 85 characters, including spaces, per bullet point).

   Author response: We have followed the reviewer suggestions in our highlights.

5. Please add the following statement above references:

   Provenance and peer review

   Not commissioned, externally peer-reviewed

   Author Response: we have added it to our manuscript

Reviewer 4:

1. -As authors know, sputum taking procedure needs to perform in right time -the better is morning- and by skilled person in this regard. Explain more about that how sputum samples were taken.

   Author response: We have added it in our method.

2. Authors included too many variables for analysis. Cigarette smoking itself could change pattern of the disease and plasma factors. Considering various variables in such study with this small sample size may make deep bias in analysis.

   Author response: We have added it in our study limitation.

3. English writing needs grammatical revision.

Author response: we have used a professional translator and double-check combines Grammarly.

Highlight

1. Serum PGE_2 levels of tuberculosis patients tend to be normal even though Acid-Fast bacilli (AFB) values are high.
2. Most of the new and recurrent cases of pulmonary tuberculosis patients had normal PGE_2 levels.
3. Serum PGE_2 levels have a negative association with AFB value.

Association between Serum PGE_2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients: A Cross-sectional Study

Running head: Serum PGE_2 levels and acid-fast bacilli

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Conflict of interest

The authors declare that they have no conflict of interest.

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Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Registration of research studies

Name of the registry: Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Unique identifying number or registration ID: 1355/KEKP/VII/2019.

Hyperlink to your specific registration (must be publicly accessible and will be checked): -.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Association between Serum PGE_2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients

Abstract

Background: *Mycobacterium tuberculosis* that infected apoptotic macrophages is triggered by PGE_2. Apoptosis suppresses the growth of *Mycobacterium tuberculosis* bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria. **Objective**: Analyzing the association between serum PGE_2 levels and the positivity of AFB in the sputum of tuberculosis patients. **Methods**: A cross-sectional study was carried out from August 2019 – July 2020. Serum PGE_2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with $p < 0.05$. **Results**: The average participants' serum PGE_2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE_2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780. **Conclusion**: There is a weak negative association between serum PGE_2 levels and the degree of AFB positivity in sputum but not statistically significant.

Keywords: positivity of acid-fast bacilli, pulmonary tuberculosis, serum PGE_2 levels

Introduction

Tuberculosis (TB) is still a global health problem [1]. The increase in TB cases is accompanied by an increase in drug-resistant TB (DR TB) cases. In the Global Tuberculosis Report, WHO reported that 10 million people were suffering from TB, both new and relapsed

cases, with 558,000 of whom had DR TB [2]. Indonesia ranks third in the country with the highest TB incidence globally, both new and relapse cases. The number of new and relapsed TB cases in Indonesia in 2017 was 442,172, and 54% of them were confirmed bacteriologically by either acid-fast bacilli (AFB) sputum staining or sputum culture [3].

The pathogenesis of TB is an interaction between *Mycobacterium tuberculosis* and the host [4]. The process begins with alveolar macrophages and dendritic cells as the first cells facing *Mycobacterium tuberculosis* bacteria. Macrophages' response as the mainline in dealing with *Mycobacterium tuberculosis* infection is influenced by various inflammatory mediators [5]. The failure of macrophages to control the number of *Mycobacterium tuberculosis* will result in the significant growth of bacteria [6, 7].

This condition emphasizes the important role of the host immune system in determining the susceptibility of TB to relapse. Several studies pointed out that Prostaglandin E_2 (PGE_2) affects macrophages as the main cells in the innate immune system. PGE_2 induces apoptosis and inhibits necrosis of macrophages infected with *Mycobacterium tuberculosis* [5, 8, 9]. Macrophage apoptosis is reported to reduce the growth rate of *Mycobacterium tuberculosis*, which is very important in the elimination mechanism of bacteria that infects the lungs, whereas necrosis plays the opposite role [5, 8, 10]. When the growth of *Mycobacterium tuberculosis* cannot be inhibited, the number of bacteria will increase. The high number of bacterias is reflected in the degree of phlegm AFB positivity. The higher the value of positivity for AFB in sputum, the greater the number of *Mycobacterium tuberculosis* bacteria contained in each ml of sputum [11]. The higher the number of bacterias, the easier it is can transmit, broader lung damage, and an increased risk of resistance [12, 13].

Based on the facts above, this study further revealed the association~~relationship~~ between PGE_2, which represents the innate immune system, and the degree of phlegm AFB positivity, which represents the number of bacterias. This research is important because no

similar study was conducted in humans, so it is hoped that this research could provide further research.

Methods

Participants

Participants in this study were both new and relapsed patients with pulmonary tuberculosis. The inclusion criteria were patients diagnosed with pulmonary tuberculosis [3, 14], positive sputum examination results for AFB, aged 21-65 years, who cooperated during the research procedure. Meanwhile, the exclusion criteria included patients with risk factors for immunocompromised (AIDS, malignancy, and systemic lupus erythematosus), patients having received anti-tuberculosis drug therapy for their current illness, patients taking non-steroidal anti-inflammatory drugs and/or corticosteroids in the past ~~one~~ week.

Ethical Clearance

Participants and their families filled out the consent form before the study. Participants filled out the consent form consciously and without coercion. This study received ethical approval based on the Declaration of Helsinki and obtained the registry of research at the Health Research Ethics Committee in the Hospital.

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A cross-sectional study was carried out from August 2019 – July 2020. The number of participants in this study was 62 patients that were obtained using Ronald Fisher's classic z transformation formula. The sample collection used a consecutive sampling technique (Figure 1). Serum PGE_2 levels and levels of AFB in sputum were taken from the participants.

This study report is by the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15].

Measurement of Serum PGE_2 Level

Serum PGE_2 level is the total concentration of PGE_2 in the blood of pulmonary tuberculosis patients. This examination was carried out by taking 3-5 ml of the patient's venous blood and analyzed using the Elisa Kit PGE_2 (pg/ml). Serum PGE_2 level is categorized into high if the value is more than 400 pg/ml, normal if the value is 200-400 pg/ml, and low if the value is less than 200 pg/ml [16].

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Acid-Fast Bacilli Test

Sputum ~~culture~~ examination was conducted to determine the degree of the participant's AFB positivity. Sputum collection for participants is carried out by the patient independently in the morning [17] which the participant gets an explanation form a pulmonary specialist regarding effective deep breathing and coughing techniques [18]. The sputum is put into a tube that has been prepared previously and then taken to the laboratory for analysis. The examination of AFB in the participant's sputum used the acid-fast staining method (Ziehl Nielssen) or the rapid molecular test of sputum with the GeneXpert machine [19]. The degree of phlegm AFB positivity was assessed based on the International Union Against Tuberculosis Lung Disease (IUATLD) standards which were categorized into 2: low (1+ and scanty) and high (2+ and 3+) [19, 20].

Statistical Analysis

The analysis in this study used descriptive analysis and bivariate analysis. Descriptive analysis included the presentation of the results descriptively using the distribution table,

mean, median, standard deviation, maximum value, and minimum value. Meanwhile, bivariate analysis was used to assess the association between two variables. The association between variables was analyzed using the Chi-Square test and assessed the association strength using the Spearman correlation test. The analysis was declared significant if $p < 0.05$. The analysis was assisted by IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Characteristic of Participant

Most participants were male who was 43.37 ± 12.58 years old. Meanwhile, the median of participants' age was 44.5 years, with the lowest age being 21 years and the highest being 64 years. Some patients had a smoking habit (56.5%) and comorbidity of diabetes mellitus (32.3%). A total of 37 participants were new tuberculosis patients and the rest were relapsed, tuberculosis patients. Most participants had a body mass index (BMI) in the skinny category as much as 53.2% (table 1). The average BMI value was 19.46 ± 4.05 kg/m^2, with a value range of 14.20 – 38.28 kg/m^2.

Distribution of Serum PGE_2 Levels in Tuberculosis Patients

Most participants had normal serum PGE_2 levels (62.9%; Table 1). The average participants had serum PGE_2 levels of 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. The lowest and highest value of the participants' serum PGE_2 levels were 191.00 pg/ml and 2,374.00 pg/ml, respectively. The serum PGE_2 levels of smoking and non-smoking participants was 228.80 (191.0 – 2,3374.0) pg/ml and 214.40 (198.3 – 1,724.0) pg/ml, respectively. Most serum PGE_2 levels of smoking participants were normal (50%), while the serum PGE_2 levels of non-smoking participants were mostly normal (78%; p = 0.053). The

median value of serum PGE_2 levels for participants with and without diabetes mellitus was 217.30 (191.0 – 1,986.0) pg/ml and 216.80 (193.0 – 2,374.0) pg/ml, respectively. The value of serum PGE_2 levels of participants with and without diabetes mellitus were 45% and 71%, respectively, indicating that most participants had normal values (p = 0.118; Table 2).

Most of the participants' serum PGE_2 levels were normal in both groups of participants with a new diagnosis of pulmonary tuberculosis (62%) and relapsed (64%; p = 0.292). The median value of serum PGE_2 levels for participants diagnosed with new pulmonary tuberculosis was 215.70 (191.0 – 1,724.0) pg/ml and participants diagnosed with relapsed pulmonary tuberculosis was 224.40 (193.2 – 2,374.0) pg/ml. Participants' serum PGE_2 levels that were categorized by BMI were mostly normal, with 73% of skinny participants, 50% of normal participants, and 60% of fat participants (p = 0.058; Table 3). The median value of serum PGE_2 levels of participants with BMI in the skinny category was 222.60 (194.3 – 1,986.0) pg/ml, normal was 210.30 (191.0 – 2,374.0) pg/ml, and fatwas 216.40 (199.0 – 1,497.0) pg/ml.

Distribution of Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

Most participants had a high degree of AFB positivity in sputum as much as 58.1% (Table 1). Most participants who were diagnosed with new cases of pulmonary tuberculosis had a high degree of AFB positivity (68%). Meanwhile, most participants diagnosed with relapsed pulmonary tuberculosis had a low positivity degree (56%; p = 0.065). Some participants had a high degree of AFB positivity in participants with and without a history of diabetes mellitus of 65% and 55%, respectively (p = 0.455). Participants' BMI was categorized into 3, namely skinny, normal, and fast, in which some participants had a high degree of AFB positivity (p = 0.561). Most smoking (56%) and non-smoking (61%) participants had high positivity of AFB (p = 0.798; Table 3).

Association between Serum PGE_2 Levels and Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

The results showed that most participants with low (89%) and high (71%) serum PGE_2 levels had a high positivity of AFB in sputum as much as 89%. Meanwhile, participants with normal serum PGE_2 levels had a low positivity degree of AFB in sputum as much as 54% (p = 0.036). The strength of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780 (Table 4).

Discussion

PGE_2 is a derivative of arachidonic acid produced by various inflammatory cells, especially macrophages. PGE_2, as an inflammatory mediator, plays a role in regulating various cell functions, namely macrophages, T cells, etc. In addition, PGE_2 plays a role in various body functions such as blood pressure regulation, temperature regulation, gastric protection, and childbirth [21]. Under various conditions such as changes in environmental temperature, hunger conditions, stress, PGE_2 will be produced so that levels in the body will rise and fall in various ways [22].

Schoenberger et al reported an increase in serum PGE_2 levels in patients with diabetic retinopathy [23]. A study conducted by Lo et al. showed that the increase in serum PGE_2 levels was due to the upregulation of the cyclooxygenase-2 (COX_2) enzyme in patients with diabetes mellitus [24]. Kumar et al. reported differences in plasma PGE_2 levels in TB patients compared to TB-DM [16]. These results are inconsistent with various studies that reported increased levels of PGE_2 in smokers. Amadio et al. reported an increase in PGE_2 production in smokers due to the modulation of expression of tissue factors exposed to cigarette smoke

[25]. Chen et al. in their study also reported the role of cigarette smoke in increasing PGE_2 production [26].

The condition obtained in this study seemed to occur because of the patient's experience factor. In patients with relapse cases, the experience of suffering from TB in the past will make the patient who has a cough immediately come to the health facility. Meanwhile, new case –patients ignore the cough complaint that leads to accompanying complaints such as weight loss, hemoptysis, or fever. When these accompanying complaints occur, the course of TB disease would be long enough to increase the number of bacterias [1].

The profile of serum PGE_2 levels showed that the average participants had 446.23 pg/ml, with a standard deviation of 510.27 pg/ml. According to some literature, normal serum PGE_2 levels range from 200 – 400 pg/ml [16]. PGE_2 is a derivative of arachidonic acid produced mainly by inflammatory cells to face invading pathogens from outside. The effect of PGE_2 will trigger apoptosis of macrophages infected with *Mycobacterium tuberculosis* [4]. Macrophage apoptosis will have an elimination effect because *Mycobacterium tuberculosis* bacteria can be destroyed. PGE_2 also suppresses macrophage necrosis which can lead to bacterial dissemination. Increased levels of PGE_2 are associated with a decrease in the number of bacteria in the lung [7].

The negative association between serum PGE_2 levels and the degree of phlegm AFB positivity is by a study conducted by Dietzold and Amaral. Dietzold et al reported that high levels of PGE_2 and low levels of LXA_4 suppress the growth of *Mycobacterium tuberculosis* [7]. Amaral et al. also reported that PGE_2 is associated with macrophage apoptosis in vitro. Apoptotic macrophages infected with *Mycobacterium tuberculosis* will increase the elimination of these bacterias [4]. The two studies above reported a significant association between PGE_2 and the growth of *Mycobacterium tuberculosis*. The statistical analysis results of this study showed that the association between serum PGE_2 levels and the degree of AFB

positivity was not statistically significant. The main difference between this study and the two studies above is that both were carried out on mice and in vitro, whereas this study was conducted on pulmonary TB patients with various complications and uncontrollable comorbidities.

The results of this study can be used as consideration for conducting further research on the predictor factors for the positivity of AFB in pulmonary TB patients. The use of PGE_2 together with LXA_4 is expected to be able to assist clinicians in predicting the level of AFB positivity in pulmonary TB patients with specific chest X-ray images but difficulty in expectorating phlegm. In addition, in the future study it can be considered to analyze the comparison of PGE_2 in TB patients, smokers patients, smokers with tuberculosis, etc.

Nevertheless, this study has several limitations. First, extreme serum PGE_2 levels were found in some research subjects. This can be caused by various factors that can increase PGE_2 levels that cannot be controlled. Second, this study only examined PGE_2 levels in TB patients without comparing them with PGE_2 levels in healthy persons, so it cannot be used as a predictor factor for the degree of positivity of AFB with sputum.

Conclusion

The average age of new and relapsed pulmonary TB patients is 43.37 years, mostly male, have a high school education, have a smoking habit, have a low BMI, and have no history of DM. The median serum PGE_2 level of new and relapsed pulmonary TB patients was 216.95 pg/ml. The majority of new pulmonary TB patients have a high degree of positivity for AFB in sputum, but relapsed pulmonary TB patients have a low degree of positivity for AFB. This study finds a weak negative association between serum PGE_2 levels and the degree of phlegm AFB positivity but not statistically significant.

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Figure Legend

Figure 1. Participant requitement process

Association between Serum PGE_2 Levels and Degree of Acid-Fast Bacilli Positivity in Sputum of Pulmonary Tuberculosis Patients

Abstract

Background: *Mycobacterium tuberculosis* that infected apoptotic macrophages is triggered by PGE_2. Apoptosis suppresses the growth of *Mycobacterium tuberculosis* bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria. **Objective**: Analyzing the association between serum PGE_2 levels and the positivity of AFB in the sputum of tuberculosis patients. **Methods**: A cross-sectional study was carried out from August 2019 – July 2020. Serum PGE_2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with $p < 0.05$. **Results**: The average participants' serum PGE_2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE_2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780. **Conclusion**: There is a weak negative association between serum PGE_2 levels and the degree of AFB positivity in sputum but not statistically significant.

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The results showed that most participants with low (89%) and high (71%) serum PGE_2 levels had a high positivity of AFB in sputum as much as 89%. Meanwhile, participants with normal serum PGE_2 levels had a low positivity degree of AFB in sputum as much as 54% (p = 0.036). The strength of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained r = -0.036 and p-value = 0.780 (Table 4).

Discussion

PGE_2 is a derivative of arachidonic acid produced by various inflammatory cells, especially macrophages. PGE_2, as an inflammatory mediator, plays a role in regulating various cell functions, namely macrophages, T cells, etc. In addition, PGE_2 plays a role in various body functions such as blood pressure regulation, temperature regulation, gastric protection, and childbirth [21]. Under various conditions such as changes in environmental temperature, hunger conditions, stress, PGE_2 will be produced so that levels in the body will rise and fall in various ways [22].

Schoenberger et al reported an increase in serum PGE_2 levels in patients with diabetic retinopathy [23]. A study conducted by Lo et al. showed that the increase in serum PGE_2 levels was due to the upregulation of the cyclooxygenase-2 (COX_2) enzyme in patients with diabetes mellitus [24]. Kumar et al. reported differences in plasma PGE_2 levels in TB patients compared to TB-DM [16]. These results are inconsistent with various studies that reported increased levels of PGE_2 in smokers. Amadio et al. reported an increase in PGE_2 production in smokers due to the modulation of expression of tissue factors exposed to cigarette smoke

[25]. Chen et al. in their study also reported the role of cigarette smoke in increasing PGE_2 production [26].

The condition obtained in this study seemed to occur because of the patient's experience factor. In patients with relapse cases, the experience of suffering from TB in the past will make the patient who has a cough immediately come to the health facility. Meanwhile, new case patients ignore the cough complaint that leads to accompanying complaints such as weight loss, hemoptysis, or fever. When these accompanying complaints occur, the course of TB disease would be long enough to increase the number of bacterias [1].

The profile of serum PGE_2 levels showed that the average participants had 446.23 pg/ml, with a standard deviation of 510.27 pg/ml. According to some literature, normal serum PGE_2 levels range from 200 – 400 pg/ml [16]. PGE_2 is a derivative of arachidonic acid produced mainly by inflammatory cells to face invading pathogens from outside. The effect of PGE_2 will trigger apoptosis of macrophages infected with *Mycobacterium tuberculosis* [4]. Macrophage apoptosis will have an elimination effect because *Mycobacterium tuberculosis* bacteria can be destroyed. PGE_2 also suppresses macrophage necrosis which can lead to bacterial dissemination. Increased levels of PGE_2 are associated with a decrease in the number of bacteria in the lung [7].

The negative association between serum PGE_2 levels and the degree of phlegm AFB positivity is by a study conducted by Dietzold and Amaral. Dietzold et al reported that high levels of PGE_2 and low levels of LXA_4 suppress the growth of *Mycobacterium tuberculosis* [7]. Amaral et al. also reported that PGE_2 is associated with macrophage apoptosis in vitro. Apoptotic macrophages infected with *Mycobacterium tuberculosis* will increase the elimination of these bacterias [4]. The two studies above reported a significant association between PGE_2 and the growth of *Mycobacterium tuberculosis*. The statistical analysis results of this study showed that the association between serum PGE_2 levels and the degree of AFB

positivity was not statistically significant. The main difference between this study and the two studies above is that both were carried out on mice and in vitro, whereas this study was conducted on pulmonary TB patients with various complications and uncontrollable comorbidities.

The results of this study can be used as consideration for conducting further research on the predictor factors for the positivity of AFB in pulmonary TB patients. The use of PGE_2 together with LXA_4 is expected to be able to assist clinicians in predicting the level of AFB positivity in pulmonary TB patients with specific chest X-ray images but difficulty in expectorating phlegm. In addition, in the future study it can be considered to analyze the comparison of PGE_2 in TB patients, smokers patients, smokers with tuberculosis, etc.

Nevertheless, this study has several limitations. First, extreme serum PGE_2 levels were found in some research subjects. This can be caused by various factors that can increase PGE_2 levels that cannot be controlled. Second, this study only examined PGE_2 levels in TB patients without comparing them with PGE_2 levels in healthy persons, so it cannot be used as a predictor factor for the degree of positivity of AFB with sputum.

Conclusion

The average age of new and relapsed pulmonary TB patients is 43.37 years, mostly male, have a high school education, have a smoking habit, have a low BMI, and have no history of DM. The median serum PGE_2 level of new and relapsed pulmonary TB patients was 216.95 pg/ml. The majority of new pulmonary TB patients have a high degree of positivity for AFB in sputum, but relapsed pulmonary TB patients have a low degree of positivity for AFB. This study finds a weak negative association between serum PGE_2 levels and the degree of phlegm AFB positivity but not statistically significant.

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Figure Legend

Figure 1. Participant requitement process

Table and Legend

Table 1. Characteristic of participant

Variable	n (%)
Sex	
Male	36 (58.1)
Female	26 (41.9)
Education	
Elementary School	8 (12.9)
Junior High School	12 (19.4)
Senior High School	34 (54.8)
College	7 (11.3)
Not attending school	1 (1.6)
History of Diabetes Mellitus	
Yes	20 (32.3)
No	42 (67.7)
History of Tuberculosis Treatment	
New case	37 (59.7)
Relapse	25 (40.3)
Smoking Habit	
Smoking	35 (56.5)
No smoking	27 (43.5)
Degree of Acid-Fast Bacilli Positivity	
Low	26 (41.9)
High	36 (58.1)
Serum PGE_2 Level	
Low	9 (14.5)
Normal	39 (62.9)
High	14 (22.6)
Body Mass Index	
Skinny (<18.5 kg/m^2)	33 (53.2)
Normal (18.5 – 25.0 kg/m^2)	24 (38.7)
Fat (>25.0 kg/m^2)	5 (8.1)

Table 2. Distribution of Serum PGE_2 Levels in Tuberculosis Patients

Variable	Serum PGE_2 Levels			*p*
	Low	Normal	High	
Pulmonary				
Tuberculosis	6 (16)	23 (62)	8 (22)	0.292
New case	3 (12)	16 (64)	6 (24)	
Relapse case				
Diabetes mellitus				
Yes	4 (20)	9 (45)	7 (35)	0.118
No	5 (12)	30 (71)	7 (17)	
BMI				
Skinny	1 (3)	24 (73)	8 (24)	0.058
Normal	7 (29)	12 (50)	5 (21)	
Fat	1 (20)	3 (60)	1 (20)	
Smoking				

Yes	6 (18)	17 (50)	11 (32)	0.053
No	3 (11)	22 (78)	3 (11)	

Abbreviation: BMI = body mass index

Table 3. Distribution of Positivity of Acid-Fast Bacilli in Sputum of Tuberculosis Patients

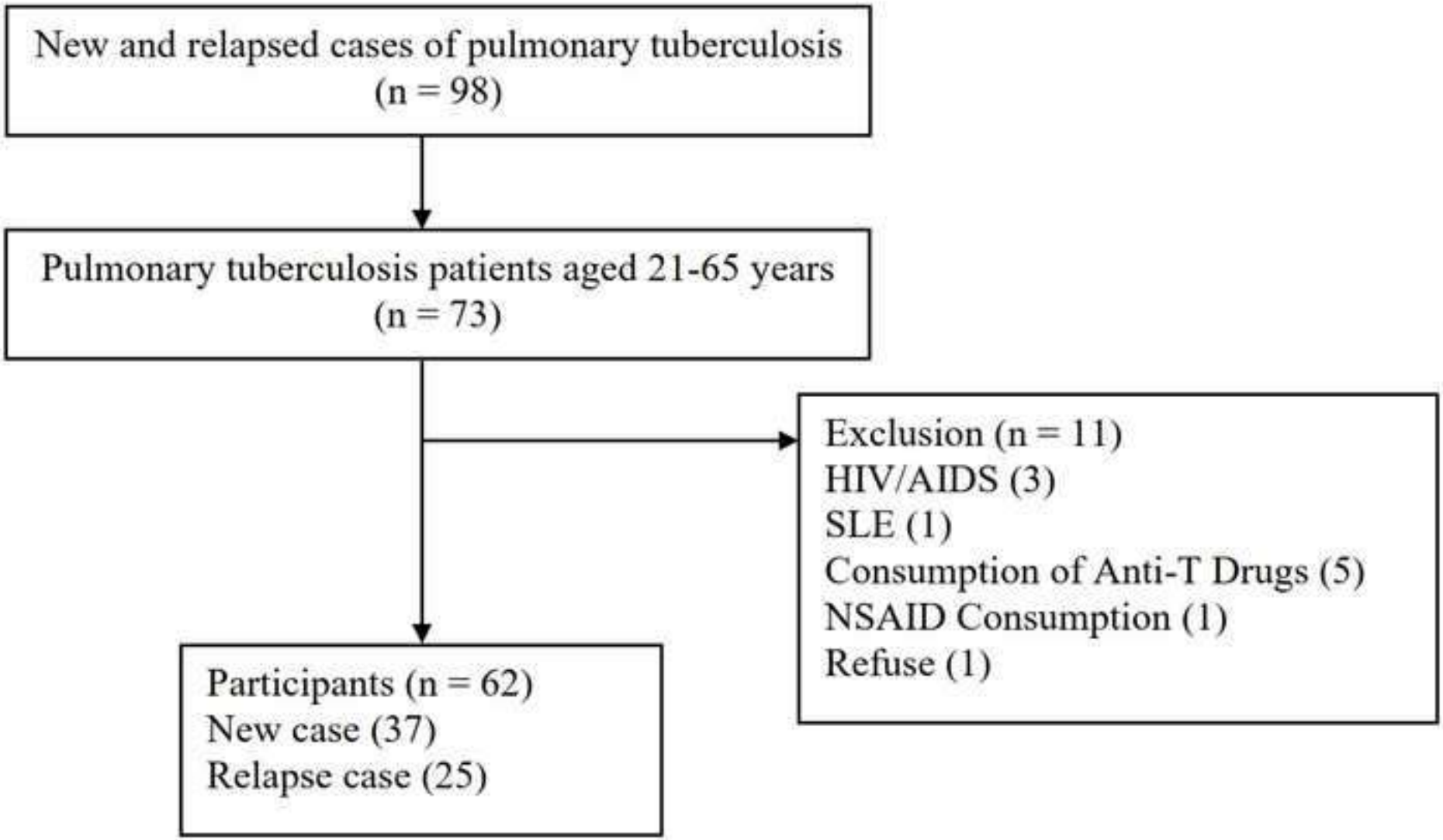
Variable	Degree of Acid-Fast Bacilli Positivity		*p*
	Low (%)	High	
Pulmonary Tuberculosis			
New case	12 (32)	25 (68)	0.065
Relapse case	14 (56)	11 (44)	
Diabetes Mellitus			
Yes	7 (35)	13 (65)	0.455
No	19 (45)	23 (55)	
BMI			
Skinny	15 (45)	18 (55)	0.561
Normal	10 (42)	14 (58)	
Fat	1 (20)	4 (80)	
Smoking			
Yes	15 (45)	19 (56)	0.798
No	11 (39)	17 (61)	

Abbreviation: BMI = body mass index

Table 4. Association between PGE_2 Levels and Positivity of Acid-Fast Bacilli in the Sputum of Tuberculosis Patients

Variable	Tuberculosis Positivity		p^a	*r*	p^b
	Low	High			
PGE_2 Levels					
Low	1 (11)	8 (89)	0.036	-0.036	0.780
Normal	21 (54)	18 (46)			
High	4 (29)	10 (71)			

Note: p^a = Chi-square test; p^b = Spearman's correlation test.



The STROCSS 2019 Guideline		
Item no.	**Item description**	**Page**
TITLE		
1	Title: - The word cohort or cross-sectional or case-controlled is included - The area of focus is described (e.g. disease, exposure/intervention, outcome) - Key elements of study design are stated (e.g. retrospective or prospective)	1
ABSTRACT		
2a	Introduction: the following points are briefly described - Background - Scientific Rationale for this study	1
2b	Methods: the following areas are briefly described - Study design (cohort, retro-/prospective, single/multi-centred) - Patient populations and/or groups, including control group, if applicable - Interventions (type, operators, recipients, timeframes) - Outcome measures	1
2c	Results: the following areas are briefly described - Summary data (with statistical relevance) with qualitative descriptions, where appropriate	1
2d	Conclusion: the following areas are briefly described - Key conclusions - Implications to practice - Direction of and need for future research	1
3	Introduction: the following areas are described in full - Relevant background and scientific rationale - Aims and objectives - Research question and hypotheses, where appropriate	1-2
4a	Registration and ethics - Research Registry number is stated, in accordance with the declaration of Helsinki* - All studies (including retrospective) should be registered before submission *"*Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject*" (this can be obtained from: ResearchRegistry.com or ClinicalTrials.gov or ISRCTN)	9
4b	Ethical Approval: the following areas are described in full - Necessity for ethical approval - Ethical approval, with relevant judgement reference from ethics committees - Where ethics was unnecessary, reasons are provided	9
4c	Protocol: the following areas are described comprehensively - Protocol (*a priori* or otherwise) details, with access directions - If published, journal mentioned with the reference provided	3

4d	Patient Involvement in Research - Describe how, if at all, patients were involved in study design e.g. were they involved on the study steering committee, did they provide input on outcome selection, etc.	3
5a	Study Design: the following areas are described comprehensively - 'Cohort' study is mentioned - Design (e.g. retro-/prospective, single/multi-centred)	3-4
5b	Setting: the following areas are described comprehensively - Geographical location - Nature of institution (e.g. academic/community, public/private) - Dates (recruitment, exposure, follow-up, data collection)	3-4
5c	Cohort Groups: the following areas are described in full - Number of groups - Division of intervention between groups	3
5d	Subgroup Analysis: the following areas are described comprehensively - Planned subgroup analyses - Methods used to examine subgroups and their interactions	3
6a	Participants: the following areas are described comprehensively - Eligibility criteria - Recruitment sources - Length and methods of follow-up	3
6b	Recruitment: the following areas are described comprehensively - Methods of recruitment to each patient group - Period of recruitment	3
6c	Sample Size: the following areas are described comprehensively - Margin of error calculation - Analysis to determine study population - Power calculations, where appropriate	3
7a	Pre-intervention Considerations: the following areas are described comprehensively - Patient optimisation (pre-surgical measures) - Pre-intervention treatment (hypothermia/-volaemia/-tension; ICU care; bleeding problems; medications)	4
7b	Intervention: the following areas are described comprehensively - Type of intervention and reasoning (e.g. pharmacological, surgical, physiotherapy, psychological) - Aim of intervention (preventative/therapeutic) - Concurrent treatments (antibiotics, analgaesia, anti-emetics, NBM, VTE prophylaxis) - Manufacturer and model details where applicable	4
7c	Intra-Intervention Considerations: the following areas are described comprehensively - Administration of intervention (location, surgical details, anaesthetic, positioning, equipment needed, preparation, devices, sutures, operative time) - Pharmacological therapies include formulation, dosages, routes and durations - Figures and other media are used to illustrate	4

7d	Operator Details: the following areas are described comprehensively - Training needed - Learning curve for technique - Specialisation and relevant training	4
7e	Quality Control: the following areas are described comprehensively - Measures taken to reduce variation - Measures taken to ensure quality and consistency in intervention delivery	4
7f	Post-Intervention Considerations: the following areas are described comprehensively - Post-operative instructions and care - Follow-up measures - Future surveillance requirements (e.g. imaging, blood tests)	4
8	Outcomes: the following areas are described comprehensively - Primary outcomes, including validation, where applicable - Definitions of outcomes - Secondary outcomes, where appropriate - Follow-up period for outcome assessment, divided by group	4
9	Statistics: the following areas are described comprehensively - Statistical tests, packages/software used, and interpretation of significance - Confounders and their control, if known - Analysis approach (e.g. intention to treat/per protocol) - Sub-group analysis, if any	4
10a	Participants: the following areas are described comprehensively - Flow of participants (recruitment, non-participation, cross-over and withdrawal, with reasons) - Population demographics (prognostic features, relevant socioeconomic features, and significant numerical differences)	5
10b	Participant Comparison: the following areas are described comprehensively - Table comparing demographics included - Differences, with statistical relevance - Any group matching, with methods	5
10c	Intervention: the following areas are described comprehensively - Changes to interventions, with rationale and diagram, if appropriate - Learning required for interventions - Degree of novelty for intervention	5
11a	Outcomes: the following areas are described comprehensively - Clinician-assessed and patient-reported outcomes for each group - Relevant photographs and imaging are desirable - Confounders to outcomes and which are adjusted	5
11b	Tolerance: the following areas are described comprehensively - Assessment of tolerance - Loss to follow up, with reasons (percentage and fraction) - Cross-over with explanation	5
11c	Complications: the following areas are described comprehensively - Adverse events described - Classified according to Clavien-Dindo classification* - Mitigation for adverse events (blood loss, wound care, revision surgery	5

	should be specified) *Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. A New Proposal with Evaluation in a Cohort of 6336 Patients and Results of a Survey. Ann Surg. 2004; 240(2): 205-213	
12	Key Results: the following areas are described comprehensively - Key results, including relevant raw data - Statistical analyses with significance	5
13	Discussion: the following areas are described comprehensively - Conclusions and rationale - Reference to relevant literature - Implications to clinical practice - Comparison to current gold standard of care - Relevant hypothesis generation	6-8
14	Strengths and Limitations: the following areas are described comprehensively - Strengths of the study - Limitations and potential impact on results - Assessment of bias and management	8
15	Implications and Relevance: the following areas are described comprehensively - Relevance of findings and potential implications to clinical practice are detailed - Future research that is needed is described, with study designs detailed	8
16	Conclusions: - Key conclusions are summarised - Key directions for future research are summarised	8
17a	Conflicts of interest - Conflicts of interest, if any, are described	9
17b	Funding - Sources of funding (e.g. grant details), if any, are clearly stated	9

Cross-sectional Study

Association between serum PGE_2 levels and degree of acid-fast bacilli positivity in sputum of pulmonary tuberculosis patients

Q5 The corrections made in this section will be reviewed and approved by a journal production editor.

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Abstract

***Background*:** *Mycobacterium tuberculosis* that infected apoptotic macrophages is triggered by PGE_2. Apoptosis suppresses the growth of *Mycobacterium tuberculosis* bacteria, which is shown in the results of acid-fast bacilli (AFB) in the sputum that becomes a marker of the number of bacteria.

***Objective*:** Analyzing the association between serum PGE_2 levels and the positivity of AFB in the sputum of tuberculosis patients.

***Methods*:** A cross-sectional study was carried out from August 2019–July 2020. Serum PGE_2 levels and AFB levels in sputum were collected from participants. Data analysis used the Chi-square test and Spearman's correlation with $p < 0.05$.

***Results*:** The average participants' serum PGE_2 levels were 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. Most participants had normal serum PGE_2 levels (62.9%). Most participants had a high positivity of AFB in sputum (58.1%). Analysis of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained $r = -0.036$ and p-value = 0.780.

***Conclusion*:** There is a weak negative association between serum PGE_2 levels and the degree of AFB positivity in sputum but not statistically significant.

Keywords:

Positivity of acid-fast bacilli, Pulmonary tuberculosis, Serum PGE_2 levels

Abbreviations

No keyword abbreviations are available

1 Introduction

Tuberculosis (TB) is still a global health problem [1]. The increase in TB cases is accompanied by an increase in drug-resistant TB (DR TB) cases. In the Global Tuberculosis Report, WHO reported that 10 million people were suffering from TB, both new and relapsed cases, with 558,000 of whom had DR TB [2]. Indonesia ranks third in the country with the highest TB incidence globally, both new and relapse cases. The number of new and relapsed TB cases in Indonesia in 2017 was 442,172, and 54% of them were confirmed bacteriologically by either acid-fast bacilli (AFB) sputum staining or sputum culture [3].

The pathogenesis of TB is an interaction between *Mycobacterium tuberculosis* and the host [4]. The process begins with alveolar macrophages and dendritic cells as the first cells facing *Mycobacterium tuberculosis* bacteria. Macrophages' response as the mainline in dealing with *Mycobacterium tuberculosis* infection is influenced by various inflammatory mediators [5]. The failure of macrophages to control the number of *Mycobacterium tuberculosis* will result in the significant growth of bacteria [6,7].

This condition emphasizes the important role of the host immune system in determining the susceptibility of TB to relapse. Several studies pointed out that Prostaglandin E_2 (PGE_2) affects macrophages as the main cells in the innate immune system. PGE_2 induces apoptosis and inhibits necrosis of macrophages infected with *Mycobacterium tuberculosis* [5,8,9]. Macrophage apoptosis is reported to reduce the growth rate of *Mycobacterium tuberculosis,* which is very important in the elimination mechanism of bacteria that infects the lungs, whereas necrosis plays the opposite role [5,8,10]. When the growth of *Mycobacterium tuberculosis* cannot be inhibited, the number of bacteria will increase. The high number of bacterias is reflected in the degree of phlegm AFB positivity. The higher the value of positivity for AFB in sputum, the greater the number of *Mycobacterium tuberculosis* bacteria contained in each ml of sputum [11]. The higher the number of bacterias, the easier it is can transmit, broader lung damage, and an increased risk of resistance [12,13].

Based on the facts above, this study further revealed the association between PGE_2, which represents the innate immune system, and the degree of phlegm AFB positivity, which represents the number of bacterias. This research is important because no similar study was conducted in humans, so it is hoped that this research could provide further research.

2 Methods

2.1 Participants

Participants in this study were both new and relapsed patients with pulmonary tuberculosis. The inclusion criteria were patients diagnosed with pulmonary tuberculosis [3,14], positive sputum examination results for AFB, aged 21–65 years, who cooperated during the research procedure. Meanwhile, the exclusion criteria included patients with risk factors for immunocompromised (AIDS, malignancy, and systemic lupus erythematosus), patients having received anti-tuberculosis drug therapy for their current illness, patients taking non-steroidal anti-inflammatory drugs and/or corticosteroids in the past week.

2.2 Ethical clearance

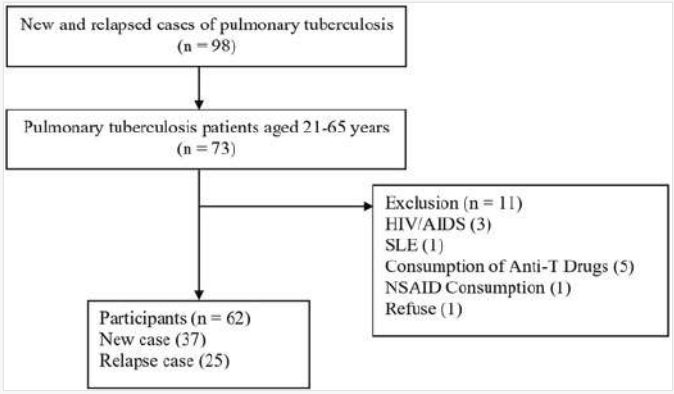
Participants and their families filled out the consent form before the study. Participants filled out the consent form consciously and without coercion. This study received ethical approval based on the Declaration of Helsinki and obtained the registry of research at the Health Research Ethics Committee in the Hospital.

2.3 Study design

A cross-sectional study was carried out from August 2019–July 2020. The number of participants in this study was 62 patients that were obtained using Ronald Fisher's classic z transformation formula. The sample collection used a consecutive sampling technique (Fig. 1). Serum PGE_2 levels and levels of AFB in sputum were taken from the participants. This study report is by the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15].

alt-text: Fig. 1

Fig. 1



Participant requitement process.

2.4 Measurement of serum PGE_2 level

Serum PGE_2 level is the total concentration of PGE_2 in the blood of pulmonary tuberculosis patients. This examination was carried out by taking 3–5 ml of the patient's venous blood and analyzed using the Elisa Kit PGE_2 (pg/ml). Serum PGE_2 level is categorized into high if the value is more than 400 pg/ml, normal if the value is 200–400 pg/ml, and low if the value is less than 200 pg/ml [16].

2.5 Acid-fast bacilli test

Sputum examination was conducted to determine the degree of the participant's AFB positivity. Sputum collection for participants is carried out by the patient independently in the morning [17] which the participant gets an explanation form a pulmonary specialist regarding effective deep breathing and coughing techniques [18]. The sputum is put into a tube that has been prepared previously and then taken to the laboratory for analysis. The examination of AFB in the participant's sputum used the acid-fast staining method (Ziehl Nielssen) or the rapid molecular test of sputum with the GeneXpert machine [19]. The degree of phlegm AFB positivity was assessed based on the International Union Against Tuberculosis Lung Disease (IUATLD) standards which were categorized into 2: low (1+ and scanty) and high (2+ and 3+) [19,20].

2.6 Statistical analysis

The analysis in this study used descriptive analysis and bivariate analysis. Descriptive analysis included the presentation of the results descriptively using the distribution table, mean, median, standard deviation, maximum value, and minimum value. Meanwhile, bivariate analysis was used to assess the association between two variables. The association between variables was analyzed using the Chi-Square test and assessed the association strength using the Spearman correlation test. The analysis was declared significant if $p < 0.05$. The analysis was assisted by IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Characteristic of participant

Most participants were male who was 43.37 ± 12.58 years old. Meanwhile, the median of participants' age was 44.5 years, with the lowest age being 21 years and the highest being 64 years. Some patients had a smoking habit (56.5%) and comorbidity of diabetes mellitus (32.3%). A total of 37 participants were new tuberculosis patients and the rest were relapsed, tuberculosis patients. Most participants had a body mass index (BMI) in the skinny category as much as 53.2% (Table 1). The average BMI value was 19.46 ± 4.05 kg/m^2, with a value range of 14.20–38.28 kg/m^2.

alt-text: Table 1

Table 1

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Characteristic of participant.

Variable	n (%)
Sex	
Male	36 (58.1)

Female	26 (41.9)
Education	
Elementary School	8 (12.9)
Junior High School	12 (19.4)
Senior High School	34 (54.8)
College	7 (11.3)
Not attending school	1 (1.6)
History of Diabetes Mellitus	
Yes	20 (32.3)
No	42 (67.7)
History of Tuberculosis Treatment	
New case	37 (59.7)
Relapse	25 (40.3)
Smoking Habit	
Smoking	35 (56.5)
No smoking	27 (43.5)
Degree of Acid-Fast Bacilli Positivity	
Low	26 (41.9)
High	36 (58.1)
Serum PGE_2 Level	
Low	9 (14.5)
Normal	39 (62.9)
High	14 (22.6)
Body Mass Index	
Skinny (<18.5 kg/m^2)	33 (53.2)
Normal (18.5–25.0 kg/m^2)	24 (38.7)
Fat (>25.0 kg/m^2)	5 (8.1)

3.2 Distribution of serum PGE_2 levels in tuberculosis patients

Most participants had normal serum PGE_2 levels (62.9%; Table 1). The average participants had serum PGE_2 levels of 446.37 ± 510.27 pg/ml, with a median value of 216.95 pg/ml. The lowest and highest value of the participants' serum PGE_2 levels were 191.00 pg/ml and 2374.00 pg/ml, respectively. The serum PGE_2 levels of smoking and non-smoking participants was 228.80 (191.0–2,3374.0) pg/ml and 214.40 (198.3–1724.0) pg/ml, respectively. Most serum PGE_2 levels of smoking participants were normal (50%), while the serum PGE_2 levels of non-smoking participants were mostly normal (78%; p = 0.053). The median value of serum PGE_2 levels for participants with and without diabetes mellitus was 217.30 (191.0–1986.0) pg/ml and 216.80 (193.0–2374.0) pg/ml, respectively. The value of serum PGE_2 levels of participants with and without diabetes mellitus were 45% and 71%, respectively, indicating that most participants had normal values (p = 0.118; Table 2).

alt-text: Table 2

Table 2

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Distribution of serum PGE_2 levels in tuberculosis patients.

Variable	Serum PGE_2 Levels			p
	Low	Normal	High	
Pulmonary Tuberculosis				

New case	6 (16)	23 (62)	8 (22)	0.292
Relapse case	3 (12)	16 (64)	6 (24)	
Diabetes mellitus				
Yes	4 (20)	9 (45)	7 (35)	0.118
No	5 (12)	30 (71)	7 (17)	
BMI				
Skinny	1 (3)	24 (73)	8 (24)	0.058
Normal	7 (29)	12 (50)	5 (21)	
Fat	1 (20)	3 (60)	1 (20)	
Smoking				
Yes	6 (18)	17 (50)	11 (32)	0.053
No	3 (11)	22 (78)	3 (11)	

Abbreviation: BMI = body mass index.

Most of the participants' serum PGE_2 levels were normal in both groups of participants with a new diagnosis of pulmonary tuberculosis (62%) and relapsed (64%; $p = 0.292$). The median value of serum PGE_2 levels for participants diagnosed with new pulmonary tuberculosis was 215.70 (191.0–1724.0) pg/ml and participants diagnosed with relapsed pulmonary tuberculosis was 224.40 (193.2–2374.0) pg/ml. Participants' serum PGE_2 levels that were categorized by BMI were mostly normal, with 73% of skinny participants, 50% of normal participants, and 60% of fat participants ($p = 0.058$; Table 3). The median value of serum PGE_2 levels of participants with BMI in the skinny category was 222.60 (194.3–1986.0) pg/ml, normal was 210.30 (191.0–2374.0) pg/ml, and fatwas 216.40 (199.0–1497.0) pg/ml.

alt-text: Table 3

Table 3

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Distribution of positivity of acid-fast bacilli in sputum of tuberculosis patients.

Variable	Degree of Acid-Fast Bacilli Positivity		*p*
	Low (%)	High	
Pulmonary Tuberculosis			
New case	12 (32)	25 (68)	0.065
Relapse case	14 (56)	11 (44)	
Diabetes Mellitus			
Yes	7 (35)	13 (65)	0.455
No	19 (45)	23 (55)	
BMI			
Skinny	15 (45)	18 (55)	0.561
Normal	10 (42)	14 (58)	
Fat	1 (20)	4 (80)	
Smoking			
Yes	15 (45)	19 (56)	0.798
No	11 (39)	17 (61)	

Abbreviation: BMI = body mass index.

3.3 Distribution of positivity of acid-fast bacilli in sputum of tuberculosis patients

Most participants had a high degree of AFB positivity in sputum as much as 58.1% (Table 1). Most participants who were diagnosed with new cases of pulmonary tuberculosis had a high degree of AFB positivity (68%). Meanwhile,

most participants diagnosed with relapsed pulmonary tuberculosis had a low positivity degree (56%; $p = 0.065$). Some participants had a high degree of AFB positivity in participants with and without a history of diabetes mellitus of 65% and 55%, respectively ($p = 0.455$). Participants' BMI was categorized into 3, namely skinny, normal, and fast, in which some participants had a high degree of AFB positivity ($p = 0.561$). Most smoking (56%) and non-smoking (61%) participants had high positivity of AFB ($p = 0.798$; Table 3).

4 Association between serum PGE_2 levels and positivity of acid-fast bacilli in sputum of tuberculosis patients

The results showed that most participants with low (89%) and high (71%) serum PGE_2 levels had a high positivity of AFB in sputum as much as 89%. Meanwhile, participants with normal serum PGE_2 levels had a low positivity degree of AFB in sputum as much as 54% ($p = 0.036$). The strength of the association between serum PGE_2 levels and the degree of AFB positivity in sputum obtained $r = -0.036$ and p-value = 0.780 (Table 4).

alt-text: Table 4

Table 4

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Association between PGE_2 levels and positivity of acid-fast bacilli in the sputum of tuberculosis patients.

Variable	Tuberculosis Positivity		p^a	r	p^b
	Low	High			
PGE_2 Levels					
Low	1 (11)	8 (89)	0.036	−0.036	0.780
Normal	21 (54)	18 (46)			
High	4 (29)	10 (71)			

Note: p^a = Chi-square test; p^b = Spearman's correlation test.

5 Discussion

PGE_2 is a derivative of arachidonic acid produced by various inflammatory cells, especially macrophages. PGE_2, as an inflammatory mediator, plays a role in regulating various cell functions, namely macrophages, T cells, etc. In addition, PGE_2 plays a role in various body functions such as blood pressure regulation, temperature regulation, gastric protection, and childbirth [21]. Under various conditions such as changes in environmental temperature, hunger conditions, stress, PGE_2 will be produced so that levels in the body will rise and fall in various ways [22].

Schoenberger et al. reported an increase in serum PGE_2 levels in patients with diabetic retinopathy [23]. A study conducted by Lo et al. showed that the increase in serum PGE_2 levels was due to the upregulation of the cyclooxygenase-2 (COX_2) enzyme in patients with diabetes mellitus [24]. Kumar et al. reported differences in plasma PGE_2 levels in TB patients compared to TB-DM [16]. These results are inconsistent with various studies that reported increased levels of PGE_2 in smokers. Amadio et al. reported an increase in PGE_2 production in smokers due to the modulation of expression of tissue factors exposed to cigarette smoke [25]. Chen et al. in their study also reported the role of cigarette smoke in increasing PGE_2 production [26].

The condition obtained in this study seemed to occur because of the patient's experience factor. In patients with relapse cases, the experience of suffering from TB in the past will make the patient who has a cough immediately come to the health facility. Meanwhile, new case patients ignore the cough complaint that leads to accompanying complaints such as weight loss, hemoptysis, or fever. When these accompanying complaints occur, the course of TB disease would be long enough to increase the number of bacterias [1].

The profile of serum PGE_2 levels showed that the average participants had 446.23 pg/ml, with a standard deviation of 510.27 pg/ml. According to some literature, normal serum PGE_2 levels range from 200 to 400 pg/ml [16]. PGE_2 is a derivative of arachidonic acid produced mainly by inflammatory cells to face invading pathogens from outside. The effect of PGE_2 will trigger apoptosis of macrophages infected with *Mycobacterium tuberculosis* [4]. Macrophage apoptosis will have an elimination effect because *Mycobacterium tuberculosis* bacteria can be destroyed. PGE_2 also suppresses macrophage necrosis which can lead to bacterial dissemination. Increased levels of PGE_2 are associated with a decrease in the number of bacteria in the lung [7].

The negative association between serum PGE_2 levels and the degree of phlegm AFB positivity is by a study conducted by Dietzold and Amaral. Dietzold et al. reported that high levels of PGE_2 and low levels of LXA_4 suppress the growth of *Mycobacterium tuberculosis* [7]. Amaral et al. also reported that PGE_2 is associated with macrophage apoptosis in vitro. Apoptotic macrophages infected with *Mycobacterium tuberculosis* will increase the elimination of these bacterias [4]. The two studies above reported a significant association between PGE_2 and the growth of *Mycobacterium tuberculosis*. The statistical analysis results of this study showed that the association between serum PGE_2 levels and the degree of AFB positivity was not statistically significant. The main difference between this study and the two studies above is that both were carried out on mice and in vitro, whereas this study was conducted on pulmonary TB patients with various complications and uncontrollable comorbidities.

The results of this study can be used as consideration for conducting further research on the predictor factors for the positivity of AFB in pulmonary TB patients. The use of PGE_2 together with LXA_4 is expected to be able to assist clinicians in predicting the level of AFB positivity in pulmonary TB patients with specific chest X-ray images but difficulty in expectorating phlegm. In addition, in the future study it can be considered to analyze the comparison of PGE_2 in TB patients, smokers patients, smokers with tuberculosis, etc.

Nevertheless, this study has several limitations. First, extreme serum PGE_2 levels were found in some research subjects. This can be caused by various factors that can increase PGE_2 levels that cannot be controlled. Second, this study only examined PGE_2 levels in TB patients without comparing them with PGE_2 levels in healthy persons, so it cannot be used as a predictor factor for the degree of positivity of AFB with sputum.

6 Conclusion

The average age of new and relapsed pulmonary TB patients is 43.37 years, mostly male, have a high school education, have a smoking habit, have a low BMI, and have no history of DM. The median serum PGE_2 level of new and relapsed pulmonary TB patients was 216.95 pg/ml. The majority of new pulmonary TB patients have a high degree of positivity for AFB in sputum, but relapsed pulmonary TB patients have a low degree of positivity for AFB. This study finds a weak negative association between serum PGE_2 levels and the degree of phlegm AFB positivity but not statistically significant.

Please state any sources of funding for your research

None.

Ethical approval

We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Consent

Written informed consent was obtained from the patient.

Author contribution

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Registration of research studies

1. Name of the registry: Health Research Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
2. Unique Identifying number or registration ID: 1355/KEKP/VII/2019.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):-.

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The authors declare that they have no conflict of interest.

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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.103008.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Highlights

- Serum PGE_2 levels of tuberculosis patients tend to be normal even though ~~A~~acid-~~F~~fast bacilli (AFB) values are high.
- Most of the new and recurrent cases of pulmonary tuberculosis patients had normal PGE_2 levels.
- Serum PGE_2 levels have a negative association with AFB value.

Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

alt-text: Multimedia component 1

Queries and Answers

Q1

Query: Please review the given names and surnames to make sure that we have identified them correctly and that they are presented in the desired order. Carefully verify the spelling of all authors' names as well. If changes are needed, please provide the edits in the author section.

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Answer: Yes

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