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Indole Propionic Acid (IPA), A Gut Microbiota-Derived Metabolite, in Active Pulmonary Tuberculosis (TB) Infection: A *Case Control* Study

Novi Maulina[1,2], Zinatul Hayati[2,*], Kartini Hasballah[3] and Zulkarnain Zulkarnain[4]

[1]Doctorate Student of Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh 23116, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh 23116, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh 23116, Indonesia
[4]Department of Physiology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh 23116, Indonesia

([*]Corresponding author's e-mail: hayatikarmil@usk.ac.id)

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Abstract

Introduction: A metabolomics study revealed that the tryptophan (Trp) pathway is upregulated in tuberculosis (TB) infection. *Indole propionic acid* (IPA) is a gut microbiota-derived tryptophan metabolite with antimycobacterial properties through TrpE inhibition and as ligand for AhR in *in vitro* and *in vivo* studies. TB infection is associated with gut dysbiosis. However, plasma IPA levels and related factors in active TB patients have not yet been explored. This study aimed to determine the differences and correlations between plasma IPA levels and patient characteristics (age, gender, BMI and GeneXpert result), IPA-producing gut microbiota (*Clostridium sporogenes, Lactobacillus* sp., and *Peptostreptococcus anaerobius*), and IFN-γ concentrations in active TB patients and healthy subjects. Materials and methods: A *case-control* study was performed in 29 active TB patients and 30 healthy controls (HCs) in Aceh, Indonesia. The plasma IPA and IFN-γ levels were measured by ELISA, and the relative abundance of gut microbiota was determined by quantitative PCR (qPCR). The results and discussion: Increased plasma IPA levels (median (IQR) of 22.2 pmol/L (21.3 - 23.9) vs. 18.9 pmol/L (17 - 22.8), p 0.000) were associated with active TB according to the *adjusted* model (AOR 1.36 (95 % CI 0.37 - 5.1)). Increase of plasma IPA level in higher mycobacterial load patients was found. Higher plasma IPA was positively associated with female, normo- and overweight status, and higher relative abundance of *C. sporogenes*. The metabolite could be used to discriminate active TB disease at a *cutoff* of 21.40 pmol/L (72 % sensitivity, 70 % specificity and AUC 0.77). Dysbiosis in IPA-producing bacteria was showed in active TB patients. Conclusion: Plasma IPA could be considered as TB biomarker, and it may also influence disease progression. Future works are needed to validate plasma IPA as biomarker in LTBI and other respiratory infection, as well as its role as biomarker of treatment monitoring response.
Keywords: *Indole propionic acid, Tuberculosis, Tryptophan, Gut microbiota*

Introduction

Tuberculosis (TB) remains a leading infectious disease responsible for millions of deaths globally. In 2022, 10.6 million people were infected, and 1.3 million deaths worldwide were reported due to this disease

[1]. Since nearly everyone with active TB can be cured with prompt diagnosis and treatment, the number of TB deaths is intolerably high. Thus, every effort to combat tuberculosis needs to be stepped up. The World Health Organization (WHO) has encouraged the development of a feasible nonsputum diagnostic assay to identify new cases of TB, especially in pediatric, elderly, and extra-pulmonary TB where sputum was difficult to gain. For point-of-care testing (POC), *host* biomarker-based samples derived from nonsputum samples may meet the requirements of target product profiles [1,2].

Host metabolism is intricately related to the immune system, especially in infectious diseases. A metabolomics study revealed that the tryptophan (Trp) pathway is highly regulated in TB infection via an unbiased approach [3]. An increase in the activity of indoleamine 2,3-dioxygenase (IDO), an IFN γ–inducible cytosolic enzyme, catalyzes Trp to kynurenine (Kyn) and causes a decrease in local Trp in TB. Tryptophan (Trp) is an essential amino acid required for *host* cellular protein synthesis. The *mycobacterium tuberculosis* (Mtb) also needed Trp for its intracellular activity to colonize and survive in *host* macrophage alveolar against stress from CD4 T cell [4]. Mtb infection will stimulate macrophages and enhances their oxidative defense mechanisms by increase production of IFN-γ as the primary cytokine responsible for protection against Mtb. Increase of IFN-γ during TB infection has a protective effect for host [5]. Intracellular pathogens, including *Chlamydophila psittaci*, *Chlamydia trachomatis* [6], *Streptococcus agalactiae, Leishmania donovani, and Francisella novicida* [7], are susceptible to a decrease in Trp since they are natural auxotrophs for Trp synthesis. However, Mtb is capable of synthesizing Trp itself and counteracting IDO activity without hampering bacillus replication or causing disease. Inhibiting the Mtb Trp biosynthetic pathway may cause Mtb cell death through Trp starvation and significantly affect the *host* defense response [4,8].

Indole propionic acid (IPA) is a human metabolite of the aromatic amino acid Trp produced by gut bacteria. This low-molecular-weight metabolite is produced in the intestine and enters the circulation and cerebrospinal fluid [9]. This molecule is mimicking the Trp structure and blocking the *anthranilate synthase* TrpE enzyme in the Mtb Trp biosynthesis pathway [10]. Metabolites of Tryptophan, such as IPA, also have strong capabilities to activate the Aryl hydrocarbon Receptor (AhR), which plays a crucial role in controlling both innate and adaptive immune responses. AhR is a ligand-activated transcription factor in complex with other protein found in the cytosol of human cell. Once activated, it will translocate to cell nucleus and regulate expression of various immune response genes, including increase of IL-22 production. This cytokine stimulates the production of antimicrobial peptides, inhibits the growth of mycobacteria within macrophages by increasing phagolysosomal fusion, and induces production of Th17 T cells, which produce IL-17 that provides protection against mycobacterial infection [11-13]. Studies have shown that AhR plays a crucial role in controlling mycobacterial infections, as double knock-out AhR−/− mice are highly susceptible to Mtb [1]. These findings suggest that IPA role as AhR ligand may hold potential in controlling mycobacterial diseases by reducing excessive inflammation and enhancing the eradication of mycobacteria [11,14].

Several anaerobic gram-positive bacteria have tryptophan catabolizing enzyme (*tryptophanase* (TNA) and *tryptophan decarboxylase* (TrpD)) to produce IPA [15-17]. Well studied IPA-producing gut microbiota are *Clostridium sporogenes*, *Lactobacillus* sp., and *Peptostreptococcus anaerobius.* Studies also revealed that TB patients showed dysbiosis by lower ratio of the *Firmicutes* to *Bacteroidetes* phyla [10-13]. However, whether dysbiosis affects specific gut bacteria related to IPA production and difference of plasma IPA concentration between active TB and healthy subjects have not yet been explored. Therefore, this study aimed to determine the difference and performance of plasma IPA levels in differentiating between active TB patients and healthy subjects and to investigate the correlation between IPA, patient characteristics, immunological status (Interferon-γ/IFN-γ) and IPA-producing gut microbiota. The results

of this study are essential for understanding the missing functional link between specific gut microbiota in TB patients and the ability of IPA to differentiate active TB patients from healthy individuals and the factors affecting this ability.

Materials and methods

Study design and subject recruitment

Fifty-nine participants were recruited using a purposive sampling method in Banda Aceh and Aceh Besar Districts, Indonesia during Mai to December 2023. Sample size was calculated with Slovin formula and number of new TB cases detected in study location was used as population number. Twenty-nine adult patients with newly diagnosed TB confirmed bacteriologically using a sputum test by Gene-Xpert MTB/RIF were recruited. These TB patients were from province referral hospital and public health centers in Aceh Besar District. Patients receiving TB drugs for >2 weeks and those receiving TB-DM or TB-HIV were excluded. Patient characteristic including GeneXpert positivity result was noted as very low, low, medium and high. Thirty age- and sex-matched healthy adult subjects, who were of the same age, sex, and living area as the patients with no history of respiratory symptoms (cough, bloody cough in sputum, fever for > 2 weeks, dyspneu, weight loss, night sweats) and no family history of TB and HIV (to exclude Latent TB), were included as controls. All the patients were subjected to blood and fresh fecal examination for the study.

Ethical approval

This study was approved by the Ethical Committee of Dr. Zainoel Abidin Hospital and the Faculty of Medicine, Aceh, Indonesia (No. 122/Etik-RSUDZA/2023). All participants signed the form of written informed consent before enrollment, and their characteristics were collected.

Fecal sample collection and DNA extraction

One g of fresh fecal sample from each subject was weighed and stored immediately at −80 °C with a DNA/RNA shieldTM (Zymo Research Sample collection product, California, US) for DNA extraction. DNA was extracted from fecal samples using the Quick-DNA TM Fecal/Soil Microbe Miniprep Kit (Zymo Research DNA Collection Product, California, US) following the manufacturer's instructions. The concentration of DNA was quantified by a Maestrogen MaestroNano Pro Spectrophotometer (GMI Spectrophotometer UV VIS products, Minnesota, USA).

Primer design

Specific primers for gut bacteria-related IPA were designed for Qrt-PCR based on the National Center for Biotechnology Information (NCBI) database, and we used references for other primers (**Table 1**). The specificity of the primers was considered by comparing all available sequences using the BLAST search program (http://www.ncbi. nlm.nih.gov/BLAST) to verify the complementarity of the proposed primers to the target species. A universal primer was used as a reference that targets the V3 variable regions of all known bacteria (located at positions 339 - 539 in the *E. coli* gene) [14,15]. The primers were matched on average 99.1 ± 0.88 % of a total of 931 - 412 good-quality (> 1200 bp) 16S rRNA gene sequences for *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia*, which were acquired from RDP *(Ribosomal Database Project*).

Table 1 Real-time PCR primers for specific gut bacteria.

Microorganism	Primer sequence (5'-3')	Annealing temp. (°C)	Amplicon size (bp)	Ref.
Clostridium sporogenes	FP: TGCCGAGTTCCCTAAAGAAA RP: AGTAGGAACGGTGTGCCAAG	53	198	This Study
Lactobacillus spp.	FP: AGCAGTAGGGAATCTTCCA RP: CACCGCTACACATGGAG	51	341	[18,19]
Peptostreptococcus anaerobius	FP: GCTCGGTGCCTTCACTAACG RP: AGCCCCGAAGGGAAGGTGTG	59.5	390	[20]
Universal Bacteria	FP: ACTCCTACGGGAGGCAGCAGT RP: GTATTACCGCGGCTGCTGGCAC	62.5	174 - 179	[21]

Real-time PCR assays

RT-PCR was used to quantify the relative changes in the abundances of *C. sporogenes*, *Lactobacillus* sp., *and Peptostreptococcus anaerobius* in the 2 groups. Gene amplification was performed in 96-well plates in a CFX96 Thermocycler (*Bio-Rad*, *California*, *USA*), and fluorescence resonance energy transfer with a SYBR Green 490 fluorophore was used for detection. A final volume of 20 µl (10 µl of commercial SYBR Green Master mix (Bioline, USA), 1.6 µl of forward and reverse primers, 5 µl of DNA template and 3.4 µl of nuclease-free water) was used. The amplification process was as follows: initial denaturation at 95 °C (3 min), followed by 40 cycles of 95 °C for 5 s (denaturation), 10 s for annealing and 72 °C for 20 s (elongation). The master mix without the DNA template was used as a negative control.

Data analysis was performed using the 2−ΔCt and 2−ΔΔCt methods [22]. The specific bacteria were determined as targets, and universal bacteria were used as a reference [23]. The calculation was performed based on the following formula:

Relative abundance of target bacteria with respect to universal bacteria:

$$2^{-\Delta Ct} = 2^{-(\text{Ct of target bacteria} - \text{Ct of universal bacteria})} \quad (1)$$

Fold change in the relative abundance of target bacteria in TB patients compared to healthy controls:

$$2^{-\Delta\Delta Ct} = 2^{-[\Delta Ct \text{ of target bacteria of healthy controls or TB} - \text{mean } \Delta Ct \text{ of target bacteria of HC group}]} \quad (2)$$

Blood sample collection and ELISA

EDTA-anticoagulated plasma was stored at −80 °C and used for ELISA to measure the concentrations of IPA (*Indolisa, Indogen Intertama Company, Indonesia*) and IFN-γ (*Bioassay Technology Lab (BT Lab), Zhejiang, China*). For the plasma IPA assay, competitive inhibition was observed between HRP-labeled IPA and free IPA in samples with the anti-IPA antibody. After washing, the substrate solution was added and incubated. A blue color is produced, and when the stop solution is added, a yellow color appears.

For the plasma IFN-γ assay, the sample containing IFN-γ binds to well-coated antibodies. Biotinylated human IFN-γ antibody was added to IFN-γ in the sample. Then, streptavidin-HRP was added to bind to the biotinylated IFN-γ antibody. Unbound streptavidin-HRP was washed away after incubation. The addition of the substrate solution produced a color based on the amount of IFN-γ, and the addition of the stop solution terminated the reaction. The intensity of the yellow color was measured by a spectrophotometer (Ao Absorbance Microplate Reader; *Azure Biosystems, Dublin, CA, USA*) and read at a

wavelength of 450 nm. The IPA and IFN-γ concentrations of the samples were obtained by adjusting the OD to a standard curve using polynomial cubic regression and the 4-parameter logistic function, respectively.

Statistical analysis

Univariate and bivariate analyses were performed. We used the mean, standard deviation (SD) or median, interquartile range (IQR) for numeric variables and percentages for categorical variables as measures of central tendency. For unadjusted comparisons between 2 groups, we used the Mann-Whitney-Wilcoxon test and the chi-square test for numeric and categorical variables, respectively. A receiver operating characteristic (ROC) curve was generated to provide the diagnostic accuracy of the test by area under ROC curve (AUC) and the optimal cut-point value. We determined the cut off points by Liu method [24], which defines the optimal cut-point as the point maximizing the product of sensitivity and specificity. We generated Table with sensitivity and specificity value and draw a graph to determine the intersection of these 2 values and determined this as cut-off value. ANOVA test was performed to compare means of plasma IPA level in 4 groups of GeneXpert positivity result.

For multivariable analysis, we used ordinal logistic regressions with IPA level as the dependent variable and age, sex, BMI, IFN-γ level, and relative abundance of gut bacteria (*C. sporogenes, Lactobacillus* sp*., and P. anaerobius*) as independent variables in the final models. The results of ordinal logistic regressions are reported as adjusted odds ratios accompanied by 95 % confidence intervals (95 % CIs). Ordinal logistic regression with the relative abundance of gut bacteria and plasma IFN-γ level as dependent variables was also performed. The means and standard deviations (SDs) of the log-transformed IPA levels for the active TB and HC groups were calculated and compared using the T test. All the statistical analyses were conducted and visualized using SPSS version 23.0 (*SPSS, Inc., Chicago, IL, USA)* and *GraphPad Prism version 10.1.0*.

Results

Patient characteristics

Blood and fecal samples were collected from 29 TB patients (24 males and 5 females) with a mean age of 50.7 ± 17.8 years and from 30 healthy subjects (22 males and 8 females) with a mean age of 45.1 ± 12.9 years. The data on the subjects' characteristics are shown in **Table 2**.

Table 2 Subject demographic data.

Parameters	TB Patients (n = 31)	HC (n = 30)	*p*-value
Age, mean ± SD	50.7 ± 17.8	45.1 ± 12.9	0.17
Male Sex, n (%)	24 (82)	22 (73)	0.53
BMI, mean ± SD	19.8 ± 3.8	25.5 ± 3.9	0.000*
Underweight (BMI < 18,5)	15 (88.2)	2 (11.8)	
Normoweight (BMI 18.5 - 24.9)	12 (46.2)	14 (53.8)	
Overweight (BMI 25 – 29.9)	1 (9.1)	10 (90.9)	
Obese (BMI > 30)	1 (20)	4 (80)	
GeneXpert Result, n (%)			
Very Low	5 (17.2)	-	
Low	6 (20.6)	-	
Medium	11 (37.9)	-	
High	7 (24.1)	-	

Higher plasma IPA concentrations were associated with more active TB patients

Compared with healthy subjects, active TB patients had significantly greater plasma IPA concentrations according to the unadjusted model (median (IQR); 22.2 pmol/L (21.3 - 23.9) vs. 18.9 pmol/L (17 - 22.8), *p*-value 0.000), as shown in **Figure 1(a)**. A significant difference in the mean plasma IPA between the 2 groups after logarithmic transformation was also detected (mean ± SD; TB: 1.36 ± 0.06 vs. 1.28 ± 0.09; t test, *p*-value < 0.05; median (IQR); 1.34 (1.32 - 1.37) vs. 1.27 (1.23-1.35)).

According to the ROC analysis, a *cutoff* plasma IPA of 21.40 pmol/L had a sensitivity of 72 % (95 % CI 54.3 - 85.3 %, a specificity of 70 % (95 % CI 52.1 - 83.34 %), and an AUC of 77 % (95 % CI 0.64 - 0.89, *p* 0.000), with a likelihood ratio of 2.41, for differentiating active TB patients from healthy subjects, as shown in **Figure 1(b)** and Supplementary **Table 1**.

Figure 2 shows plasma IPA level in 4 GeneXpert positivity results. It shows that plasma IPA level is increased following higher bacterial load indicated by CT value of GeneXpert test, but it was not significantly different (*p* 0.44).

A positive association was shown when the plasma IPA level was adjusted to the patient's body mass index (BMI), but this association was not statistically significant (AOR 1.36 (95 % CI 0.37 - 5.1)), as shown in Model 1 **Table 3**. According to the multivariate analysis, active TB was associated with increased plasma IPA level after adjusting for age, sex, BMI, plasma IFN-γ levels and the relative abundances of the gut microbiota (AOR 1.18 (95 % CI 0.3 - 4.5)), as shown in **Table 3** (model 2). In this model, a higher plasma IPA concentration was positively correlated with female, normo- and overweight status, and higher relative abundance of *C. sporogenes.* In terms of body mass index, underweight status was negatively correlated with plasma IPA levels compared to obese status according to the *unadjusted* and *adjusted* models.

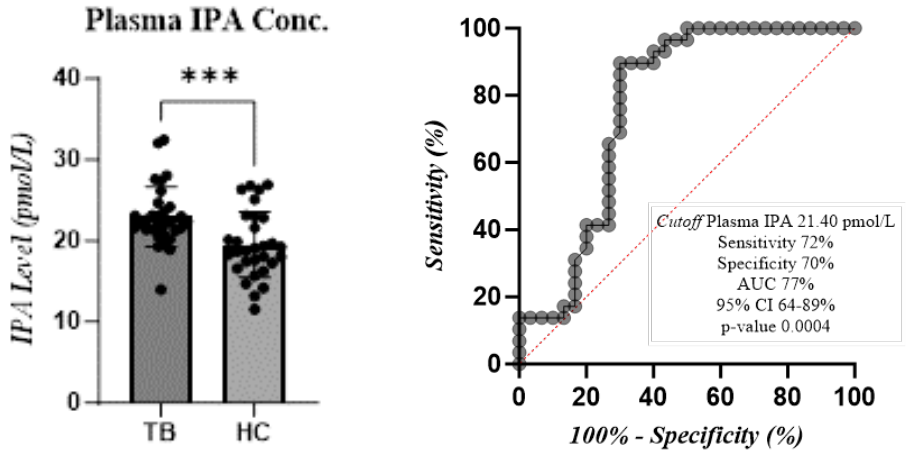

Figure 1 (a) Differences in plasma IPA concentrations between groups in *the unadjusted* model; 1(b). ROC curves of plasma IPA concentrations for discriminating active TB patients from healthy subjects.

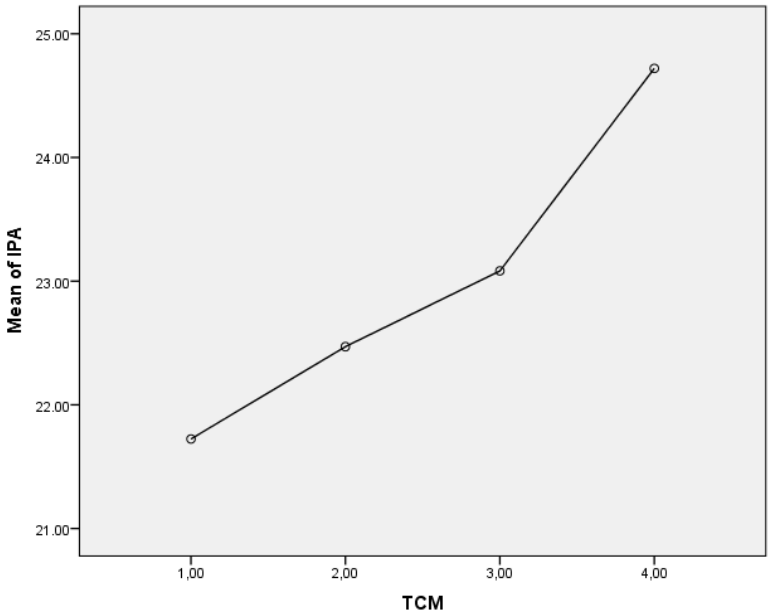

Figure 2 Means of Plasma IPA level in Active TB patients in 4 groups of GeneXpert Positivity Result (1 = Very Low, 2 = Low, 3 = Medium, 4 = High; *p* 0.44)).

Table 3 Ordinal logistic regression for plasma *indole propionic acid (IPA)* concentration as the dependent variable.

Model	Variable	AOR (95 % CI)	*p*-value
1	Active TB	1.36 (0.37 - 5.1)	0.64
	BMI (Ref, Obese)		
	- Underweight	0.7 (0.04 - 9.9)	0.78
	- Normoweight	3.7 (0.35 - 36.6)	0.27
	- Overweight	3.4 (0.3 - 42.5)	0.33
2	Active TB	1.18 (0.3 - 4.5)	0.81
	Age in years	1.03 (0.9 - 2.2)	0.12
	Female	1.5 (0.3 - 6.6)	0.59
	BMI (Ref, Obese)		
	Underweight	0.5 (0.02 - 8.1)	0.60
	Normoweight	4.0 (0.32 - 49.4)	0.27
	Overweight	3.3 (0.24 - 44.7)	0.36
	↓ plasma IFN-γ	1.09 (0.28 - 4.1)	0.89
	↓ Rel Ab of *C.spo*	0.6 (0.15 - 2.2)	0.42
	↓ Rel Ab of *Lacto*	2.4 (0.4 - 13.5)	0.32
	↓ Rel Ab of *P.an*	1.8 (0.3 - 8.7)	0.47

Relative abundance of gut bacteria in active TB patients and healthy subjects

The relative abundances of the bacteria were quantified, and the median was used to assess whether the decrease in specific bacteria was related to TB. **Table 4** shows the ordinal logistic regression analysis results for the relative abundance of the gut microbiota (*C. sporogenes, Lactobacillus* sp.*,* and *P. Anaerobius*) as the dependent variable with BMI adjusted.

An increase in TB activity was associated with a decrease in the relative abundance of *C. sporogenes, Lactobacillus* sp.*,* and *P. anaerobius* and a decrease in BMI, but these differences were not statistically significant (AOR 0.8 (95 % CI 0.2 - 3.2), AOR 0.6 (95 % CI 0.13 - 3.3), and AOR 0.5 (95 % CI 0.1 - 2.2), respectively). The results also showed that compared with obesity, normoweight status had a positive association with the relative abundance of bacteria, and overweight status had an inverse association with the relative abundance of bacteria compared to obese status according to the unadjusted and adjusted models.

Table 4 Ordinal logistic regression analysis for the relative abundance of gut microbiota related to IPA (*C. sporogenes, Lactobacillus* sp.*,* and *P. Anaerobius*) as the dependent variable with BMI adjusted.

Dependent var	Variable	AOR (95 % CI)	*p*-value
C.sporogenes	Active TB	0.8 (0.2 - 3.2)	0.82
	BMI (Ref, Obese)		
	Underweight	1.84 (0.14 - 24.5)	0.64
	Normoweight	3.5 (0.3 - 36.6)	0.28
	Overweight	0.88 (0.06 - 12.2)	0.92
Lactobacillus sp.	Active TB	0.6 (0.13-3.3)	0.62
	BMI (Ref, Obese)		
	Underweight	---	---
	Normoweight	1.6 (0.15 - 18.2)	0.68
	Overweight	0.8 (0.06 - 12.2)	0.90
P.anaerobius	Active TB	0.5 (0.1 - 2.2)	0.37
	BMI (Ref, Obese)		
	Underweight	1.4 (0.09 - 20.08)	0.82
	Normoweight	1.7 (0.16 - 18.17)	0.64
	Overweight	0.4 (0.02 - 8.08)	0.52

Circulating IFN-γ levels in patients with active TB and healthy patients

Compared with healthy subjects, patients with active TB had greater plasma IFN-γ levels according to the BMI-adjusted model (AOR 1.5 (95 % CI 0.2 - 9.9), as shown in **Table 5**).

Table 5 Ordinal logistic regression analysis for plasma IFN-γ level as the dependent variable with BMI adjusted.

Variable	AOR (95 % CI)	*p*-value
Active TB	1.5 (0.2 - 9.9)	0.65
BMI (Ref, Obese)		
- Underweight	0.5 (0.06 - 4.9)	0.65
- Normoweight	0.5 (0.07 - 3.9)	0.55
- Overweight	1.8 (0.2 - 14.8)	0.59

Discussion

The gut microbiota contributes to host physiology by producing a myriad of metabolites, such as signaling molecules and substrates for metabolic reactions [25-27]. *Indole propionic acid* (IPA) is an indole metabolite produced by gut bacteria through Trp catabolism. The metabolite might impair Mtb growth by inhibit TrpE in Mtb Trp biosynthesis and as a ligand for AhR [10,12,28]. To the best of our knowledge, this is the first study exploring plasma IPA levels in TB patients. Another study investigating the altered gut microbiota and fecal metabolomic profiles in TB patients revealed that among 33 significantly different metabolites, IPA enriched in TB patients had the strongest association with the gut microbiota [16].

Plasma IPA level in 2 groups

Our study showed that the plasma IPA level was significantly greater in active TB patients in the unadjusted model and after log transformation, which implies that highly regulated Trp catabolism in active TB occurred not only in the kynurenine pathway [29], but also in the bacterial pathway. Active TB also showed an association with higher plasma IPA levels in the adjusted model (**Table 3**). ROC analysis showed that at a *cutoff* of 21.40 pmol/L, a sensitivity of 72 % and a specificity of 70 % with AUC 0.77 and a *likelihood* ratio of 2.41 suggested that the plasma IPA level could potentially be used to discriminate active TB disease.

To understand whether IPA might influence TB infection and progression, we analyze plasma IPA level to positivity results of GeneXpert which is indicated by very low (CT value > 28), low (CT value 22 - 28), medium (CT value 16 - 22), and high (CT value < 16) of bacterial load. **Figure 2** showed that plasma IPA level increased following high bacterial load. This may emphasize the protective role of IPA in immune response against Mtb through AhR binding and inhibition of Trp biosynthesis in Mtb.

Dysbiosis of gut microbiota in TB

The association of active TB with a lower relative abundance of gut bacteria (*C. sporogenes, Lactobacillus sp.,* and *Peptostreptococcus anaerobius*) is shown in **Table 4**. It is relevant to studies suggesting that dysbiosis in TB patients are characterized by a lower ratio of the *Firmicutes* to *Bacteroidetes* phyla [15,16,32]. [4-6] The bacteria measured in this study were included the *Firmicutes* phylum, the second greatest phylum in the gut, which was decreased in TB patients [33,34]. Decreases in the *Firmicutes* and *Bacteroides* (F/B) ratio also occur during respiratory virus infection [35], obesity [36], diabetes and other diseases.

An adjusted model of plasma IPA is shown in **Table 3**. A higher plasma IPA was positively associated with relative abundance of *C. sporogenes*. Thus, the presence of *C. sporogenes* was found to be positively associated with plasma IPA levels. However, the decrease in these bacteria in active TB patients in this

study did not reflect the plasma IPA level in active TB patients. This might be due to functional redundancy in gut microbiota niche and because the ability to produce metabolites is shared with that of other gut-derived anaerobic bacteria [37]. An *in vitro* study revealed that the *phenyllactate dehydratase* (fldAIBC) gene cluster was a reliable marker for IPA production, which was found in some bacteria, including *C. sporogenes, C. cadaveris, C. caloritolerans, C. botulinum, C. cylindrosporum, P. anaerobius, P. russelliii, P. stomatis, and P. asaccharolyticus* [38]. The investigation of IPA-producing bacteria and the determination of host IPA content are areas that require further exploration in upcoming research. Consequently, a more extensive analysis of the relationship between IPA content and the gut microbiota is necessary to pinpoint potential IPA-producing bacteria within the associated microbiota. The comprehensive analysis by metabolomics study coupled with *in vitro culture* and bioreactor techniques will provide better determination of other IPA-producing microbiota in future works. In addition to diet and the gut microbiota, genome-wide association studies revealed that *acyl-CoA* produced by the *mitochondrial xenobiotic/medium-chain fatty acid:CoA ligases* (ACSM2A) gene is an essential factor affecting plasma IPA concentration in human [39,40].

Gut microbiota, plasma IPA level and body mass index (BMI)

The composition of the gut microbiota is affected by factors such as ethnicity, geographical location, diet, lifestyle, age, and sex. In this study, normoweight status had a positive association with the higher relative abundance of bacteria compared to obese, according to the unadjusted and adjusted models (**Table 4**). These findings are relevant to studies exploring the gut microbiota at different body mass indices [36,41]. Higher plasma IPA level was also positively associated in normoweight and negatively associated with underweight, compared to obese, as shown in **Table 3**. It was relevant to active TB patients in this study with lower BMI that showed lower plasma IPA level compared to healthy subjects. Chronic wasting state is occurred in TB where amino acids are separated for protein synthesis and result in oxidation mechanism and body protein pool loss (anabolic block) [42].

Plasma IFN-γ level

Active TB was positively associated with higher plasma IFN-γ levels than healthy subjects according to the BMI-adjusted model in **Table 5** (AOR 1.5 (95 % CI 0.2 - 9.9)). A similar result was also reported by other studies [13,30]. Increased production of IFN-γ modulated by IL-12 aims to induce and activate macrophages to destroy intracellular Mtb and protect the host. Interferon-γ has a direct correlation with mycobacterial burdens and is indicated as a secondary feature of disease severity and disease extent [43]. However, the plasma IFN-γ concentration was not associated with the plasma IPA according to the adjusted model in **Table 3** (AOR 1.09 (95 % CI 0.28 - 4.1)). Higher IPA levels in patients with active TB might reflects either a lower bacterial load or upregulation of the immune response via AhR, to impair its intracellular growth. This study showed higher plasma IPA and IFN-γ levels in active TB patients, which might describe a synergistic immune response in Mtb infection.

Briefly, the results of the present study highlighted the capability of plasma IPA level in 2 groups and its correlation with specific gut microbiota and host immune markers (IFN-γ). As candidate of TB biomarker, plasma is an easy collected clinical sample and ELISA assay is an uncomplicated method to perform. Nevertheless, more extensive analysis of the relationship between the gut microbiota and IPA in different spectra of TB disease in multiple settings are crucial to delve deeper into the intricate human mechanisms and to discover the role of IPA metabolites as biological markers or host-supported therapies.

Study limitations include small sample size and limited study groups included. Future studies should include group of latent TB infection (LTBI) patients to evaluate whether plasma IPA level could be used

to differ active and latent TB. The potency of IPA as biomarker for treatment monitoring response also could be addressed. Furthermore, analysis of samples from people with other respiratory infections is required before it could be used for TB clinical test.

Conclusions

This study presented significant different of plasma IPA level in both groups and good capability of plasma IPA level as TB biomarker (AUC 0.77, sensitivity of 72 % and specificity of 70 %). Plasma IPA level may also influence disease progression by GeneXpert positivity results. Additional studies are needed to validate plasma IPA as biomarker in LTBI and other respiratory infection, as well as its role as biomarker of treatment monitoring response.

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