Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 4
August 2023

Published by:

PERI
The Prodia Education & Research Institute

Secretariat: Prodia Tower 9th Floor
Jl. Kramat Raya No.150,
Jakarta 10430, Indonesia
Tel.: +62-21-3144182
Fax.: +62-21-3144181
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org

#200/M/KPT/2020

Included in:
Scopus DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
EMERGING SOURCES CITATION Clarivate Analytics

RESEARCH ARTICLES

Electric Field Stimulation of Stem Cell Culture Media Increases Oxygen Radical Uptake Capacity
Yoshikawa M, Oshita M, Gotoh T, Tawa Y, Dan K

Oxidative Stress Mediates the Association between Thyroid Dysfunction and Breast Cancer
Mahmood M, Mushtaq M, Malik A, Khurshid R, Aamir F, Javed A

Antibodies to Glutamic Acid Decarboxylase-65 is Associated with Total Daily Dose of Insulin Requirement in Children with Type 1 Diabetes
Faisal, Rochmah N, Faizi M, Novina, Nuraeni E

Sleeve Gastrectomy Decrease Body Weight, Fasting Blood Glucose, and Gene Expression of TNF-α and IL-1 in The Abdominal Aorta of Rats with Obesity and Diabetes Mellitus
Saputro A, Prasetyo SA, Sobirin MA, Mughni A, Prajoko YW

Ocimum sanctum Leaves Prevent Neuronal Cell Apoptosis Through Reduction of Caspase-3 and -9 Expressions and Inhibition of β-amyloid Oligomerization
Aliffia D, Ramadhani DA, Wasityastuti W, Sari DRT, Kustiati U, Wihadmadyatami H, Kusindarta DL

Polyscias scutellaria Aqueous Leaves Extract Increases Insulin Levels and Improves Mammary Gland Histology in Lactating Rats
Budiono B, Pertami SB, Kasiati, Herawati T, Pujiastuti N, Arifah SN, Atho'illah MF

2-(3-(chloromethyl)benzoyloxy)benzoic Acid Increases $CD4^+$ Regulatory T-Cell Population and FoxP3 Expression in Lipopolysaccharide-induced Mice
Tjahjono Y, Caroline, Nugraha J, Foe K, Karnati S, Ergün S, Jafet N, Chrisantia O, Kusuma IMAB, Wijaya H, Hadinugroho W, Nugrahaningsih DAA, Kusindarta DL, Wilianto YR, Esar SY, Wihadmadyatami H

Rosmarinus officinalis Essential Oil Increases Hair Length and Follicle Diameter of Ultraviolet B-exposed Mice Through VEGF
Luziani S, Darwinata AE, Praharsini IGAA, Wijaya VO

A Double-Blind, Randomized Controlled Trial of Hydroxychloroquine for Cognitive Dysfunction and Inflammatory Biomarkers in Systemic Lupus Erythematosus Patients in Indonesia
Suntoko B, Hadisaputro S, Kalim H, Hadi S, Warlisti IV

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients
Indrasari ND, Simadibrata M, Rustamadji P, Yusra, Kekalih A , Suhendro, Harahap AR, Wibowo H, Parwati I, Sandra F

Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 4, August 2023

Contact Address
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Tel.: +62-21-3144182, ext. 3872
Fax.: +62-21-3144181
WhatsApp No.: +62 877-3616-3117
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org

Focus & Scope
The Indonesian Biomedical Journal (InaBJ) is an open access, peer-reviewed journal that encompasses all fundamental and molecular aspects of basic medical sciences, emphasizing on providing the molecular studies of biomedical problems and molecular mechanisms.

InaBJ is dedicated to publish original research and review articles covering all aspects in biomedical sciences. The editors will carefully select manuscript to present only the most recent findings in basic and clinical sciences. All professionals concerned with biomedical issues will find this journal a most valuable update to keep them abreast of the latest scientific development.

Section Policies
Review Article
Review Article should consist of no more than 10,000 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than eight figures and/or tables in total and no more than 250 references. Only invited authors are allowed to submit review article.

Research Article
Research Article should consist of no more than 3,500 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than six figures and/or tables in total and no more than 40 references.

Peer Review Process
All manuscripts submitted to InaBJ will be selected and double-blind peer-reviewed by two or more reviewers to present valuable and authentic findings in biomedical sciences. At least, an external reviewer will be included as the reviewer in each manuscript reviewing process.

Author can suggest reviewer/s that not having publication together within five years and should not be member/s of the same research institution. However, reviewers will be selected independently by Section Editor based on their expertise, specialties, and independencies to fit the topic. Section Editor will ensure that the reviewers will be not from the same institution as the author.

Manuscript will be reviewed comprehensively, including appropriate title; content reflecting abstract; concise writing; clear purpose, study method and figures and/or tables; and summary supported by content. Supplementary data will also be sent to reviewer. The reviewing process will take generally 2-3 months depends on sufficiency of information provided.

Decisions are ultimately made by the Section Editor based on the peer-reviewing results. Therefore, Section Editor will consider thoroughly, if necessary Section Editor can invite another one or more reviewer/s to conclude the final decision.

Publication Frequency
InaBJ is published bimonthly (in February, April, June, August, October, and December).

Open Access Policy
InaBJ provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Print ISSN: 2085-3297, Online ISSN: 2355-9179

Content

The Indonesian Biomedical Journal
Volume 15 Number 4, August 2023

RESEARCH ARTICLE

Electric Field Stimulation of Stem Cell Culture Media Increases Oxygen Radical Uptake Capacity
Yoshikawa M, Oshita M, Gotoh T, Tawa Y, Dan K
p.296-302

Oxidative Stress Mediates the Association between Thyroid Dysfunction and Breast Cancer
Mahmood M, Mushtaq M, Malik A, Khurshid R, Aamir F, Javed A
p.303-9

Antibodies to Glutamic Acid Decarboxylase-65 is Associated with Total Daily Dose of Insulin Requirement in Children with Type 1 Diabetes
Faisal, Rochmah N, Faizi M, Novina, Nuraeni E
p.310-5

Sleeve Gastrectomy Decrease Body Weight, Fasting Blood Glucose, and Gene Expression of TNF-α and IL-1 in The Abdominal Aorta of Rats with Obesity and Diabetes Mellitus
Saputro A, Prasetyo SA, Sobirin MA, Mughni A, Prajoko YW
p.316-21

***Ocimum sanctum* Leaves Prevent Neuronal Cell Apoptosis Through Reduction of Caspase-3 and -9 Expressions and Inhibition of β-amyloid Oligomerization**
Aliffia D, Ramadhani DA, Wasityastuti W, Sari DRT, Kustiati U, Wihadmadyatami H, Kusindarta DL
p.322-32

RESEARCH ARTICLE

***Polyscias scutellaria* Aqueous Leaves Extract Increases Insulin Levels and Improves Mammary Gland Histology in Lactating Rats**
Budiono B, Pertami SB, Kasiati, Herawati T, Pujiastuti N, Arifah SN, Atho'illah MF
p.333-8

2-(3-(chloromethyl)benzoyloxy)benzoic Acid Increases CD4+ Regulatory T-Cell Population and FoxP3 Expression in Lipopolysaccharide-induced Mice
Tjahjono Y, Caroline, Nugraha J, Foe K, Karnati S, Ergün S, Jafet N, Chrisantia O, Kusuma IMAB, Wijaya H, Hadinugroho W, Nugrahaningsih DAA, Kusindarta DL, Wilianto YR, Esar SY, Wihadmadyatami H
p.339-46

***Rosmarinus officinalis* Essential Oil Increases Hair Length and Follicle Diameter of Ultraviolet B-exposed Mice Through VEGF**
Luziani S, Darwinata AE, Praharsini IGAA, Wijaya VO
p.347-53

A Double-Blind, Randomized Controlled Trial of Hydroxychloroquine for Cognitive Dysfunction and Inflammatory Biomarkers in Systemic Lupus Erythematosus Patients in Indonesia
Suntoko B, Hadisaputro S, Kalim H, Hadi S, Warlisti IV
p.354-61

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients
Indrasari ND, Simadibrata M, Rustamadji P, Yusra, Kekalih A , Suhendro, Harahap AR, Wibowo H, Parwati I, Sandra F
p.362-8

RESEARCH ARTICLE

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Nuri Dyah Indrasari[1,2], Marcellus Simadibrata[3], Primariadewi Rustamadji[4], Yusra[2], Aria Kekalih[5], Suhendro[3], Alida Roswita Harahap[2], Heri Wibowo[6], Ida Parwati[7], Ferry Sandra[8,*]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[5]Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No.16, Jakarta 10430, Indonesia
[6]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia
[8]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: May 20, 2023; Revised date: Aug 18, 2023; Accepted date: Aug 21, 2023

Abstract

BACKGROUND: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

METHODS: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological test were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

RESULTS: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

CONCLUSION: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

Indones Biomed J. 2023; 15(4): 362-8

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).(1,2) The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was ≤9.9%. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3-16%.(3-5)

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.(3,6-10) The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.(11)

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.(11,12) Elevated ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.(12,13) The activity of ADA differs significantly between patients with pulmonary TB and the normal healthy people.(14,15)

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them are leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and erythrocyte sedimentation rate (ESR).(3,6,16,17) At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December 2022, with the inclusion criteria as follows; for the ITB group: ≥18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (No. KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.(16)

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in non-anticoagulated tubes were

allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzen, China) with enzymatic colorimetry principle test used. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry for measurement of leukocyte, basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistical Analysis

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p<0.05$.

The calculation of cut-off and area under the curve (AUC) was obtained from the receiver operating characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

Results

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI: 0.542-0.849) was obtained

Table 1. Characteristics of ITB and non-ITB groups.

Characteristics	ITB (n=16)	Non-ITB (n=127)
Age (years), mean±SD	42.19±18.28	43.53 ±16.65
Gender, n (%)		
Male	10 (62.5%)	40 (31.5%)
Female	6 (37.5%)	87 (68.5%)
Clinical manifestation, n (%)		
Weight loss	13 (81.3%)	69 (54.3%)
Night sweat	1 (6.3%)	5 (3.9%)
Cough	2 (12.5%)	5 (3.9%)
Fever	5 (31.3%)	16 (12.6%)
Loss of appetite	9 (56.3%)	41 (32.3%)
Non-specific abdominal pain	12 (75.0%)	111 (87.4%)
Chronic diarrhea	13 (81.3%)	73 (57.5%)
Constipation	13 (81.3%)	82 (64.6%)
Blood in stool	8 (50.0%)	69 (54.3%)
Mucus in stool	8 (50.0%)	70 (55.1%)
Blood and mucus in stool	8 (50.0%)	68 (53.5%)
TB history, n (%)		
History of contact with TB patient	1 (6.3%)	1 (0.8%)
History of pulmonary and non-pulmonary TB	2 (12.5%)	10 (7.9%)

Table 2. Laboratory results of ITB and non-ITB groups.

Parameter	ITB (n=16)	Non-ITB (n=127)	*p*- value
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10^3/µL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Tested with Mann-Whitney U test, *significant if $p<0.05$. Data were presented in median (min-max).

with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI: 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (95% CI: 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).(1,18)

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity

Table 3. ADA results of ITB and non-ITB groups with cut-off=12.56 IU/L.

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
Sensitivity	= 75%		
Specificity	= 57%		
Positive Predictive Value (PPV)	= 18%		
Negative Predictive Value (NPV)	= 95%		

Table 4. ESR results of ITB and non-ITB groups with cut-off=32.5 mm/hour.

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
Sensitivity	= 81%		
Specificity	= 62%		
Positive Predictive Value (PPV)	= 21%		
Negative Predictive Value (NPV)	= 96%		

and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.(15,19) The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L) (19) and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L) (20).

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.(21)

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.(22)

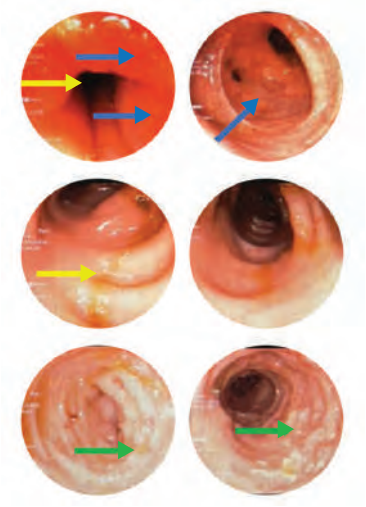


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.(23)

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar (22) and contradictive findings (24) in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.(25) In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.(25-27)

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

Conclusion

ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

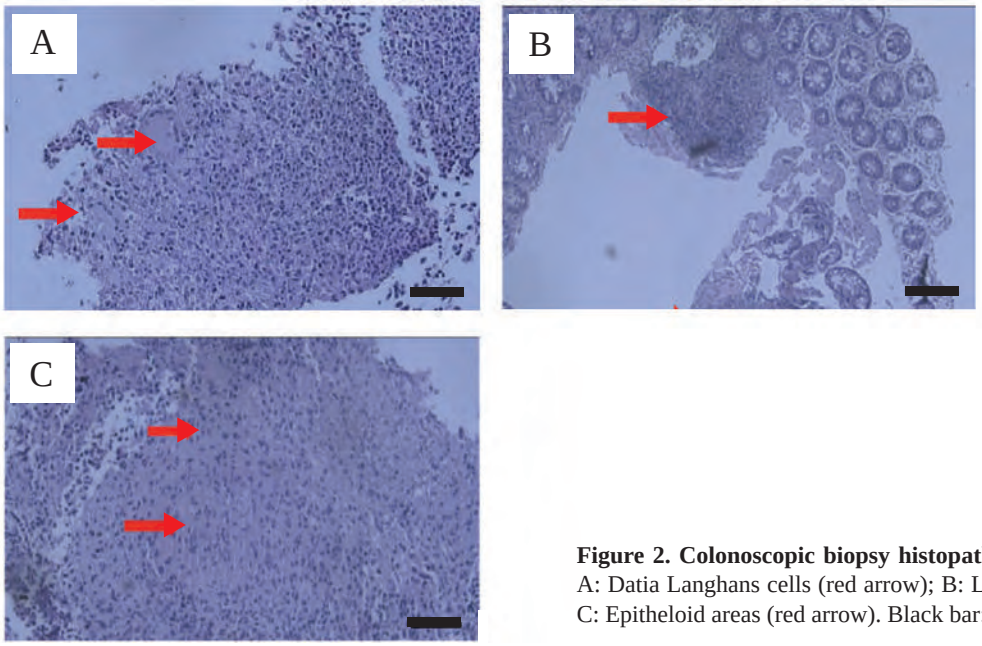


Figure 2. Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 µm.

Acknowledgements

The authors would thank to the patients, clinician staffs, nurse staffs, technician's laboratory, and the expert committee for their participation in this study.

Authors Contribution

NDI, MS, PR, Y, AK, S, ARH, HW, and IP were involved in concepting and planning the research. NDI, MS, PR, AK, ARH, HW, and IP performed the data acquisition/collection. NDI, MS, PR, Y, AK, and S calculated the experimental data and performed the analysis. NDI, MS, PR, Y, AK, ARH, HW, IP, and FS drafted the manuscript. NDI, MS, AK and S designed the figures and tables. NDI, MS, PR, Y, AK, S, ARH, HW, IP, and FS aided in interpreting the results. NDI, MS, PR, Y, AK, and FS took parts in giving critical revision of the manuscript.

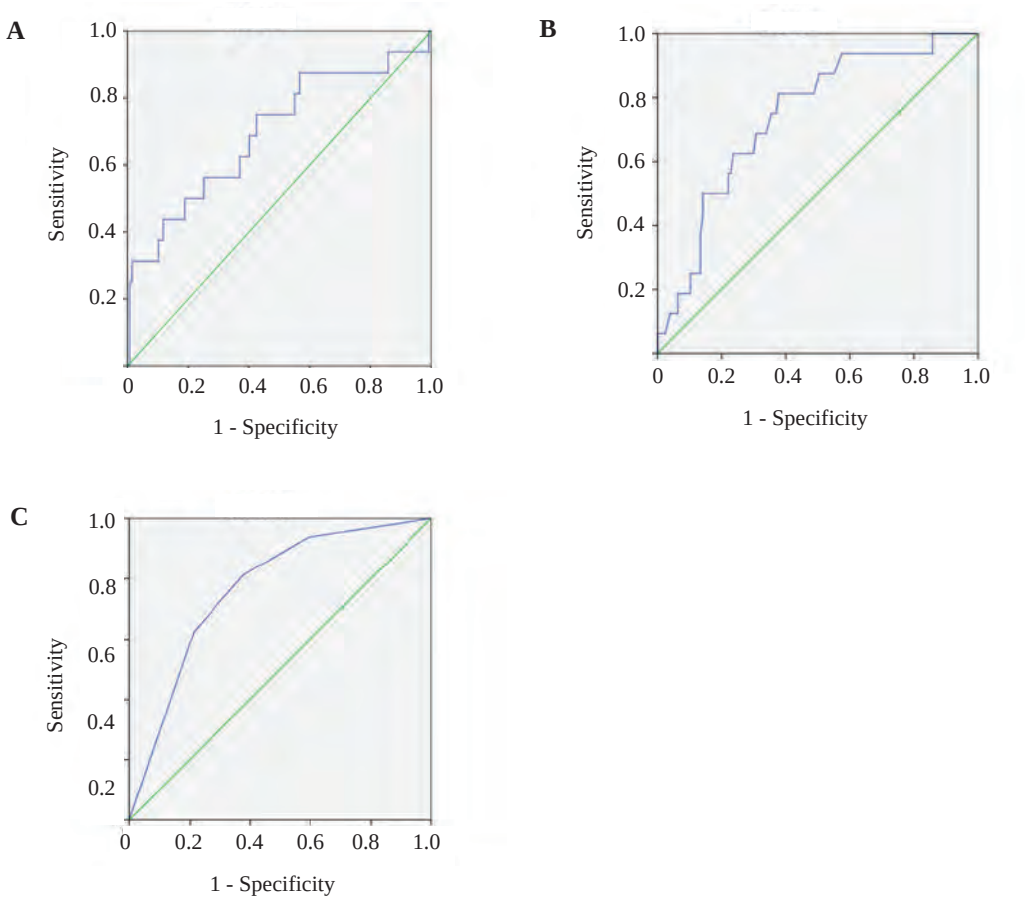
References

1. World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
2. Yunda DK, Witjaksono F, Nurwidya F. Correlation between protein intake, fat free mass, and total lymphocyte count with quality of life in pulmonary tuberculosis patients undergoing intensive phase treatment in Pekanbaru, Riau Province. Mol Cell Biomed Sci. 2020; 4(3): 128-34.
3. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, *et al.* Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010; 11(3): 143-9.
4. Evans RP, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016; 18(12): 45. doi: 10.1007/s11908-016-0546-5.
5. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015; 17(1): 3-11.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. J Penyakit Dalam Indones. 2016; 3(3): 165-73.
7. Shi XC, Zhang LF, Zhang YQ, Liu XQ, Fei GJ. Clinical and laboratory diagnosis of intestinal tuberculosis. Chin Med J. 2016; 129(11): 1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—An update on the diagnosis, treatment and drug resistance. J. Respir. 2021; 1(2): 141-64.
9. World Health Organization. Implementing Tuberculosis Diagnostics: Policy Framework. Geneva: World Health Organization; 2015.
10. Lehman DC. Mycobacterium tuberculosis and nontuberculous mycobacteria. In: Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. p. 563-88.
11. Jha DK, Pathiyil MM, Sharma V. Evidence-based approach to diagnosis and management of abdominal tuberculosis. Indian J Gastroenterol. 2023; 42(1): 17-31.
12. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010; 88(2): 279-90.
13. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006; 387(3): 319-21.
14. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. Int J Res Health Sci. 2015; 3(4): 451-6.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary

tuberculosis. Jundishapur J Microbiol. 2015; 8(3): e21760. doi: 10.5812/jjm.21760.

16. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016; 17(2): 134-40.
17. Purnomo A, Bintoro UY, Sedana MP, Ashariati A. Association between Hasford scoring system and hematologic response in chronic and accelerated phase of chronic myelocytic leukemia patient with imatinib for three months. Mol Cell Biomed Sci. 2019; 3(2): 88-94.
18. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: A cross-sectional study. Pan Afr Med J. 2022; 42: 262-71.
19. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, *et al.* Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal IMAB. 2011; 17(1): 130-4.
20. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, *et al.* Serum adenosine deaminase: A novel biomarker tool for the diagnosis of tuberculosis. Biosci Biotechnol Res Asia. 2016; 13(1): 551-6.
21. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Tuberc Res Treat. 2018; 2018: 5740951. doi: 10.1155/2018/5740951.
22. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, *et al.* The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020; 20(1): 609. doi: 10.1186/s12879-020-05331-w.
23. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher neutrophil-lymphocyte ratio in TB/HIV co-infection compared to pulmonary tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
24. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. Medicine. 2022; 101(44): e31539. doi: 10.1097/MD.0000000000031539.
25. Bashir AB, Ageep Ali K, Abufatima AS, Mohamedani AA. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J Med Lab Diagn. 2020; 5(3): 29-34.
26. Khalil MM, Halim HAA, Abdelazeem MS. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. Egypt J Chest Dis Tuberc. 2020; 69(3): 458-65.
27. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: A comparative study. Med J Malaysia. 2011; 66(4): 318-21.

Supplementary 1.



ROC curve for (A) ADA test, (B) ESR test, and (C) ADA+ ESR tests.

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Ferry Sandra
Assignment title:	SIJALI 9
Submission title:	InaBJ V15N410 - High Adenosine Deaminase Level and Eryth...
File name:	V15N4A10.pdf
File size:	417.84K
Page count:	7
Word count:	3,910
Character count:	21,345
Submission date:	17-Jan-2024 08:44AM (UTC+0700)
Submission ID:	2272238753

DOI: 10.18585/inabj.v15i4.2406

RESEARCH ARTICLE

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Nuri Dyah Indrasari[1,2], Marcellus Simadibrata[3], Primariadewi Rustamadji[4], Yusra[2], Aria Kekalih[5], Suhendro[3], Alida Roswita Harahap[2], Heri Wibowo[6], Ida Parwati[7], Ferry Sandra[8,*]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[5]Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No.16, Jakarta 10430, Indonesia
[6]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia
[8]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: May 20, 2023; Revised date: Aug 18, 2023; Accepted date: Aug 21, 2023

Abstract

BACKGROUND: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

METHODS: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological test were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

RESULTS: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

CONCLUSION: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

Indones Biomed J. 2023; 15(4): 362-8

InaBJ V15N410 - High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

by Ferry Sandra

Submission date: 17-Jan-2024 08:44AM (UTC+0700)
Submission ID: 2272238753
File name: V15N4A10.pdf (417.84K)
Word count: 3910
Character count: 21345

RESEARCH ARTICLE

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Nuri Dyah Indrasari[1,2], Marcellus Simadibrata[3], Primariadewi Rustamadji[4], Yusra[2], Aria Kekalih[5], Suhendro[3], Alida Roswita Harahap[2], Heri Wibowo[6], Ida Parwati[7], Ferry Sandra[8,*]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
[5]Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No.16, Jakarta 10430, Indonesia
[6]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia
[8]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: May 20, 2023; Revised date: Aug 18, 2023; Accepted date: Aug 21, 2023

Abstract

BACKGROUND: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

METHODS: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological test were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

RESULTS: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group (p<0.05). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

CONCLUSION: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

Indones Biomed J. 2023; 15(4): 362-8

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).(1,2) The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was ≤9.9%. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3-16%.(3-5)

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.(3,6-10) The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.(11)

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.(11,12) Elevated ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.(12,13) The activity of ADA differs significantly between patients with pulmonary TB and the normal healthy people.(14,15)

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them are leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and erythrocyte sedimentation rate (ESR).(3,6,16,17) At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December 2022, with the inclusion criteria as follows; for the ITB group: ≥18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (No. KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.(16)

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in non-anticoagulated tubes were

allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzen, China) with enzymatic colorimetry principle test used. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry for measurement of leukocyte, basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistical Analysis

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p<0.05$.

The calculation of cut-off and area under the curve (AUC) was obtained from the receiver operating characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

Results

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI: 0.542-0.849) was obtained

Table 1. Characteristics of ITB and non-ITB groups.

Characteristics	ITB (n=16)	Non-ITB (n=127)
Age (years), mean±SD	42.19±18.28	43.53 ±16.65
Gender, n (%)		
Male	10 (62.5%)	40 (31.5%)
Female	6 (37.5%)	87 (68.5%)
Clinical manifestation, n (%)		
Weight loss	13 (81.3%)	69 (54.3%)
Night sweat	1 (6.3%)	5 (3.9%)
Cough	2 (12.5%)	5 (3.9%)
Fever	5 (31.3%)	16 (12.6%)
Loss of appetite	9 (56.3%)	41 (32.3%)
Non-specific abdominal pain	12 (75.0%)	111 (87.4%)
Chronic diarrhea	13 (81.3%)	73 (57.5%)
Constipation	13 (81.3%)	82 (64.6%)
Blood in stool	8 (50.0%)	69 (54.3%)
Mucus in stool	8 (50.0%)	70 (55.1%)
Blood and mucus in stool	8 (50.0%)	68 (53.5%)
TB history, n (%)		
History of contact with TB patient	1 (6.3%)	1 (0.8%)
History of pulmonary and non-pulmonary TB	2 (12.5%)	10 (7.9%)

Table 2. Laboratory results of ITB and non-ITB groups.

Parameter	ITB (n=16)	Non-ITB (n=127)	*p*- value
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10^3/µL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Tested with Mann-Whitney U test, *significant if $p<0.05$. Data were presented in median (min-max).

with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI: 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (95% CI: 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).(1,18)

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity

Table 3. ADA results of ITB and non-ITB groups with cut-off=12.56 IU/L.

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
Sensitivity	= 75%		
Specificity	= 57%		
Positive Predictive Value (PPV)	= 18%		
Negative Predictive Value (NPV)	= 95%		

Table 4. ESR results of ITB and non-ITB groups with cut-off=32.5 mm/hour.

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
Sensitivity	= 81%		
Specificity	= 62%		
Positive Predictive Value (PPV)	= 21%		
Negative Predictive Value (NPV)	= 96%		

and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.(15,19) The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L) (19) and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L) (20).

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.(21)

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.(22)

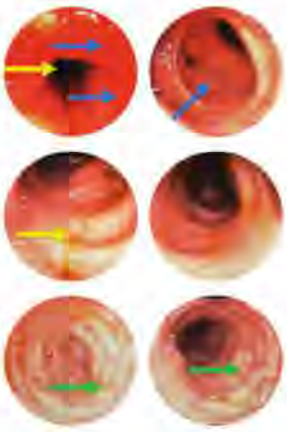


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.(23)

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar (22) and contradictive findings (24) in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.(25) In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.(25-27)

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

Conclusion

ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

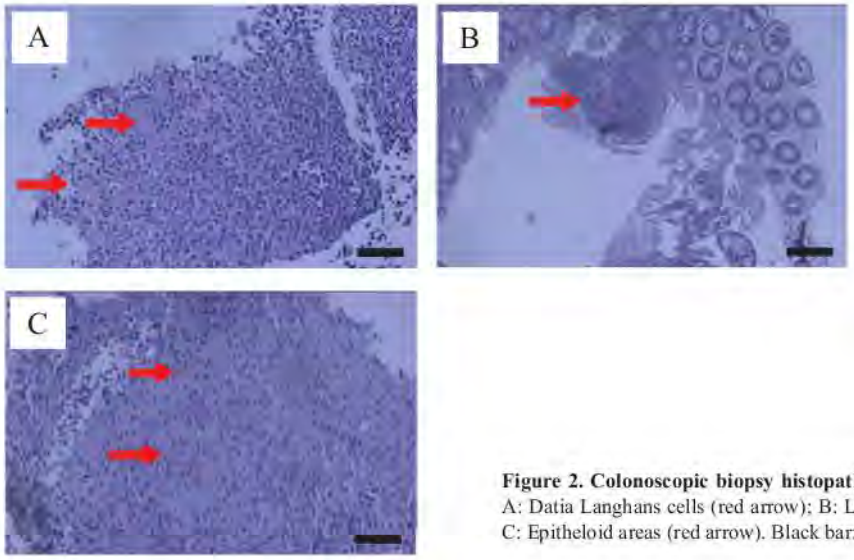


Figure 2. Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 µm.

Acknowledgements

The authors would thank to the patients, clinician staffs, nurse staffs, technician's laboratory, and the expert committee for their participation in this study.

Authors Contribution

NDI, MS, PR, Y, AK, S, ARH, HW, and IP were involved in concepting and planning the research. NDI, MS, PR, AK, ARH, HW, and IP performed the data acquisition/collection. NDI, MS, PR, Y, AK, and S calculated the experimental data and performed the analysis. NDI, MS, PR, Y, AK, ARH, HW, IP, and FS drafted the manuscript. NDI, MS, AK and S designed the figures and tables. NDI, MS, PR, Y, AK, S, ARH, HW, IP, and FS aided in interpreting the results. NDI, MS, PR, Y, AK, and FS took parts in giving critical revision of the manuscript.

References

1. World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
2. Yunda DK, Witjaksono F, Nurwidya F. Correlation between protein intake, fat free mass, and total lymphocyte count with quality of life in pulmonary tuberculosis patients undergoing intensive phase treatment in Pekanbaru, Riau Province. Mol Cell Biomed Sci. 2020; 4(3): 128-34.
3. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, *et al.* Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010; 11(3): 143-9.
4. Evans RP, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016; 18(12): 45. doi: 10.1007/s11908-016-0546-5.
5. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015; 17(1): 3-11.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. J Penyakit Dalam Indones. 2016; 3(3): 165-73.
7. Shi XC, Zhang LF, Zhang YQ, Liu XQ, Fei GJ. Clinical and laboratory diagnosis of intestinal tuberculosis. Chin Med J. 2016; 129(11): 1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—An update on the diagnosis, treatment and drug resistance. J. Respir. 2021; 1(2): 141-64.
9. World Health Organization. Implementing Tuberculosis Diagnostics: Policy Framework. Geneva: World Health Organization; 2015.
10. Lehman DC. Mycobacterium tuberculosis and nontuberculous mycobacteria. In: Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. p. 563-88.
11. Jha DK, Pathiyil MM, Sharma V. Evidence-based approach to diagnosis and management of abdominal tuberculosis. Indian J Gastroenterol. 2023; 42(1): 17-31.
12. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010; 88(2): 279-90.
13. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006; 387(3): 319-21.
14. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. Int J Res Health Sci. 2015; 3(4): 451-6.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary

tuberculosis. Jundishapur J Microbiol. 2015; 8(3): e21760. doi: 10.5812/jjm.21760.

16. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016; 17(2): 134-40.
17. Purnomo A, Bintoro UY, Sedana MP, Ashariati A. Association between Hasford scoring system and hematologic response in chronic and accelerated phase of chronic myelocytic leukemia patient with imatinib for three months. Mol Cell Biomed Sci. 2019; 3(2): 88-94.
18. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: A cross-sectional study. Pan Afr Med J. 2022; 42: 262-71.
19. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, *et al.* Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal IMAB. 2011; 17(1): 130-4.
20. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, *et al.* Serum adenosine deaminase: A novel biomarker tool for the diagnosis of tuberculosis. Biosci Biotechnol Res Asia. 2016; 13(1): 551-6.
21. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Tuberc Res Treat. 2018; 2018: 5740951. doi: 10.1155/2018/5740951.
22. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, *et al.* The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020; 20(1): 609. doi: 10.1186/s12879-020-05331-w.
23. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher neutrophil-lymphocyte ratio in TB/HIV co-infection compared to pulmonary tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
24. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. Medicine. 2022; 101(44): e31539. doi: 10.1097/MD.0000000000031539.
25. Bashir AB, Ageep Ali K, Abufatima AS, Mohamedani AA. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J Med Lab Diagn. 2020; 5(3): 29-34.
26. Khalil MM, Halim HAA, Abdelazeem MS. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. Egypt J Chest Dis Tuberc. 2020; 69(3): 458-65.
27. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: A comparative study. Med J Malaysia. 2011; 66(4): 318-21.

InaBJ V15N410 - High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

ORIGINALITY REPORT

5%	6%	6%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.karger.com Internet Source	1%
2	Bethoux, F., N. Boulis, S. McClelland, M. A. Willis, M. Hussain, A. Machado, L. Mychkovsky, D. Stough, M. Sutliff, and E. P. Pioro. "Use of Intrathecal Baclofen for Treatment of Severe Spasticity in Selected Patients With Motor Neuron Disease", Neurorehabilitation and Neural Repair, 2013. Publication	1%
3	dspace.library.uu.nl Internet Source	1%
4	assets.cureus.com Internet Source	1%
5	S. Rathod, Veena. "Serum Adenosine Deaminase in Pulmonary Tuberculosis and Common Respiratory Diseases", Rajiv Gandhi University of Health Sciences (India), 2023 Publication	1%

6	www.frontiersin.org Internet Source	1%
7	perpustakaan.poltekkes-malang.ac.id Internet Source	<1%
8	pubmed.ncbi.nlm.nih.gov Internet Source	<1%

Exclude quotes	On	Exclude matches	< 15 words
Exclude bibliography	On		

InaBJ V15N410 - High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

GRADEMARK REPORT

FINAL GRADE

/15

GENERAL COMMENTS

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 1 - Resubmit for Review

Secretariat of InaBJ <secretariatinabj@gmail.com> Mon, Jun 26, 2023 at 8:02 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis**".

Our decision is: **Resubmit for Review.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **July 3, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/2406, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,
--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org

4 attachments

- **Round 1 Reviewer 1 - F09 Manuscript Review Form.pdf** 174K
- **Round 1 Reviewer 1 - Manuscript.docx** 4996K
- **Round 1 Reviewer 2 - F09 Manuscript Review Form.pdf** 494K
- **Round 1 Reviewer 3 - F09 Manuscript Review Form.pdf** 176K

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Manuscript Review Form

Reviewer	:	Reviewer 1
Manuscript #	:	M2023128
Manuscript Title	:	Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?	**V**	
	Notes:		
2.	Are the title and abstract of the manuscript appropriate?		V
	Notes:		
3	Do the title and abstract reflect the study result/content?		v
	Notes: Part of the title does not reflect the content		
4.	Is the significance of the study well explained at the Background?		V
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?		V
	Notes:		
6.	Are the results, ideas, and data presented in this manuscript important enough for publication?		V

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

	Notes:		
7.	Are all figures and tables necessarily presented?		V
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?		V
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?		V
	Notes:		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?		V
	Notes:		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	V
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Date and Sign:
June 9, 2023

Reviewer 1

Need to include this new reference

Evidence-based approach to diagnosis and management of abdominal **tuberculosis**. Jha DK, Pathiyil MM, Sharma V.Indian J Gastroenterol. 2023 Feb;42(1):17-31. doi: 10.1007/s12664-023-01343-x.

Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis

Abstract

Background: Currently, laboratory diagnosis of ITB???? is limited and opens an ~~still~~ opportunity for other?? laboratory tests. There is not many data about adenosine deaminase (ADA) and haematology tests in patients with ITB. This study aimeds to determine the role of ADA and haematology tests in patients with ITB.

Method: →**PAST TENSE!**

The subjects are adult patients aged ≥18 years with suspected ITB, who come to the Gastroenterology Outpatient Clinic and undergo a colonoscopy and histopathology examinations. Activity of ADA is examined by using Mindray BS-200 analyzers. Haematology tests are performed by Sysmex XN-2000 Haematology Analyzer and ESR by Starrsed RS ESR.

Result: There were 143 subjects, consist of 16 (11.2%) subjects with ITB and 127 (88.8%) subjects non-ITB →SUSPECT OF ???? CROHN??? . ADA activity of ~~all subjects~~, ITB group, and non-ITB group were ~~12.15 (2.95-45.57),~~ 21.38 (± 12.79), and 12.11 (2.95-45.57) IU/L. ADA activity in the ITB and non-ITB groups was higher significant (p= 0.01). AUC of ADA was 0.695 with a cut-off of 12.56 IU/L, sensitivity of 75%, and specificity of 57%. There were no significant differences in leukocyte count, differential count (basophile, eosinophile, neutrophile, lymphocyte, and monocyte), NLR, and MLR between ITB and non-ITB groups. ESR result mention the meanin the ITB and non-ITB groups was higher

Formatted: English (U.S.)

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

Formatted: English (U.S.)

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

Formatted: Font: (Default) Times New Roman, 12 pt, Strikethrough

Formatted: Font: (Default) Times New Roman, 12 pt, Strikethrough

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

significant (p=0.00). AUC of ESR was 0.741 with a cut-off of 32.5 mm/hour, sensitivity of 81%, and specificity of 62%.

Conclusion: ADA activity and ESR tests can be used potential candidates for laboratory tests in suspected ITB patients.

Keyword: intestinal tuberculosis, adenosine deaminase, haematology tests

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia is on the third rank of the countries with highest burden of tuberculosis (TB).[1] The global prevalence of extrapulmonary TB is 14% and the prevalence in Indonesia is ≤ 9.9%. Intestinal tuberculosis (ITB) as part of abdominal TB has prevalence of approximately 3–16%.[2-4]

Currently, ITB diagnosis is established based on clinical manifestation, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.[2,6-10] The limitations of the respective laboratory tests make it difficult to diagnose ITB, and hence, providing opportunities for various other laboratory tests to diagnose ITB.

Adenosine deaminase (ADA) is an enzyme that is involved in purine metabolism to catalyze the conversion of adenosine to inosine. Proliferation and differentiation of lymphocytes and maturation of monocytes require ADA enzymes.[11,12] In TB disease, an increase of ADA in the blood or body fluid is reported due to stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.[11,12] Varma et al[13] and Salmanzadeh et al[14] reported activity of ADA with significant difference in patients with pulmonary TB and normal controls.

In ITB, there is acute or chronic inflammation due to infection process. Haematological parameters as markers of inflammation or infection include leukocyte count, leukocyte differential count (basophile, eosinophile, neutrophile, lymphocyte, and monocyte), Neutrophile to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR). Haematology tests are often requested by clinicians in patients suspected of having ITB.[2,6,15] Currently, some inflammatory markers have been known, such as Neutrophile to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).

The role of ADA serum and haematology tests in ITB patients are still not widely known yet. The objective of this study is to determine the role of ADA and haematology tests in patients suspected with ITB. Should there be any significant result of ADA and haematology tests, it is hoped that they can be used to assist in diagnosing ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of XXX. The study was conducted from December 2020 until December 2022. The inclusion criteria of the study subjects were adult patients aged ≥18 years suspected of ITB, who came to the Gastroenterology Outpatient Clinic, Department of Internal Medicine in Gastrointestinal Endoscopy Center XXX, and/or histopathology test. The exclusion criteria of the study subjects were subjects who underwent treatment with anti-tuberculosis drugs > 3 months or post-treatment with anti-tuberculosis drugs < 6 months. Diagnosis of suspected ITB was made by an internal medicine specialist based on the medical history and physical examination, i.e., if there are 3 of 4 main clinical findings and 1 of 3 additional history. Main clinical complaints include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. Additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (< 6 months of treatment), and positive contact with TB patients. The study subjects were given an explanation and asked for their willingness to participate by signing an informed consent form. History taking was performed to determine whether the subject complies with the inclusion and exclusion criteria.

Colonoscopy Examination

A colonoscopy examination was performed by a clinician of Gastroenterology Division, Department of Internal Medicine in Gastrointestinal Endoscopy Center XXX. Before a colonoscopy examination, the patient is asked to empty the digestive tract by consuming laxatives at night or in the morning. Laxatives have been prescribed by a doctor. Patients are given sedative drugs and pain relievers. The patient lies in a supine position and the knees are bent towards the chest. Next, the doctor will insert a colonoscope into the anus while pumping air into the large intestine to expand the intestine, so that the intestinal wall can be seen clearly. Patients are also asked to fasting until the colonoscopy is completed. The colonoscopy procedure takes about 30–60 minutes. The doctor will take the necessary pictures. If any abnormalities are found, the doctor will take a sample of intestinal tissue for histopathological examination. Colonoscopy in patient suspected of having ITB may find various features, including ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. The intestinal area affected by ITB can be found from ileum to caecum, in the form of continuous lesions or single or skip lesions.[16] If abnormalities found in the colonoscopy, a biopsy will be done and the specimen obtained will be sent to the Anatomic Pathology Laboratory of XXX for histopathology test.

Histopathology Examination

Histopathology examination is using biopsy material from colonoscopy. In ITB, the gut is harvested from at least 5 granulomas from biopsies originating from one segment. Histopathological procedure were tissue cutting, tissue fixation, grossing, embedding, trimming, fishing/plating, staining of tissue with hematoxylin (Epredia, Kalamzoo, US) and eosin (Merck, Darmstadt, Germany), and finally mounting. Prepared tissue slides will be read by an anatomic pathology specialist. The histopathological result in ITB is formed of

submucosal granulomatous inflammatory features. TB granuloma is characterized by lesions in the submucosa in the form of epitheloid cells, Datia Langhans giant cells, lymphocytes at the edges of granulomas, and caseous necrosis.[17]

ADA Test

ADA and haematology tests were performed in the Clinical Pathology Laboratory of XXX. Vein blood sample was used for ADA and haematology tests. 9 mL of venous blood was taken from the cubital vein which was collected in a 3 mL EDTA tube for complete hematological examination and ESR, 6 mL non-anticoagulant tube for ADA examination. Blood in a tube non-anticoagulant, allowed to stand at room temperature for about 30 minutes, until a clot forms. Then immediately centrifuge 3,000 rotates per minute (rpm) for 15 minutes to separate the serum contained in the top layer of the tube.

Quantitative test of blood ADA was performed by Mindray BS-200 automatic clinical chemistry. The principle of ADA test is enzymatic colorimetric method. Adenosine deaminase irreversibly converts adenosine into inosine. Inosine is then released from ribose by purine nucleoside phosphorylase (PNP) so that hypoxanthine is formed. Hypoxanthine is oxidized by xanthine oxidase (XOD) to uric acid and hydrogen peroxide (H_2O_2). Hydrogen peroxide along with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl aniline (EHSPT) and 4-amino and imipramine Lin (4-AAP) is converted into coloured benzoquinone by peroxidase (POD). The oxidation rate of benzoquinone is equivalent to the activity of ADA in the sample, which is determined by measuring the increase of absorbance photometrically at a wavelength of 546 nm.[18]

Haematology Test

Haematology test was performed by Sysmex XN-2000 haematology analyzer with semiconductor laser flowcytometry principle. K3EDTA blood is diluted by reagent that lyses erythrocytes and make holes in the cytoplasmic membrane of all nucleated cells, so that fluorescence dye can enter the cells and bind to the DNA/RNA of the cells. The diluted sample goes into a central core whose outer envelope is coated by a very strong fluid flow (sheath fluid). Afterward, the laser beam will be fired towards the cell from 3 sides, i.e., Forward Scattered Light (FSC), Side Scattered Light (SSC), and Side Fluorescent Light (SFL). Forward Scattered Light (FSC) informs the size of the cell. Side Scattered Light (SSC) informs the complexity or characteristics of the cell. Side Fluorescent Light (SFL) informs the contents of nucleic acids and cell organelles.[19] ESR inspection was performed using Starrsed RS ESR automatic analyzer.[20]

The results of colonoscopy, histopathology, and response towards treatment with anti-tuberculosis drugs are used as the gold standard in diagnosing ITB. This study was approved by the Health Research Ethics Committee – XXX with Ethical Clearance number XXX.

Statistics

Data processing using statistical product and service solution (SPSS) program version 20 and Microsoft Excel 2016. The characteristics of the study subject are presented descriptively. The data distribution is checked by Kolmogorov-Smirnov test. The distribution of ADA activity and haematology data were abnormal, therefore the data were presented in medians and ranges. Bivariate analysis to investigate the relationship between two variable. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann Whitney U Test. The level of significance used is $\alpha = 0.05$.

Formatted: Font: (Default) Times New Roman, 12 pt, Highlight

The calculation of cut-off and Area Under the Curve (AUC) is obtained from the ROC (Receiver Operating Characteristic) curve, and then the sensitivity and specificity of the examination to the gold standard were calculated (combined results of histopathology, colonoscopy, and therapeutic response).

RESULTS

Baseline Characteristics

A total of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB. The other baseline characteristics are summarized in Table 1.

Table 1. Baseline Characteristics of Subjects

Characteristics	~~Total (N=143, 100%)~~		ITB (N=16, 11.2%)		Non-ITB (N=127, 88.8%)		*p**
Age (years)	~~43 (± 16.8)~~		37 (± 17.9)		44 (± 16.6)		0.12
Sex							
- Male, N(%)	~~50~~	~~35.0%~~	10	7.0%	40	28.0%	0.02
- Female, N(%)	~~93~~	~~65.0%~~	6	4.2%	87	60.8%	
Clinical manifestation							
- Weight loss N(%)	~~82~~	~~57.3%~~	13	9.1%	69	48.3%	0.06
- Night sweat N(%)	~~6~~	~~4.2%~~	1	0.7%	5	3.5%	0.51
- Cough N(%)	~~7~~	~~4.9%~~	2	1.4%	5	3.5%	0.17
- Fever N(%)	~~21~~	~~14.7%~~	5	31%	16	12,6%	0.06
- Loss of appetite N(%)	~~50~~	~~35.0%~~	9	6.3%	41	28.7%	0.09
- Non-specific abdominal pain N(%)	~~123~~	~~86.0%~~	12	8.4%	111	77.6%	0.24
- Chronic diarrhea N(%)	~~86~~	~~60.0%~~	13	9.1%	73	51.0%	0.10
- Constipation N(%)	~~95~~	~~66.4%~~	13	9.1%	82	57.3%	0.26
- Diarrhea and constipation N(%)	~~85~~	~~59.4%~~	13	9.1%	72	50.3%	0.06
- Blood in stool N(%)	~~77~~	~~53.8%~~	8	5.6%	69	48.3%	0.79
- Mucus in stool N(%)	~~78~~	~~54.5%~~	8	5.6%	70	49.0%	0.79
- Blood and mucus in stool N(%)	~~76~~	~~53.1%~~	8	5.6%	68	47.6%	0.79
TB history							
- History of contact with TB patient N(%)	~~2~~	~~1.4%~~	1	0.7%	1	0.7%	0.21
- History of pulmonary and non-pulmonary TB N(%)	~~12~~	~~8.4%~~	2	1.4%	10	7.0%	0.62

**p value: ITB group to non-ITB group*

Formatted: Strikethrough

The results of laboratory test in ITB and non-ITB groups are summarized in Table 2. There were significant differences in blood ADA activity and ESR, with p values from Mann Whitney U test were 0.01 and 0.00, respectively.

Table 2. The Results of Laboratory Test of Subjects in ITB and Non-ITB Groups

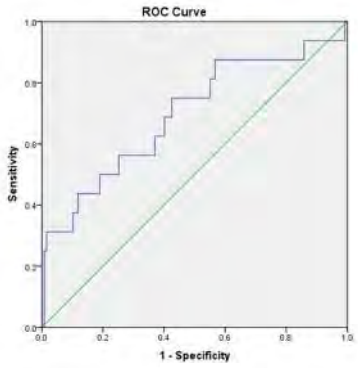
➔ THIS IS MEDIAN, need to mention here

	~~Total~~ n =143, 100%	~~Diagnosed with~~ ITB n =16, 11.2%	Non-ITB n =127, 88.8%	*p**
ADA level in blood (IU/L)	~~12.15 (2.95-45.57)~~	16.51(5.76-44.89)	12.11 (2.95-45.57)	0.01
Leukocyte (10^3/µL)	~~6.99 (2.09-23.84)~~	7.63(2.09-11.85)	6.98(2.90-23.84)	0.65
Basophil (%)	~~0.5 (0-2)~~	0.50(0.00-1.40)	0.5 (0.00-2.00)	0.35
Eosinophil (%)	~~1.9 (0-24)~~	1.80(0.00-8.10)	1.9 (0-24)	0.94
Neutrophil (%)	~~61.30(33.10-87.70)~~	63.85(38.50-86.60)	61.00(33.10-87.70)	0.52
Lymphocyte (%)	~~28.30(5.60-53.20)~~	25.70(7.00-51.60)	29.10(5.60-53.20)	0.52
Monocyte (%)	~~6.6 (3.3-15.9)~~	7.20(3.30-12.90)	6.60(3.50-15.90)	0.67
NLR	~~2.15(0.67-15.37)~~	2.48(0.75-11.40)	2.12(0.67-15.37)	0.49
MLR	~~0.22 (0.09-2.84)~~	0.29(0.09-1.79)	0.22 (0.09-2.84)	0.40
ESR (mm/hour)	~~28 (1-140)~~	65(9-140)	26(1.00-134.00)	0.00

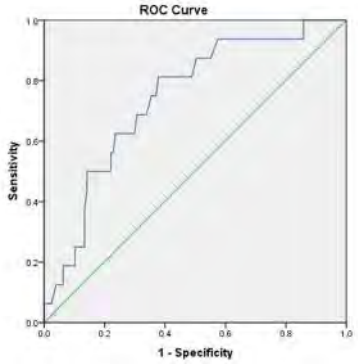
**p value: ITB group to non-ITB group*

The Result of Blood ADA Test

The median of ADA activity in blood of all subjects, ITB group, and non-ITB group were 12.15 (2.95-45.57) IU/L, 21.38 (± 12.79) IU/L, and 12.11 (2.95-45.57) IU/L, respectively. Blood ADA activity in ITB and non-ITB groups were higher and significantly different (p=0.01). In the ROC curve analysis, and AUC of 0.695 was obtained with a cut-off of 12.56 IU/L, sensitivity of 75%, and specificity of 57% (Figure 1(a) and Table 3).

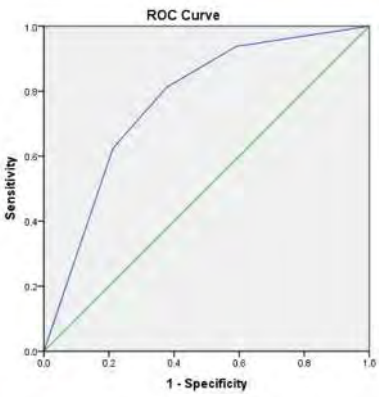


(a)



(b)

Formatted: Highlight
Formatted: Bulleted + Level: 1 + Aligned at: 0,63 cm + Indent at: 1,27 cm
Formatted: English (U.S.), Highlight
Formatted: Font: 10 pt
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough
Formatted: Strikethrough



(c)

Figure 1. ROC curve for (a) blood ADA test, (b) ESR test, and (c) ADA+ ESR tests

Table 3. Blood ADA Diagnostic Test

Result of Blood ADA (cut-off 12.56 IU/L)	Gold Standard		
	ITB	**Non-ITB**	**Total**
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

Haematology Test Results

The result of leukocyte count from all subjects, ITB group, and non-ITB group showed the following median and mean respectively 6.99 (2.09-23.84) $10^3/\mu L$, 7.6 (± 3.2) $10^3/\mu L$, and 7.7 (2.9-23.84) $10^3/\mu L$ (Table 2). There was no significant difference between the ITB and non-ITB groups in leukocyte count, leukocyte differential count (for basophil, eosinophil, neutrophil, lymphocyte, monocyte), NLR and MLR with p value 0.65, 0.35, 0.94, 0.52, 0.52, 0.67, 0.49 and 0.40, respectively (Table 2).

The median of ESR of all subjects, ITB group, and non-ITB group were 28(1-140) mm/hour, 65.87(± 38.1) mm/hour, and 36.75(± 33) mm/hour, respectively (Table 2). ESR result in ITB and non-ITB groups were higher and significantly different (p=0.00). In the

ROC curve analysis, an AUC of 0.741 was obtained with a cut-off of 32.5 mm/hour, sensitivity of 81%, and specificity of 62% (Figure 1 (b) and Table 4).

Table 4. Blood ESR Diagnostic Test

ESR Test Result (cut-off 32.5 mm/hour)	Gold Standard		
	ITB	**Non-ITB**	**Total**
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
- Sensitivity	= 81%		
- Specificity	= 62%		
- Positive Predictive Value (PPV)	= 21%		
- Negative Predictive Value (NPV)	= 96%		

Combination of Blood Biomarkers

From the blood ADA and ESR tests, a combination of tests was performed to diagnose ITB with a cut-off of 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and an AUC of 0.768 (CI 0.656-0.880) (Figure 1 (c)), sensitivity of 81.3%, and specificity of 62.2 % were obtained.

Histopathological Result

The histopathological result several patients with confirmed ITB showed complete overview of ITB markers in the form of granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2), while some other subjects showed incomplete and varied overview.

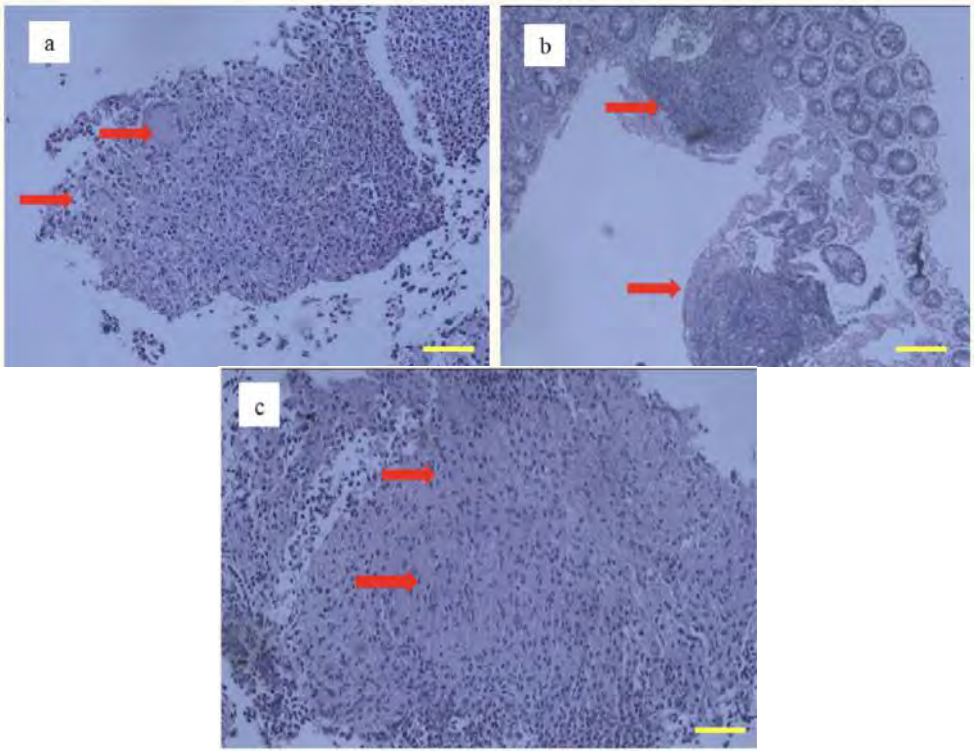


Figure 2. Histopathological result of subjects with confirmed ITB a. Overview of Datia Langhans cells (red arrows) b. Overview of lymphoid aggregates (red arrows) c. Overview of epitheloid areas (red arrows). Yellow bar: 20x magninification.

Colonoscopy Result

The colonoscopy result of patients with ITB in this study showed multiple ulceration, oedema, and hyperaemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum as seen in Figure 3.

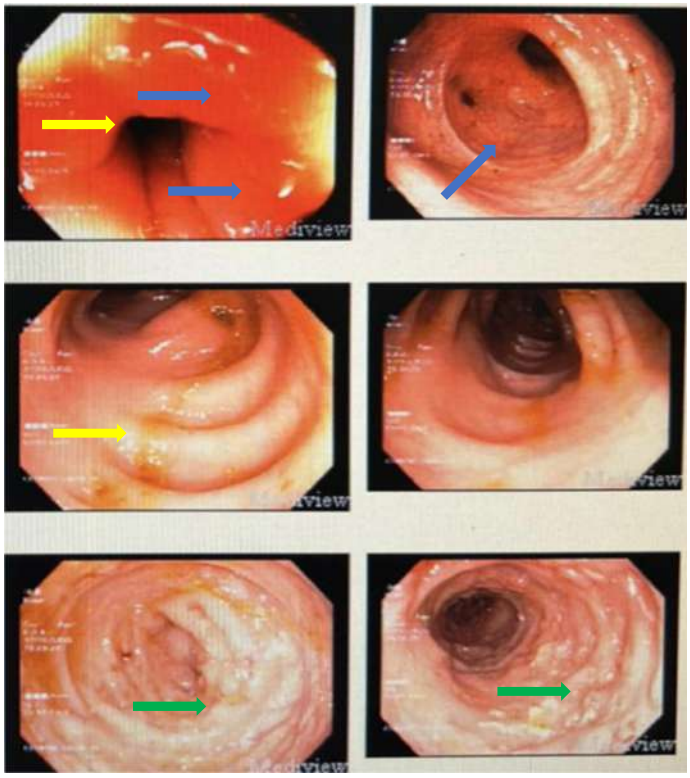


Figure 3. Colonoscopy result in patient with ileocolitis who were suspected of having TB showed multiple ulceration (green arrow), oedema (yellow arrow), and hyperaemic intestinal mucosa (blue arrow).

Discussion

A total of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were non-ITB. The proportion of ITB in this study was 11.2%, which is accordance with the ITB prevalence of 3-16%.[1] The proportion of ITB in XXX in this study is almost the ~~same as Suparmin et al study~~, i.e., 8/60 (13.3%).[5]

Formatted: Strikethrough

The age and sex characteristics of the subjects in this study is almost the same as Suparmin et al. The mean age of the subjects in TB and non-TB colitis groups in the study conducted by Suparmin et al were 38.6 (± 15.76) years and 49.7 (±17.96) years. The mean age of the subjects of this study in TB and non-TB colitis groups were 37 (± 17.9) years and 44 (± 16.6) years. The majority of sex characteristic in this study were females (65%), same with Suparmin et al (55%)[5].

In this study, there were significant differences in blood ADA enzyme activity between ITB and non-ITB groups (p= 0.011) with ADA activity of 21.38 (± 12.79) IU/L and 12.11 (2.95-45.57) IU/L, respectively. The sensitivity and specificity of blood ADA test were 75% and 57%, respectively, with a cut-off of 12.56 U/L. There are limited reports regarding ADA test in patients with ITB. Most reports were reporting ADA test in patients with pulmonary TB and abdominal TB with ascites (Table 5).

The comparison of sensitivity and specificity of blood ADA test in this study and other studies can be seen in Table 5. The result of sensitivity and specificity of blood ADA test in this study are in line with the result obtained by Salmanzadeh et al[14] in pulmonary TB cases, i.e., 77% and 70% with ADA cut-off point of 15.5 U/L. However, the result of sensitivity and specificity in this study are different with Stevanovic et al[21], i.e., 56% and 89% in extrapulmonary TB subjects with a cut-off of 24 U/L. The differences might be Stevanovic et al used the reference value from the test kit (24 U/L) and did not use a new cut-off that might be optimal for ADA test in extrapulmonary TB.

Table 5. Comparison of ADA activity in various TB patients

DISCUSSION WITH TABLE?

Formatted: Font: 10 pt, Highlight

Formatted: English (U.S.)

No.	Study	Patient	Specimen	*Cut-off* (U/L)	Sensitivity	Specificity
1	This study	Intestinal TB	Serum	12.56	75%	57%
2	Salmanzadeh et al[14]	Pulmonary TB	Serum	15.5	77%	70%
3	Stevanovic et al [21]	Extrapulmonary TB (Peritonitis TB. Spondylitis TB, lymph node TB, ovarian, Tuba fallopi, renal, etc.)	Serum	24	56%	89%
4	Pandey R et al[24]	Pulmonary TB, extrapulmonary TB, healthy controls	Serum	25	90.7%	100%

ADA consists of 2 isoenzymes, namely ADA1 and ADA2. ADA1 is an intracellular protein that is expressed mainly by T, B lymphocytes, and natural killer cells. ADA1 plays a role in the immune mechanism. ADA2 is an intracellular protein that is expressed primarily by monocytes and macrophages. ADA2 activity is increased in tuberculous pleural effusion, cerebrospinal fluid, and autoimmune disease. ADA activity in T lymphocytes was 10-12 times higher than in B lymphocytes. ADA activity was reported to increase in TB patients due to T cell stimulation by MTB antigen.[22.23]

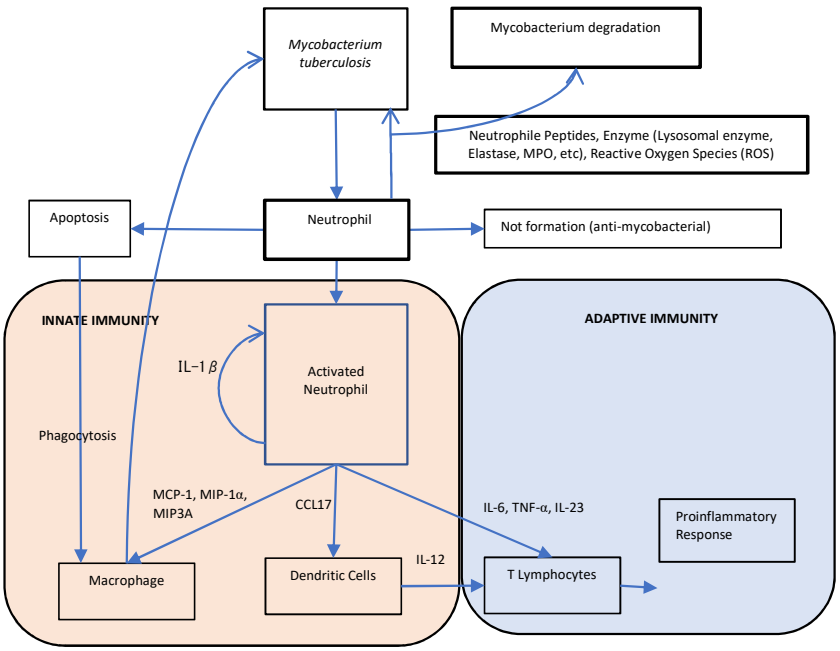
In ITB patients, when granulomas rupture and release MTB, macrophages in intestinal tissue will phagocytize MTB and present MTB antigen to T lymphocytes. Subsequently, T lymphocytes will be activated to produce ADA at the time of proliferation and differentiation of T lymphocytes. Apart from T lymphocytes, ADA is also produced by monocytes in the process of maturation into macrophages. Therefore, ADA can be used to diagnose ITB.

The cut-off value of ADA test have a lot of varies. Therefore, it is need to be determine a suitable cut-off value according to the patient population. The cut-off value of the ADA kit in this study was the same with Stevanovic et al[21], i.e., 24 U/L, while Salmazadeh et al[14] and this study obtained cut-off values of 15.5 U/L and 12.56 U/L, respectively. Pandey et al[24] used a higher cut-off value for ADA serum, i.e., 25 U/L.

There were no significant differences in leukocyte count, leukocyte differential count, neutrophil to lymphocyte ratio (NLR), and monocyte to lymphocyte ratio (MLR) between ITB and non-ITB groups. Reports on leukocyte count, leukocyte differential count, NLR, and MLR in ITB cases are still rare. Most reports on leukocyte count, leukocyte differential count, NLR, and MLR are in pulmonary TB cases. The results of leukocyte count and leukocyte differential count in this study are in line with Abay et al.[25] The results of leukocyte count and leukocyte differential count that reported by Abay et al were not significantly different.[25]

The NLR result of this study did not show any significant difference between ITB and non-ITB groups. It could possibly because in this study, the ITB cases were chronic which were supported by high ESR results of the subjects, while NLR plays more role in early cases of acute inflammation/infection or early TB infection. The results of this study is in line with those obtained by Rees et al[26] in subjects with pulmonary TB, but it is not in accordance with the report of Sulastri et al[27] who got a higher and significant NLR in the pulmonary TB group.

Neutrophil to lymphocyte ratio (NLR) is an indicator of acute inflammation. Neutrophil plays a role in the MTB killing process in the early days of infection. MTB germs that enter the body will stimulate neutrophils and phagocyte MTB. Neutrophils kill MTB by secreting various enzymes and peptides that are present in its granules (lysosomal enzymes, neutrophil peptides, and reactive oxygen species (ROS)). Activated neutrophils produce IL-8, IL-1β, and IFN-γ cytokines which in turn activate monocytes/ macrophages, dendritic cells, and lymphocytes.Neutrophils will release Neutrophil Extracellular Traps (NET) to the circulation which will trap MTB germs. Neutrophil response is related to the degree of inflammation and tissue damage. Inadequate neutrophil phagocytosis of MTB will result in damage to host cells causing tissue necrosis. Inflammatory processes that last a long time will result in chronic inflammation and damage to body tissues. (Figure 4).[28]



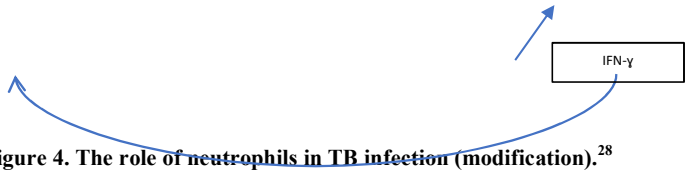


Figure 4. The role of neutrophils in TB infection (modification).[28]

There were no significant differences in MLR result between ITB and non-ITB groups in this study. The results of this study are in line with those reported by Rees et al[26] who did not find any significant differences in MLR. However, a systematic review by Adane et al[31] reported significant differences of MLR in patients with TB. These varied results of MLR studies depended on the origin of subject's location, existence of any comorbidities (HIV, diabetes mellitus), age, TB treatment, and the selected control group.[26]

Monocytes play a role in natural/nonspecific immune responses, while lymphocytes play a role in specific immune responses. Monocytes or macrophages in ITB play a role in non-specific immune responses in the form of phagocytosis against MTB. Subsequently, activated macrophages secrete pro-inflammatory cytokines to recruit monocytes and dendritic cells to the site of infection.[29-32] Monocytes further differentiate into blood-derived macrophages, so that MTBs can be internalized, but not destroyed. In that phase, blood-derived macrophages accumulate, and MTB grows logarithmically.[33] Dendritic cells activate the immune response by carrying MTB to the lymph nodes and presenting it to T lymphocytes. Furthermore, IL-12 secreted by macrophages activates lymphocytes T (especially the TH1 subset of CD4+ T lymphocytes) to produce IFN-γ, so that macrophages can kill MTB (Figure 4). The accumulation of blood-derived macrophages and activation of T lymphocytes will induce the formation of granulomatous lesions in the form of epithelioid tubercles.[33]

ESR test result in the ITB group was higher and significant when compared to the non-ITB group with results of 65.87 (± 38.1) mm/hour and 36.75 (± 33) mm/hour, respectively. The sensitivity and specificity of ESR test was 81% and 62%, respectively, with a cut-off of 32.5 mm/hour. ESR test with high sensitivity (81%) can be used as a screening test in ITB. ESR report in ITB cases is still rare. Most ESR tests were reported in pulmonary TB cases. The overall ESR test results from various studies can be seen in Table 5.

Table 5. Comparison of ESR result in various TB patients

No.	Study	Patient (mm/hour)	Comparator mm/hour	*p**
1	This study	ITB patients 65.87 (± 38.1)	Non-ITB patients 36.75 (± 33)	0.00
2	Khalil et al[34]	Before pulmonary TB treatment 49.41 mm/hour	After pulmonary TB treatment 33.85 mm/hour	0.02
3	Bashir et al[35]	Pulmonary TB patients 115.17	Control 11.08	0.00
4	Chong[36]	Pulmonary TB 57± 39	Abdominal TB 46± 28	0.305

ESR test has been in use for a long time to assess acute phase proteins in chronic diseases, including TB disease. Pro-inflammatory cytokines produced in ITB disease will stimulate the liver to produce acute phase proteins, such as fibrinogen which can increase ESR result. ESR test has limitations, such as affected by anemic conditions and hypoalbuminemia which cause a false increase of ESR. Therefore, when using ESR test in patients with ITB, it is necessary to pay attention to the condition of anemia and hypoalbuminemia.[34-36]

Combination of Blood Biomarkers

The combination of ADA serum and ESR test with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not result in a significant increase in sensitivity and specificity

when compared to each of ADA or ESR. Therefore, each ADA or ESR test can be used independently to assist in diagnosing ITB.

Conclusion

Blood ADA and ESR tests were significantly higher among ITB compared to Non ITB grups. Blood ADA and ESR test have a high sensitivity value so that they are potential as screening biomarkers for intestinal TB patients. There were no significant differences in leukocyte count, leukocyte differential count, neutrophil to lymphocyte ratio (NLR), and monocyte to lymphocyte ratio (MLR) between ITB and non-ITB groups.

References

1. WHO. Global tuberculosis report 2019. [Accessed on 15 June 2020]. Available in: https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1.
2. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, et al. Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010;11:143-9.
3. Evans RPT, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016;18:1–8.
4. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015;17:3–11.
5. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: a cross-sectional study. Pan Afr Med J. 2022 Aug 10;42:262-71.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. Jurnal Penyakit Dalam Indonesia. 2016;3:165-73.
7. Shi X-C, Zhang L-F, Zhang Y-Q, Liu X-Q, Fei G-J. Clinical and laboratory diagnosis of intestinal tuberculosis. Chinese Medical Journal. 2016;129:1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary Tuberculosis—An Update on the Diagnosis, Treatment and Drug Resistance. J. Respir. 2021; 1(2):141-64.
9. WHO. Implementing tuberculosis diagnostics: policy framework. Geneva: WHO.(2015)
10. Lehman DC. Mycobacterium tuberculosis and Nontuberculous Mycobacteria. In Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. h. 563-88.
11. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010;88:279-90.
12. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006;387:319-21.

13. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. International Journal of Research in Health Sciences. 2015;3:451-6.
14. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. Jundishapur J Microbiol. 2015;8:1-5.
15. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016;17:134-40.
16. Moka P, Ahuja V, Makharia G. Endoscopic features of gastrointestinal tuberculosis and crohn's disease. Journal of Digestive Endoscopy. 2017;8:1-11.
17. Chong VH, Lim KS. Gastrointestinal Tuberculosis. Singapore Med J. 2009;50:638-45.
18. Mindray. Adenosine Deaminase/ ADA Kit (Enzymatic Colorimetric Method). Insert Kit. 2022:1-5.
19. Sysmex. XN-SERIES™ Automated Hematology Analyzer Reshaping Compact Automation. [Accessed on 2 August 2020]. Available in: www.sysmex.com/us.
20. RR Mechatronics. Starred RS User Manual. Netherland, RR Mechatronics International B.V. 2015.
21. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, et al. Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal of IMAB. 2011;17:130-4.
22. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010;88:279-90.
23. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006;387:319-21.
24. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, et al. Serum Adenosine Deaminase: A Novel Biomarker Tool for The Diagnosis of Tuberculosis. Biosci Biotechnol Res Asia. 2016;13(1):551-6.
25. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological Abnormalities of Pulmonary Tuberculosis Patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A Comparative Cross-Sectional Study. Tuberc Res Treat. 2018;574095:1-6.
26. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, Matee M, Pallangyo K, von Reyn CF. The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020 Aug 18;20(1):1-10.
27. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher Neutrophil-lymphocyte Ratio in TB/HIV Co-infection Compared to Pulmonary Tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
28. Hilda JN, Das S, Tripathy SP, Hanna LE. Role of neutrophils in tuberculosis: A bird's eye view. Innate Immun. 2020 May;26(4):240-7.
29. Pieters J. Mycobacterium tuberculosis and the macrophage: maintaining a balance. Cell Host & Microbe. 2008;3:399-407.
30. Pelaseyed T, Bergström J, Gustafsson J, Ermund A, Birchenough G, Schütte A, et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol Rev. 2014;260:8-20.
31. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-

tuberculosis treatment: systematic review and meta-analysis. Medicine. 2022, 101(44):1-8.

32. Kinnebrew MA, Pamer EG. Innate immune signaling in defense against intestinal microbes. Immunol Rev. 2012;245:113-31.
33. Van Crevel R, Ottenhoff THM, Van Der Meer JWM. Innate immunity to Mycobacterium tuberculosis. Clinical Microbiology Reviews. 2002;15:294-30.
34. Khalil MM, Halim HAA, Abdelazeem MA. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. The Egyptian Journal of Chest Diseases and Tuberculosis 2020, 69:458–65.
35. Bashir A. B, Ageep Ali K, Abufatima A. S. and Mohamedani A. A. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J. Med. Lab. Diagn. 2020;5(3):29-34.
36. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: a comparative study. The Medical Journal of Malaysia. 2011, 66(4):318-21.

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Manuscript Review Form

Reviewer	:	Reviewer 2
Manuscript #	:	M2023128
Manuscript Title	:	**Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis**

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?	√	
	Notes:		
2.	Are the title and abstract of the manuscript appropriate?	√	
	Notes:		
3	Do the title and abstract reflect the study result/content?	√	
	Notes:		
4.	Is the significance of the study well explained at the Background?	√	
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?	√	
	Notes:		
6.	Are the results, ideas, and data presented in this manuscript important	√	

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

	enough for publication?		
	Notes:		
7.	Are all figures and tables necessarily presented?		√
	Notes: Figure 1 is not necessary to be presented. The data is already covered by Table 3 and 4		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	√	
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?	√	
	Notes:		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?		√
	Notes: There are many mistakes in the text, need to be corrected		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	√
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Please ask the authors to review the text and make sure the meaning of some sentences are correct.

Date and Sign:
June 20th, 2023

Reviewer 2

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Manuscript Review Form

Reviewer	:	Reviewer 3
Manuscript #	:	M2023128
Manuscript Title	:	Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		√
	Notes: Other research that has been published: Diagnostic performance of adenosine deaminase for abdominal tuberculosis: A systematic review and meta-analysis. https://doi.org/10.3389/fpubh.2022.938544 Assessment of adenosine deaminase levels and lymphocyte counts in tuberculosis ascites. https://doi.org/10.53730/ijhs.v6nS3.7494		
2.	Are the title and abstract of the manuscript appropriate?	√	
	Notes:		
3	Do the title and abstract reflect the study result/content?	√	
	Notes:		
4.	Is the significance of the study well explained at the Background?	√	
	Notes:		

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?	√	
	Notes:		
6.	Are the results, ideas, and data presented in this manuscript important enough for publication?	√	
	Notes:		
7.	Are all figures and tables necessarily presented?	√	
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	√	
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?	√	
	Notes:		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?	√	
	Notes:		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	√
Revisions Required (Suggest changes to the manuscript as specified in this review)	
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Date and Sign:
June 14th, 2023

Reviewer 3

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 1 - Resubmit for Review

Ferry Sandra <ferry@trisakti.ac.id> Tue, Aug 15, 2023 at 9:32 AM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Please find the revision of manuscript M2023128. I sincerely apologize for the delay of the revision. I have made a major revision to the manuscript and all the comments from reviewers are incorporated accordingly.

Thank you.

Regards,
Ferry Sandra
[Quoted text hidden]
--
Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

Round 1 Revision from Author.docx
3817K

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Abstract

Background: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study is to determine the role of ADA and hematological tests in patients suspected with ITB.

Method: In this study, a total of 143 subjects were recruited. Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy and histopathological examinations, as well as ADA measurement and hematological test were performed.

Result: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation group (ESR) of ITB groups were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

Conclusion: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

Keyword: intestinal tuberculosis, adenosine deaminase, hematological tests

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).[1,2] The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was ≤ 9.9%. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3–16%.[3-5]

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.[3,6-10] The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB. [11]

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.[11,12] Elevation ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.[12,13] The activity of ADA differs significantly between patients with pulmonary TB and the control group.[14,15]

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them those are being included: leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), Neutrophil to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).[3,6,16,17] At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study is to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December 2022, with the inclusion criteria as follows: ≥18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (#KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for

histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.[16]

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test.

Blood in a non-anticoagulated tubes were allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample.

Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzen, China). The principle the test used was enzymatic colorimetry. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry. K_3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistics

Data were processed using SPSS software version 20 and Microsoft Excel 2016. Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p<0.05$.

The calculation of cut-off and Area Under the Curve (AUC) was obtained from the ROC (Receiver Operating Characteristic) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

RESULTS

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained.

Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups

Characteristics	ITB		Non-ITB	
Age (years)	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%
- Non-specific abdominal pain	12	75.0%	111	87.4%

- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups

Parameter	ITB	Non-ITB	*p*
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10^3/µL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Mann-Whitney U test; *p<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate.

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
- Sensitivity	= 81%		

- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

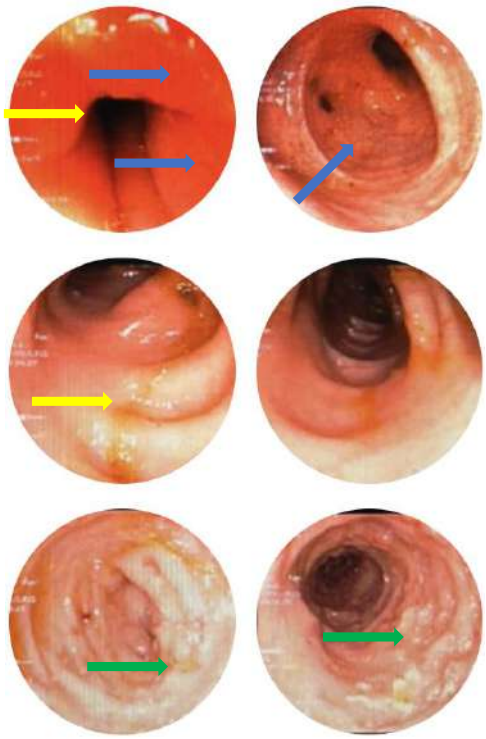


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

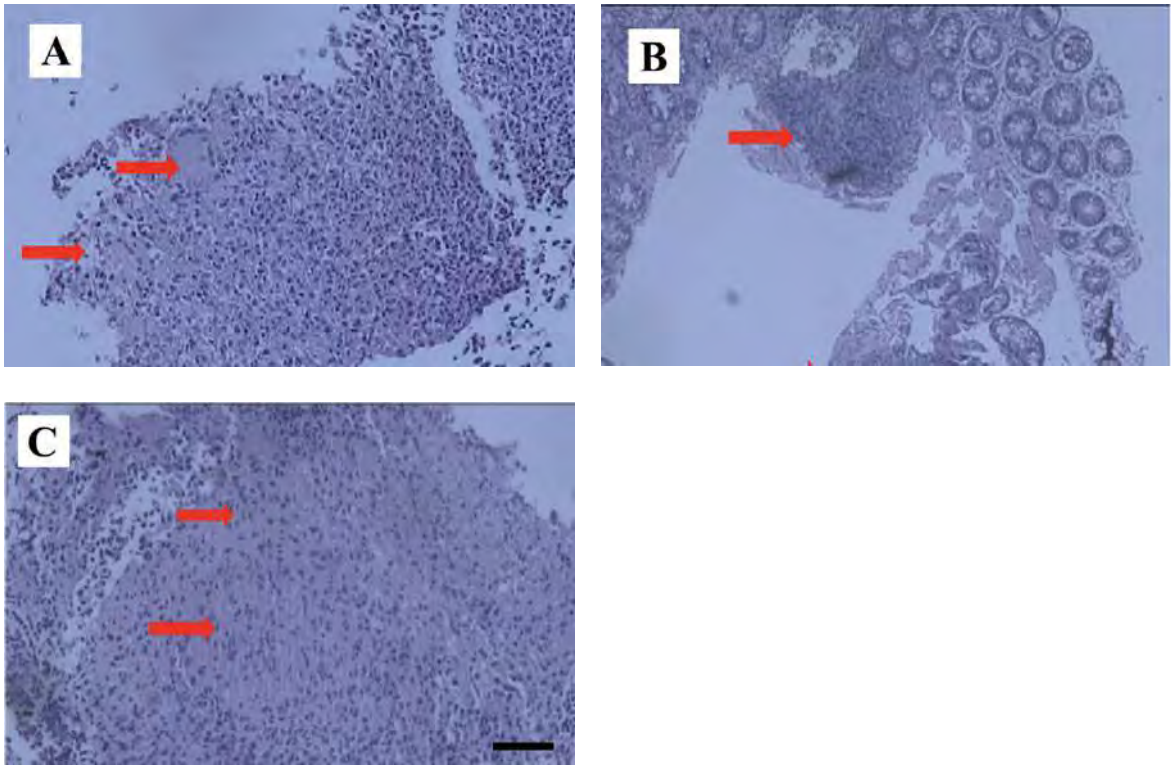


Figure 2. Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 μm.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).[1,18]

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.[15,19] The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L)[19] and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L).[20]

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.[21]

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.[22] However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.[23]

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar[22] and contradictive findings[24] in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.[22]

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.[25] In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.[25-27]

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

Conclusion

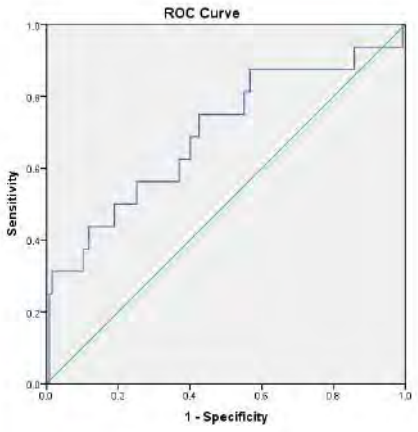
ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

References

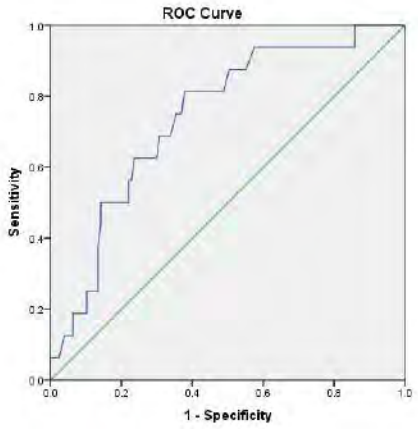
1. World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
2. Yunda DK, Witjaksono F, Nurwidya F. Correlation between protein intake, fat free mass, and total lymphocyte count with quality of life in pulmonary tuberculosis patients undergoing intensive phase treatment in Pekanbaru, Riau Province. Mol Cell Biomed Sci. 2020; 4(3): 128-34.
3. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, et al. Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010; 11(3): 143-9.
4. Evans RP, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016; 18(12): 45. doi: 10.1007/s11908-016-0546-5.
5. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015; 17(1): 3–11.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. J Penyakit Dalam Indones. 2016; 3(3): 165-73.
7. Shi XC, Zhang LF, Zhang YQ, Liu XQ, Fei GJ. Clinical and laboratory diagnosis of intestinal tuberculosis. Chin Med J. 2016; 129(11): 1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—An update on the diagnosis, treatment and drug resistance. J. Respir. 2021; 1(2): 141-64.
9. World Health Organization. Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015.
10. Lehman DC. Mycobacterium tuberculosis and Nontuberculous Mycobacteria. In: Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. p. 563-88.
11. Jha DK, Pathiyil MM, Sharma V. Evidence-based approach to diagnosis and management of abdominal tuberculosis. Indian J Gastroenterol. 2023; 42(1): 17-31.
12. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010; 88(2): 279-90.
13. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006; 387(3): 319-21.

14. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. Int J Res Health Sci. 2015; 3(4): 451-6.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. Jundishapur J Microbiol. 2015; 8(3): e21760. doi: 10.5812/jjm.21760.
16. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016;17(2):134-40.
17. Purnomo A, Bintoro UY, Sedana MP, Ashariati A. Association between Hasford scoring system and hematologic response in chronic and accelerated phase of chronic myelocytic leukemia patient with imatinib for three months. Mol Cell Biomed Sci. 2019; 3(2): 88-94.
18. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: a cross-sectional study. Pan Afr Med J. 2022 Aug 10;42:262-71.
19. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, et al. Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal IMAB. 2011; 17(1): 130-4.
20. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, et al. Serum adenosine deaminase: A novel biomarker tool for the diagnosis of tuberculosis. Biosci Biotechnol Res Asia. 2016; 13(1): 551-6.
21. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Tuberc Res Treat. 2018; 2018: 5740951. doi: 10.1155/2018/5740951.
22. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, et al. The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020; 20(1): 609. doi: 10.1186/s12879-020-05331-w.
23. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher neutrophil-lymphocyte ratio in TB/HIV co-infection compared to pulmonary tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
24. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. Medicine. 2022; 101(44): e31539. doi: 10.1097/MD.0000000000031539.
25. Bashir AB, Ageep Ali K, Abufatima AS, Mohamedani AA. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J Med Lab Diagn. 2020; 5(3): 29-34.
26. Khalil MM, Halim HAA, Abdelazeem MS. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. Egypt J Chest Dis Tuberc. 2020; 69(3): 458–65.
27. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: A comparative study. Med J Malaysia. 2011; 66(4): 318-21.

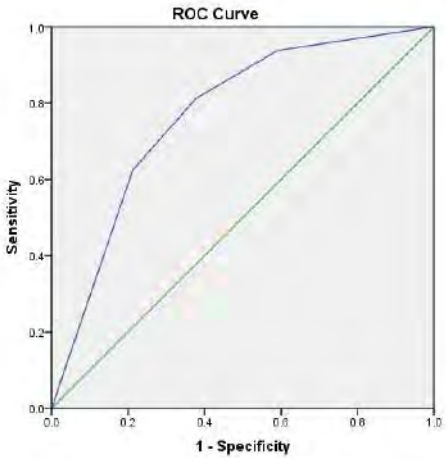
Supplementary 1.
ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests



(a)



(b)



(c)

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 2 - Revisions Required

Secretariat of InaBJ <secretariatinabj@gmail.com> Fri, Aug 18, 2023 at 7:30 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients**".

Our decision is: **Revisions Required.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **August 25, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/2406, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org

Round 2 Reviewer 4 - Manuscript.docx
3824K

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Abstract

Background: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was~~is~~ to determine the role of ADA and hematological tests in patients suspected with ITB.

Method: In this study, a total of 143 subjects were recruited. Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy and histopathological examinations, as well as ADA measurement and hematological test were performed.

Result: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate~~group~~ (ESR) of ITB group~~s~~ were significantly higher than the ones of non-ITB group (p<0.05). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

Conclusion: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

Keyword: intestinal tuberculosis, adenosine deaminase, hematological tests

Comment [NMD1]: Need some explanation regarding the association of ADA and ITB, why choosing ADA as the marker?

Comment [NMD2]: Suggestions: "In this study, suspected ITB subjects, and non-ITB subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were recruited."

No need to mention the number because in the Methods section, you did not mention the total number of section. Hence, you can mention it in the Results.

Comment [NMD3]: Mention the methods.

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).[1,2] The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was ≤ 9.9%. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3–16%.[3-5]

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.[3,6-10] The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB. [11]

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.[11,12] Elevated~~ion~~ ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.[12,13] The activity of ADA differs significantly between patients with pulmonary TB and the control group.[14,15]

Comment [NMD4]: Define the control group based on the previous study. Is it normal healthy people, or is it other type of TB patients?

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them those are~~ being~~ includinged: leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), Neutrophil to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).[3,6,16,17] At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was~~is~~ to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December 2022, with the inclusion criteria as follows: ≥18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (#KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for

Comment [NMD5]: Is this inclusion and exclusion criteria only for the ITB group? Or for all subjects?

If it's only for ITB group it should be clarified, for example by adding information "for the ITB subjects, the inclusion criteria was…."

Comment [NMD6]: This exclusion criteria can be moved after the sentences explaining the criteria for suspected ITB.

Comment [NMD7]: With similar characteristics? For example age range, etc?

histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.[16]

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in a non-anticoagulated tube~~s~~ were allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzen, China). The principle the test used was enzymatic colorimetry. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry. K_3EDTA blood was diluted by the

Comment [NMD8]: It will be better if the information about parameters examined for the hematological test are included in this sub-section as well.

reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistics

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p<0.05$.

The calculation of cut-off and Area Under the Curve (AUC) was obtained from the ~~ROC (~~Receiver Operating Characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

RESULTS

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups

Characteristics	ITB		Non-ITB	
Age (years), mean±SD	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%

Formatted: English (U.S.)

- Non-specific abdominal pain	12	75.0%	111	87.4%
- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups

Parameter	ITB	Non-ITB	*p*
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10^3/µL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Mann-Whitney U test; *p<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate. Data were presented in median (min-max).

Formatted: English (U.S.)

Table 3…?

Comment [NMD9]: No table title?

Formatted: Left

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61

Negative	3	79	82
Total	16	127	143

- Sensitivity = 81%
- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

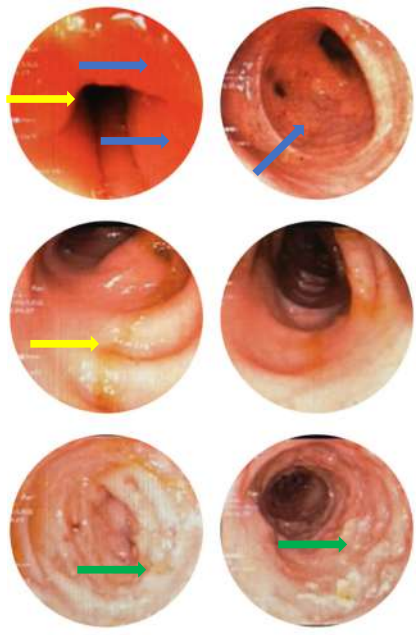


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

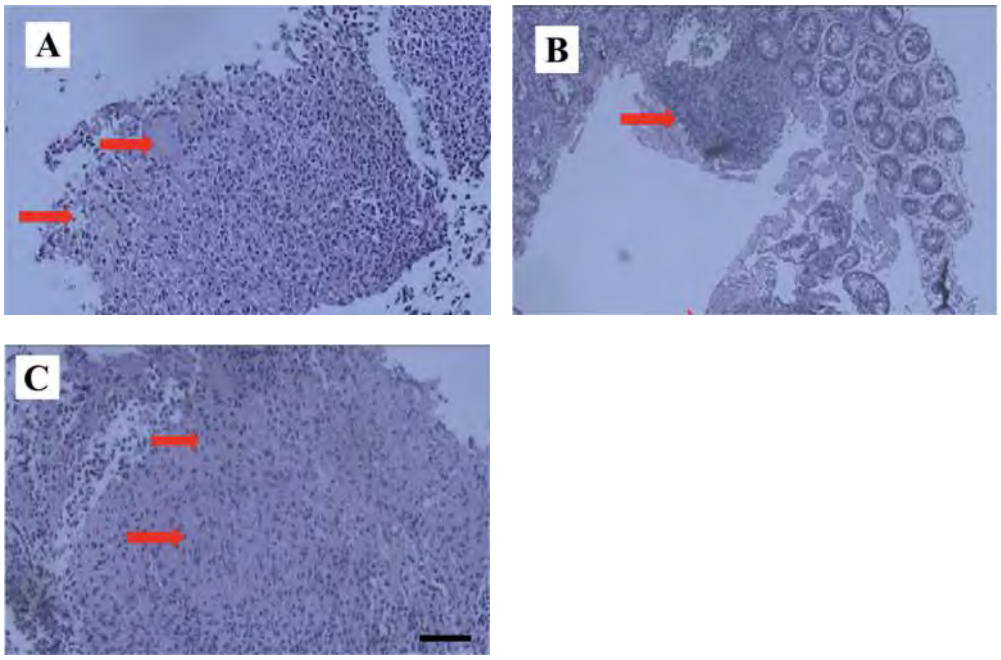


Figure 2. Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 μm.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).[1,18]

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.[15,19] The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L)[19] and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L).[20]

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.[21]

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.[22] However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.[23]

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar[22] and contradictive findings[24] in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.[22]

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.[25] In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.[25-27]

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

Conclusion

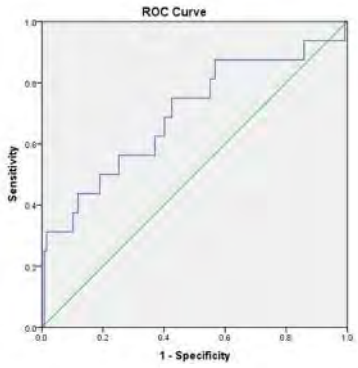
ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

References

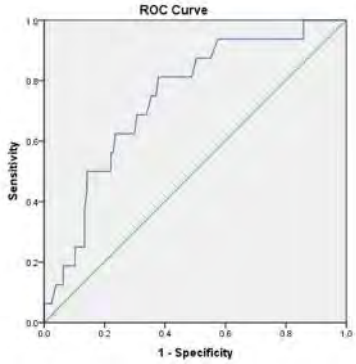
1. World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
2. Yunda DK, Witjaksono F, Nurwidya F. Correlation between protein intake, fat free mass, and total lymphocyte count with quality of life in pulmonary tuberculosis patients undergoing intensive phase treatment in Pekanbaru, Riau Province. Mol Cell Biomed Sci. 2020; 4(3): 128-34.
3. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, et al. Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010; 11(3): 143-9.
4. Evans RP, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016; 18(12): 45. doi: 10.1007/s11908-016-0546-5.
5. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015; 17(1): 3–11.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. J Penyakit Dalam Indones. 2016; 3(3): 165-73.
7. Shi XC, Zhang LF, Zhang YQ, Liu XQ, Fei GJ. Clinical and laboratory diagnosis of intestinal tuberculosis. Chin Med J. 2016; 129(11): 1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—An update on the diagnosis, treatment and drug resistance. J. Respir. 2021; 1(2): 141-64.
9. World Health Organization. Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015.
10. Lehman DC. Mycobacterium tuberculosis and Nontuberculous Mycobacteria. In: Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. p. 563-88.
11. Jha DK, Pathiyil MM, Sharma V. Evidence-based approach to diagnosis and management of abdominal tuberculosis. Indian J Gastroenterol. 2023; 42(1): 17-31.
12. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010; 88(2): 279-90.
13. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006; 387(3): 319-21.

14. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. Int J Res Health Sci. 2015; 3(4): 451-6.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. Jundishapur J Microbiol. 2015; 8(3): e21760. doi: 10.5812/jjm.21760.
16. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016;17(2):134-40.
17. Purnomo A, Bintoro UY, Sedana MP, Ashariati A. Association between Hasford scoring system and hematologic response in chronic and accelerated phase of chronic myelocytic leukemia patient with imatinib for three months. Mol Cell Biomed Sci. 2019; 3(2): 88-94.
18. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: a cross-sectional study. Pan Afr Med J. 2022 Aug 10;42:262-71.
19. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, et al. Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal IMAB. 2011; 17(1): 130-4.
20. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, et al. Serum adenosine deaminase: A novel biomarker tool for the diagnosis of tuberculosis. Biosci Biotechnol Res Asia. 2016; 13(1): 551-6.
21. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Tuberc Res Treat. 2018; 2018: 5740951. doi: 10.1155/2018/5740951.
22. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, et al. The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020; 20(1): 609. doi: 10.1186/s12879-020-05331-w.
23. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher neutrophil-lymphocyte ratio in TB/HIV co-infection compared to pulmonary tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
24. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. Medicine. 2022; 101(44): e31539. doi: 10.1097/MD.0000000000031539.
25. Bashir AB, Ageep Ali K, Abufatima AS, Mohamedani AA. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J Med Lab Diagn. 2020; 5(3): 29-34.
26. Khalil MM, Halim HAA, Abdelazeem MS. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. Egypt J Chest Dis Tuberc. 2020; 69(3): 458–65.
27. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: A comparative study. Med J Malaysia. 2011; 66(4): 318-21.

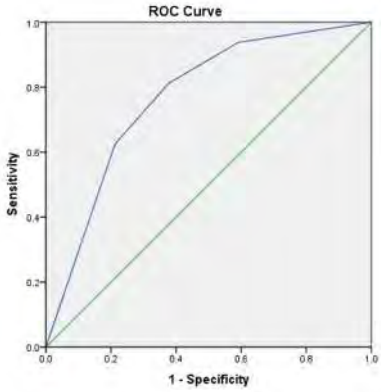
Supplementary 1.
ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests



(a)



(b)



(c)

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 2 - Revisions Required

Ferry Sandra <ferry@trisakti.ac.id> Fri, Aug 18, 2023 at 1:40 PM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Thank you for giving us the opportunity to submit our revised manuscript "High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients" for publication in the Indonesian Biomedical Journal. All reviewers' comments in the second round of the peer-review have been addressed in the attached file.

Thank you. We look forward to hearing from you.

Regards,
Ferry Sandra
[Quoted text hidden]
--
Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

Round 2 Revision from Author.docx
3818K

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Abstract

Background: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Method: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological test were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

Result: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epitheloid cells, giant cells, and lymphocyte aggregates.

Conclusion: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

Keyword: intestinal tuberculosis, adenosine deaminase, hematological tests

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).[1,2] The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was ≤ 9.9%. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3–16%.[3-5]

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.[3,6-10] The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB. [11]

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.[11,12] Elevated ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.[12,13] The activity of ADA differs significantly between patients with pulmonary TB and the normal healthy people[14,15]

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them are leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), Neutrophil to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).[3,6,16,17] At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December 2022, with the inclusion criteria as follows; for the ITB group: ≥18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (#KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for

histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.[16]

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in non-anticoagulated tubes were allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzen, China). The principle the test used was enzymatic colorimetry. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry for measurement of leukocyte,

basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K_3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistics

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p<0.05$.

The calculation of cut-off and Area Under the Curve (AUC) was obtained from the Receiver Operating Characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

RESULTS

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups

Characteristics	ITB		Non-ITB	
Age (years), mean±SD	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%

- Non-specific abdominal pain	12	75.0%	111	87.4%
- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups

Parameter	ITB	Non-ITB	*p*
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10^3/µL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Mann-Whitney U test; *p<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate. Data were presented in median (min-max).

Table 3. ADA Results of ITB (n=16) and Non-ITB (n=127) Groups with cut-off: 12.56 IU/L

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143

- Sensitivity = 81%
- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

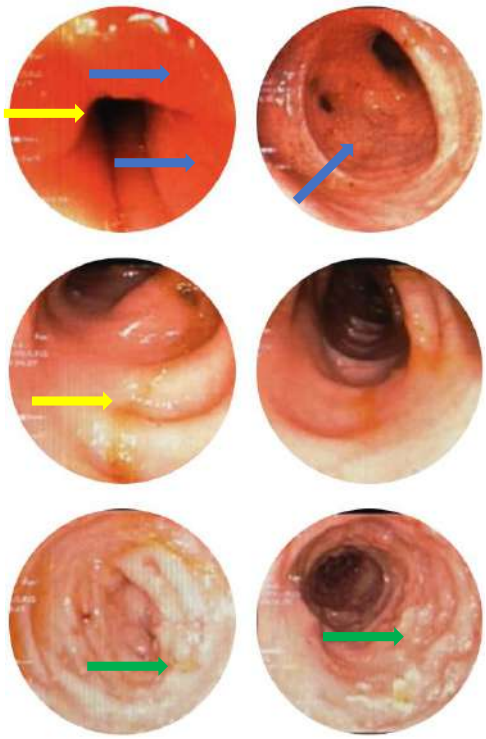


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

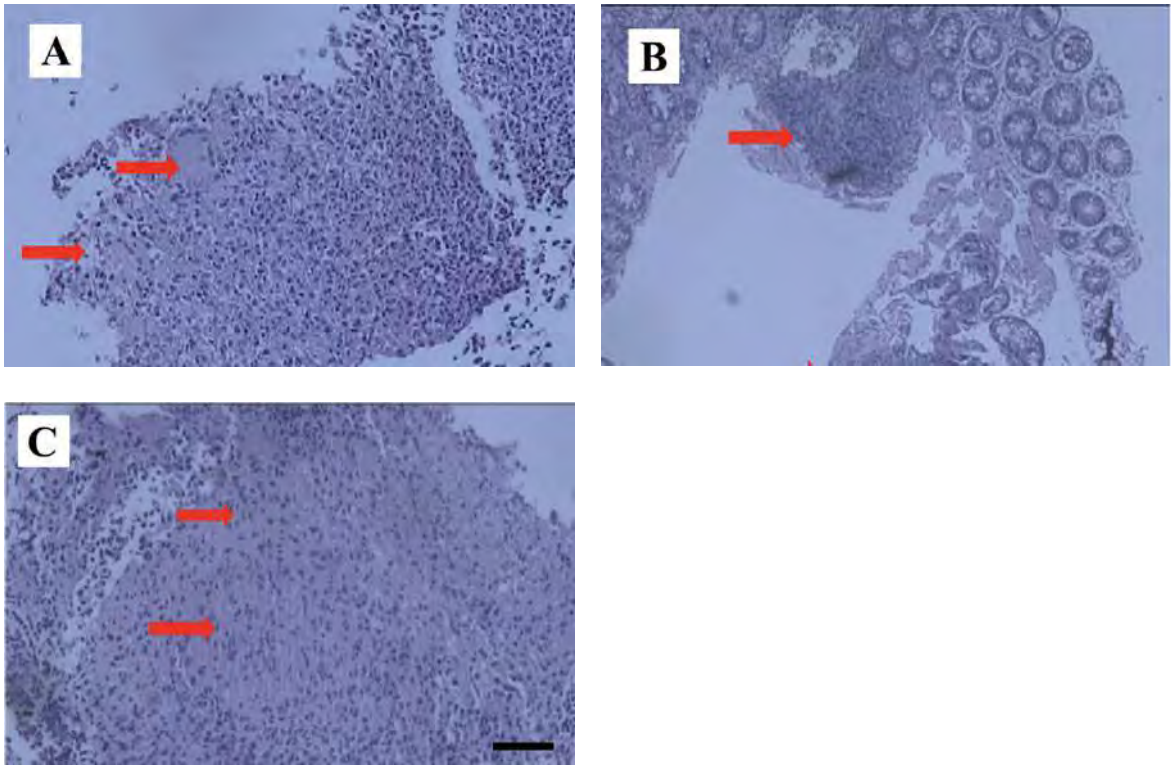


Figure 2. Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 μm.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).[1,18]

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.[15,19] The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L)[19] and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L).[20]

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.[21]

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.[22] However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.[23]

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar[22] and contradictive findings[24] in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.[22]

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.[25] In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.[25-27]

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

Conclusion

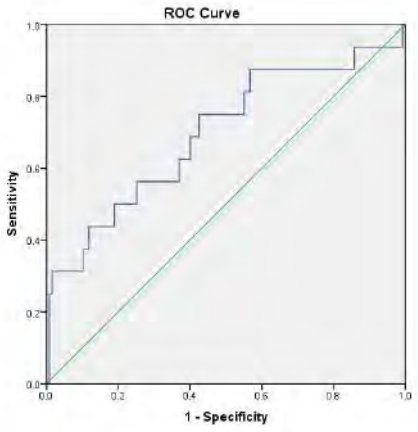
ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

References

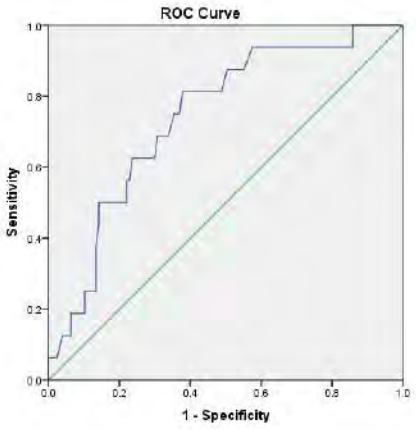
1. World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
2. Yunda DK, Witjaksono F, Nurwidya F. Correlation between protein intake, fat free mass, and total lymphocyte count with quality of life in pulmonary tuberculosis patients undergoing intensive phase treatment in Pekanbaru, Riau Province. Mol Cell Biomed Sci. 2020; 4(3): 128-34.
3. Oto BT, Fauzi A, Syam AF, Simadibrata M, Abdullah M, Makmun D, et al. Colitis tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2010; 11(3): 143-9.
4. Evans RP, Mourad MM, Dvorkin L, Bramhall SR. Hepatic and intra-abdominal tuberculosis: 2016 update. Curr Infect Dis Rep. 2016; 18(12): 45. doi: 10.1007/s11908-016-0546-5.
5. Lapausa MR, Saldana AM, Asensio AN. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015; 17(1): 3–11.
6. Murwaningrum A, Abdullah M, Makmun D. Pendekatan diagnosis dan tatalaksana tuberkulosis intestinal. J Penyakit Dalam Indones. 2016; 3(3): 165-73.
7. Shi XC, Zhang LF, Zhang YQ, Liu XQ, Fei GJ. Clinical and laboratory diagnosis of intestinal tuberculosis. Chin Med J. 2016; 129(11): 1330-3.
8. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—An update on the diagnosis, treatment and drug resistance. J. Respir. 2021; 1(2): 141-64.
9. World Health Organization. Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015.
10. Lehman DC. Mycobacterium tuberculosis and Nontuberculous Mycobacteria. In: Mahon C, Lehman D, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Missouri: Elsevier Saunders; 2015. p. 563-88.
11. Jha DK, Pathiyil MM, Sharma V. Evidence-based approach to diagnosis and management of abdominal tuberculosis. Indian J Gastroenterol. 2023; 42(1): 17-31.
12. Zavialov AV, Gracia E, Glaichenhaus N, Franco R, Zavialov AV, Lauvau G. Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. J Leukoc Biol. 2010; 88(2): 279-90.
13. Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. Biol Chem. 2006; 387(3): 319-21.

14. Varma S, Toppo A. Estimation of serum adenosine deaminase level in patients of pulmonary tuberculosis in a tertiary care hospital in Chhattisgarh. Int J Res Health Sci. 2015; 3(4): 451-6.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. Jundishapur J Microbiol. 2015; 8(3): e21760. doi: 10.5812/jjm.21760.
16. Guno TH, Putra BA, Kamelia T, Makmun D. Diagnostic and therapeutic approach in intestinal tuberculosis. Indones J Gastroenterol Hepatol Dig Endosc. 2016;17(2):134-40.
17. Purnomo A, Bintoro UY, Sedana MP, Ashariati A. Association between Hasford scoring system and hematologic response in chronic and accelerated phase of chronic myelocytic leukemia patient with imatinib for three months. Mol Cell Biomed Sci. 2019; 3(2): 88-94.
18. Suparmin S, Indrasari ND, Renaldi K, Immanuel S, Yusra Y, Br Pasaribu MM. Diagnostic role of stool TB-PCR in suspected tuberculous colitis patient at Dr Cipto Mangunkusumo National Central General Hospital: a cross-sectional study. Pan Afr Med J. 2022 Aug 10;42:262-71.
19. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I, et al. Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. Journal IMAB. 2011; 17(1): 130-4.
20. Pandey R, Tamrakar D, Jaiswal S, Sharma A, Koju S, Duwal S, et al. Serum adenosine deaminase: A novel biomarker tool for the diagnosis of tuberculosis. Biosci Biotechnol Res Asia. 2016; 13(1): 551-6.
21. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A comparative cross-sectional study. Tuberc Res Treat. 2018; 2018: 5740951. doi: 10.1155/2018/5740951.
22. Rees CA, Pineros DB, Amour M, Munseri P, Said J, Magohe A, et al. The potential of CBC-derived ratios (monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte) to predict or diagnose incident TB infection in Tanzanian adolescents. BMC Infect Dis. 2020; 20(1): 609. doi: 10.1186/s12879-020-05331-w.
23. Sulastri N, Alisjahbana B, Livia R, Sahiratmadja E. Higher neutrophil-lymphocyte ratio in TB/HIV co-infection compared to pulmonary tuberculosis. Indones Biomed J. 2021; 13(4): 375-82.
24. Adane T, Melku M, Ayalew G, Bewket G, Aynalem M, Getawa S. Accuracy of monocyte to lymphocyte ratio for tuberculosis diagnosis and its role in monitoring anti-tuberculosis treatment: Systematic review and meta-analysis. Medicine. 2022; 101(44): e31539. doi: 10.1097/MD.0000000000031539.
25. Bashir AB, Ageep Ali K, Abufatima AS, Mohamedani AA. Reactive thrombocytosis and erythrocyte sedimentation rate in patients with pulmonary tuberculosis. J Med Lab Diagn. 2020; 5(3): 29-34.
26. Khalil MM, Halim HAA, Abdelazeem MS. C-reactive protein versus erythrocyte sedimentation rate in monitoring multidrug-resistant tuberculosis. Egypt J Chest Dis Tuberc. 2020; 69(3): 458–65.
27. Chong VH. Differences in patient profiles of abdominal and pulmonary tuberculosis: A comparative study. Med J Malaysia. 2011; 66(4): 318-21.

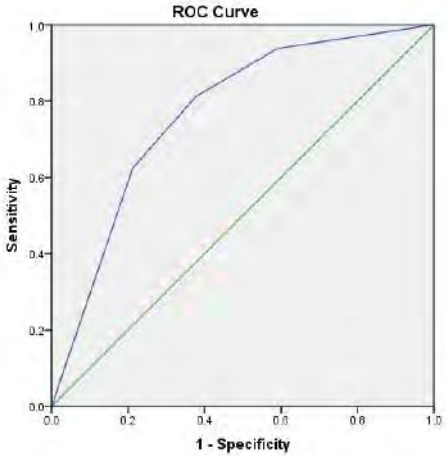
Supplementary 1.
ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests



(a)



(b)



(c)

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision - Manuscript Accepted

Secretariat of InaBJ <secretariatinabj@gmail.com> Mon, Aug 21, 2023 at 11:26 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients**."

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

Congratulations on your interesting research, and thank you for allowing us to publish this valuable material. Please let us know once you have read this email. We wish you a nice day.

Best Regards,

--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org