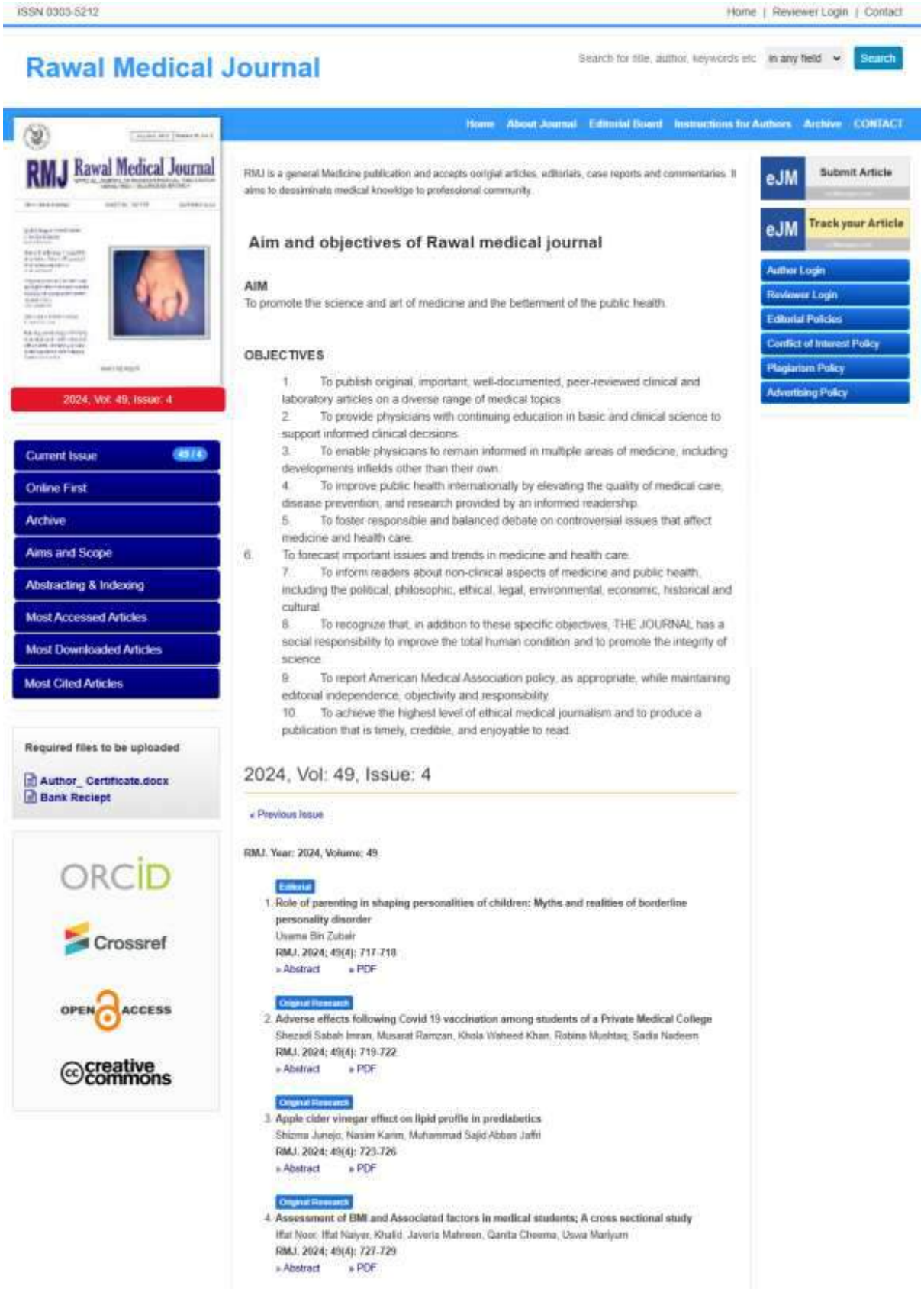
Rawal medical journal



ISSN 0303-5212

Home | Reviewer Login | Contact

Rawal Medical Journal

Search for title, author, keywords etc. in any field Search

Home About Journal Editorial Board Instructions for Authors Archive CONTACT

RMJ Rawal Medical Journal

2024, Vol: 49, Issue: 4

Current Issue 49 / 4
Online First
Archive
Aims and Scope
Abstracting & Indexing
Most Accessed Articles
Most Downloaded Articles
Most Cited Articles

Required files to be uploaded

Author_ Certificate.docx
Bank Reciept

ORCID
Crossref
OPEN ACCESS
creative commons

RMJ is a general Medicine publication and accepts original articles, editorials, case reports and commentaries. It aims to dessiminate medical knowledge to professional community.

eJM Submit Article
eJM Track your Article

Author Login
Reviewer Login
Editorial Policies
Conflict of Interest Policy
Plagiarism Policy
Advertising Policy

Aim and objectives of Rawal medical journal

AIM

To promote the science and art of medicine and the betterment of the public health.

OBJECTIVES

1. To publish original, important, well-documented, peer-reviewed clinical and laboratory articles on a diverse range of medical topics.
2. To provide physicians with continuing education in basic and clinical science to support informed clinical decisions.
3. To enable physicians to remain informed in multiple areas of medicine, including developments infields other than their own.
4. To improve public health internationally by elevating the quality of medical care, disease prevention, and research provided by an informed readership.
5. To foster responsible and balanced debate on controversial issues that affect medicine and health care.
6. To forecast important issues and trends in medicine and health care.
7. To inform readers about non-clinical aspects of medicine and public health, including the political, philosophic, ethical, legal, environmental, economic, historical and cultural.
8. To recognize that, in addition to these specific objectives, THE JOURNAL has a social responsibility to improve the total human condition and to promote the integrity of science.
9. To report American Medical Association policy, as appropriate, while maintaining editorial independence, objectivity and responsibility.
10. To achieve the highest level of ethical medical journalism and to produce a publication that is timely, credible, and enjoyable to read.

2024, Vol: 49, Issue: 4

« Previous Issue

RMJ. Year: 2024, Volume: 49

Editorial
1. Role of parenting in shaping personalities of children: Myths and realities of borderline personality disorder
Usama Bin Zubair
RMJ. 2024; 49(4): 717-718
» Abstract » PDF

Original Research
2. Adverse effects following Covid 19 vaccination among students of a Private Medical College
Shezadi Sabah Imran, Musarat Ramzan, Khola Waheed Khan, Robina Mushtaq, Sadia Nadeem
RMJ. 2024; 49(4): 719-722
» Abstract » PDF

Original Research
3. Apple cider vinegar effect on lipid profile in prediabetics
Shizma Junejo, Nasim Karim, Muhammad Sajid Abbas Jaffri
RMJ. 2024; 49(4): 723-726
» Abstract » PDF

Original Research
4. Assessment of BMI and Associated factors in medical students; A cross sectional study
Iffat Noor, Iffat Naiyer, Khalid, Javeria Mahreen, Qanita Cheema, Uswa Mariyum
RMJ. 2024; 49(4): 727-729
» Abstract » PDF

Original Research

5. **Assessment of new inflammatory marker for diabetic kidney disease in Type 2 Diabetic patients**
Syed Zakir Shah, Aimal Khan, Hamayun Mumtaz, Iffat Naiyar, Muddasar Parvaiz, Waleed Bin Abdullah
RMJ. 2024; 49(4): 730-733
» Abstract » PDF

Original Research

6. **C-reactive protein as an inflammatory marker in obese and non-obese type 2 diabetes mellitus patients with metabolic syndrome**
Rabia Siddiqui, Nighat Rukhsana, Muhammad Sajid Abbas Jafri, Shazia Shakoor, Samreen Iqbal, Muhammad Younas
RMJ. 2024; 49(4): 734-737
» Abstract » PDF

Original Research

7. **Lived experiences of type 2 diabetes patients in North Sumatra Province, Indonesia: A phenomenology study**
Mula Tarigan, Mutiara Baltina Satya Wira, Kissarawan Nilvarangkul
RMJ. 2024; 49(4): 738-741
» Abstract » PDF » doi: 10.5455/rmj.20240824061539

Original Research

8. **Nephropathological associations: Diagnostic insights into diabetic UTIs via integrated biomarker approaches**
Musaddiq Hussain Bangash, Fakhra Noureen, Naseer uddin, Ama tul Naval, Ehsan Khan, Seemi Tanvir
RMJ. 2024; 49(4): 742-745
» Abstract » PDF

Original Research

9. **Neuro-ophthalmic interface: Investigating the therapeutic potential of Glial cell-derived neurotrophic factor (GDNF) in pathology of diabetic retinopathy**
Durga Devi, Moosd Ullah Khan, Naeemullah Syed, Zain ul Abdan Bilal Ahmed, Abdul Munim, Ehsan Khan
RMJ. 2024; 49(4): 746-749
» Abstract » PDF

Original Research

10. **Prevalence of sarcopenia in patients with diabetes mellitus type II**
Aseel Kareem Tawfeeq, Waleed Ibraheem Ali
RMJ. 2024; 49(4): 750-753
» Abstract » PDF » doi: 10.5455/rmj.20240921123851

Original Research

11. **Association of fecal calprotectin and M2 pyruvate kinase with fecal pathogenic microorganisms in gastrointestinal infection**
Ade Dharmawan, Pusparini Pusparini
RMJ. 2024; 49(4): 754-758
» Abstract » PDF » doi: 10.5455/rmj.20240812023946

Original Research

12. **Correlation between information support, anxiety, and satisfaction with care among family members of patients admitted to intensive care unit**
Abdullah Usman, Bakhtayar Ali Shah, Irfan Ullah Khattak, Sardar Ali, Ijaz Arif
RMJ. 2024; 49(4): 759-762
» Abstract » PDF

Original Research

13. **Effect of methotrexate on clinical course of COVID-19 in patients with autoimmune rheumatic diseases**
Shahida Perveen, Haris Gul, Saba Samreen, Babur Salim
RMJ. 2024; 49(4): 763-768
» Abstract » PDF

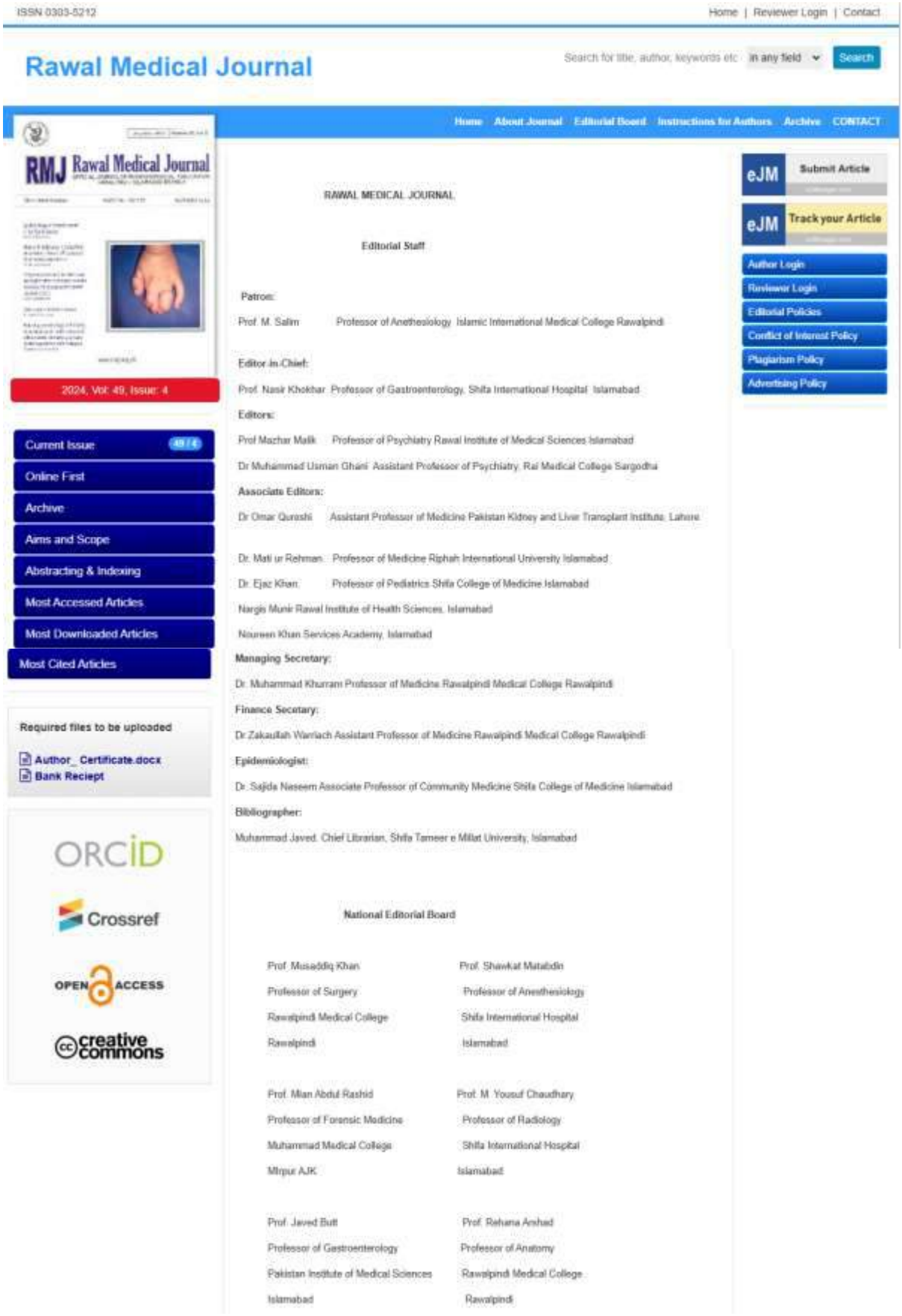
Original Research

14. **Establishing reference intervals for LDL-C and HDL-C in the Jazan region of Saudi Arabia: A population study**
Ahmed Jerah
RMJ. 2024; 49(4): 769-773
» Abstract » PDF » doi: 10.5455/rmj.20240810050420

Original Research

15. **Association of thyroid disorder with glycemic variability: a case control study**
Noor ul ain, Sheikh Abdul Saeed, Shazia Shakoor, Shah Jabeen, Sana Kashif Shahid, Shahameen Aqeel
RMJ. 2024; 49(4): 774-777
» Abstract » PDF

Editorial board



ISSN 0303-5212

Home | Reviewer Login | Contact

Rawal Medical Journal

Search for title, author, keywords etc in any field Search

Home About Journal Editorial Board Instructions for Authors Archive CONTACT

RMJ Rawal Medical Journal

2024; Vol: 49, Issue: 4

Current Issue 49 / 4
Online First
Archive
Aims and Scope
Abstracting & Indexing
Most Accessed Articles
Most Downloaded Articles
Most Cited Articles

Required files to be uploaded

Author_ Certificate.docx
Bank Reciept

ORCID
Crossref
OPEN ACCESS
creative commons

eJM Submit Article
eJM Track your Article
Author Login
Reviewer Login
Editorial Policies
Conflict of Interest Policy
Plagiarism Policy
Advertising Policy

RAWAL MEDICAL JOURNAL

Editorial Staff

Patron:

Prof. M. Salim Professor of Anesthesiology Islamic International Medical College Rawalpindi

Editor-in-Chief:

Prof. Nasir Khokhar Professor of Gastroenterology, Shifa International Hospital Islamabad

Editors:

Prof Mazhar Malik Professor of Psychiatry Rawal Institute of Medical Sciences Islamabad

Dr Muhammad Usman Ghani Assistant Professor of Psychiatry, Rai Medical College Sargodha

Associate Editors:

Dr Omar Qureshi Assistant Professor of Medicine Pakistan Kidney and Liver Transplant Institute, Lahore

Dr. Mati ur Rehman Professor of Medicine Riphah International University Islamabad

Dr. Ejaz Khan Professor of Pediatrics Shifa College of Medicine Islamabad

Nargis Munir Rawal Institute of Health Sciences, Islamabad

Noureen Khan Services Academy, Islamabad

Managing Secretary:

Dr. Muhammad Khurram Professor of Medicine Rawalpindi Medical College Rawalpindi

Finance Secretary:

Dr Zakaullah Wanlach Assistant Professor of Medicine Rawalpindi Medical College Rawalpindi

Epidemiologist:

Dr. Sajida Naseem Associate Professor of Community Medicine Shifa College of Medicine Islamabad

Bibliographer:

Muhammad Javed, Chief Librarian, Shifa Tameer e Millat University, Islamabad

National Editorial Board

Prof. Musaddiq Khan
Professor of Surgery
Rawalpindi Medical College
Rawalpindi

Prof. Shawkat Matabdin
Professor of Anesthesiology
Shifa International Hospital
Islamabad

Prof. Mian Abdul Rashid
Professor of Forensic Medicine
Muhammad Medical College
Mirpur AJK

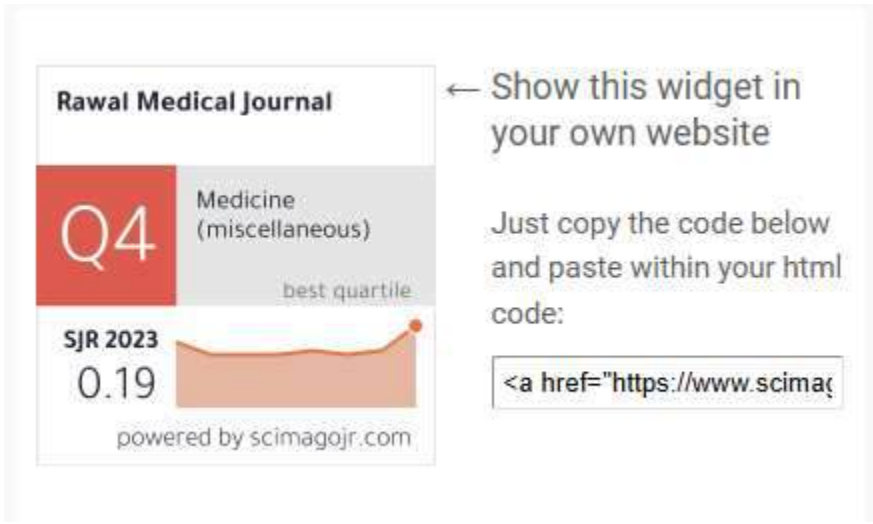
Prof. M. Yousuf Chaudhary
Professor of Radiology
Shifa International Hospital
Islamabad

Prof. Javed Butt
Professor of Gastroenterology
Pakistan Institute of Medical Sciences
Islamabad

Prof. Rehana Arshad
Professor of Anatomy
Rawalpindi Medical College
Rawalpindi

Abstracting and indexing

Scimago rank



Original Article

Association of fecal calprotectin and M2 pyruvate kinase with fecal pathogenic microorganisms in gastrointestinal infection

Ade Dharmawan,[1] Pusparini[2]

[1]Department of Microbiology, Faculty of Medicine and Health Science, Krida Wacana Christian University,
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Trisakti, Jakarta Indonesia.

Objective: To determine the association of Fecal Calprotectin (f-CP) and M2 Pyruvate Kinase **(**M2-PK) with intestinal pathogens by means of the gastrointestinal syndromic panel.

Methodology: This retrospective analytic study was conducted in the laboratory department a private hospital in Jakarta from October 2021 to September 2022. The inclusion criteria comprised patients aged ≥18 years with gastrointestinal complaints who had undergone f-CP and M2-PK examinations and gastrointestinal syndromic testing. The exclusion criteria were incomplete data on laboratory examinations, follow-up examinations or treatment evaluations. We used the Mann-Whitney test with $p<0.05$ indicating significantly different.

Results: Out of 282 subjects, 69% were women and 31% men, aged 18 to 84 years (mean 50.52±15.20). In 117 subjects, single pathogen was found in 78 specimens and ≥2 pathogens in 39. Median f-CP was 77.1 µg/g (3.68-2100) and median M2-PK 3.55 U/mL (1-72.6). Mann-Whitney test found a significant association between f-CP and intestinal pathogens (p=0.048), whereas for the M2-PK, no significant association was found with intestinal pathogens a(p=0.381).

Conclusion: f-CP increases in gastrointestinal infection with intestinal pathogens, but M2-PK does not increase. Therefore, f-CP can be considered a biomarker for gastrointestinal infections caused by microorganisms.

Keywords: Fecal calprotectin, GI-syndromic testing, gastrointestinal infection, M2-PK.

INTRODUCTION

Gastrointestinal diseases contribute significantly to the infectious disease burden throughout the world.[1] Gastrointestinal infections lead to symptoms such as diarrhea and result morbidity and mortality in certain populations.[2] The national prevalence of diarrhea is 6.8% based on the Indonesian goverment data for 2018.[3] It is of utmost importance that empirical treatment of diarrhea and preventive measures be guided by the impact and distribution of infectious agents on diarrheal disease.[4]

Clinicians frequently have difficulty in differentiating infectious from non-infectious diarrhea.[5] Faecal culture, microscopy, tests for occult blood in feces, and fecal lactoferrin assay are frequently not sensitive enough. Currently, multiplex polymerase chain reaction (PCR) based examinations are preferred, because they can detect the presence of infection in a short time.[6,7] Syndromic testing is a molecular assay that can detect several targets simultaneously, including parasites, viruses and bacteria.[6,7] Gastrointestinal infections assays for biomarkers are needed that are simple, rapid, and accurate, for the diagnosis because faeces flows along the whole intestinal mucosa where molecular markers of damage or inflammation accumulate.[5,8]

Faecal calprotectin (f-CP) and M2 pyruvate kinase (M2-PK) are gastrointestinal mucosal inflammatory biomarkers. M2-PK is the enzyme responsible for the transfer of phosphate groups in glycolysis. Both dimer M2-PK and tetramers M2-PK are found in proliferating cells of various tissues.[9] As M2-PK is also present in leukocytes, M2-PK may be found in the feces mass that is formed while in contact with the inflamed mucosa of the digestive tract. Because of the high stability of M2-PK, its faecal concentration may reflect the presence of gastrointestinal tract inflammation. The two important uses of M2-PK are the evaluation of cancer screening and the degree of inflammatory bowel disease (IBD).[9,10]

Faecal calprotectin is a Ca-binding protein, that accounts for 60% of the cytosolic proteins in neutrophils. The immunomodulatory and antiproliferative properties of calprotectin serve an important function in antibacterial neutrophil defence.[8] Neutrophil infiltration of the mucosa and migration into the intestinal lumen is indicated by f-CP concentration that correlates positively with inflammatory symptoms and epithelial damage. One study showed that f-CP concentration was higher in diarrhea caused by intestinal bacterial pathogens as compared with diarrhoea from other causes.[8] Lam et al,[8] found that a mean of 100.77 µg/g indicated a significant correlation between f-CP and intestinal pathogens, while

Sýkora et al, determined the cut-off value for differentiating acute bacterial gastroenteritis to be 103.9 µg/g.[5] Not all healthcare facilities have resources for bacterial culture, particularly syndromic testing, which is a rather expensive examination. The aim of this study was to determine the association of f-CP and M2-PK with intestinal pathogens

METHODOLOGY

This retrospective analytical study used the data of 282 subjects and was carried out from October 2021 until September 2022 in Laboratory department a private hospital in Jakarta. The study obtained ethical clearance from the Ethics Committee, Medicine and Health Sciences Faculty, University of Christian Krida Wacana, Jakarta, (Ref No. 1405/SLKE-IM/UKKW/FKIK/KE/XI/2022, dated November 30, 2022).

The criteria for inclusion were age more than 18 years, having diarrhea, and having undergone simultaneous examinations for f-CP, M2-PK, and GI-syndromic testing. The exclusion criteria were incomplete follow-up, treatment evaluation, and laboratory data.

The fecal specimens were examined for f-CP, M2-PK and GI-syndromic testing. f-CP was examined by means of ELISA (Enzyme Linked Immunosorbent Assay), using IDK® Calprotectin reagents (Immundiagnostik AG, Germany) and an Euroimmune analyzer. M2-PK was also examined by means of ELISA, using the ScheBo® M2-PK™ Stool Test reagents (ScheBoBioTech AG, Germany). GI-syndromic testing was done using Gastrointestinal panel cartridge reagents from QIAstat Dx (QIAGEN, Germany) and QIAstat Dx analyzer. With GI-syndromic testing, in the timespan of 70 minutes, 4 parasites, 6 viruses and 14 bacteria can be determined.

The assay is designed to be used with the QIAstat-Dx Analyzer 1.0 for integrated nucleic acid extraction and multiplex real-time RT-PCR detection. The Gastrointestinal panel assay followed the manufacturer's protocol. The integrated software automatically interpreted the real-time amplification signals and generated a report with results for 24 viral, bacterial, and parasite targets, along with the internal control (IC). The IC verifies the entire analysis process. A positive IC signal ensures a valid Gastrointestinal panel assay result, while a negative IC signal invalidates all results, except for identified Gastrointestinal panel assay targets.[11,12]

Statistical Analysis: Data were organized in SPSS version 26. We used the Mann-Whitney test to analyze the relationship between f-CP and GI-syndromic testing and between M2-PK and GI-syndromic testing, with $p<0.05$, as a significant difference.

RESULTS

Out of the 546 faecal specimens collected between October 2021 and September 2022, 282 specimens met the inclusion and exclusion criteria after selection. The subject characteristics and laboratory results are detailed in Table 1. EPEC and EAEC were commonest pathogen

Table 1: Characteristics of study subjects and laboratory results (n=282).

Variable	N (%)
Sex	
Male	110 (39)
Female	172 (61)
Age (years)	
Age range	**18 – 84**
Mean (SD)	**50.52±15.20**
< 60	190 (67.4)
≥ 60	92 (32.6)
GI-syndromic testing	
Pathogens found	117(41.5)
No pathogens found	165 (58.5)
Fecal calprotectin	
Median (min-max)	**77.1 (3.68-2100)**
<50 µg/g	103(36.5)
≥50 µg/g	179 (63.5)
M2-PK (U/mL)	
Median (min-max)	**3.55 (1-72.6)**
< 4 U/mL	157 (55.7)
≥ 4 U/mL	125 (44.3)

Table 2: Types of gastrointestinal pathogens (n=117).

Pathogens	N	%
EPEC	56	34.15
EAEC	30	18.29
Plesiomonas shigelloides	15	9.15
Clostridioides difficile toxin *A or B*	14	8.54
ETEC *(lt/st)*	10	6.10
EIEC*/Shigella*	8	4.88
STEC *(stx1/stx2)*	6	3.66
Campylobacter spp.	5	3.05
Salmonella spp.	4	2.43
Norovirus GII	4	2.43
Sapovirus	3	1.83
Cryptosporidium spp.	2	1.22
Entamoeba histolytica	1	0.61
Giardia lamblia	1	0.61
Cyclospora cayetanensis	1	0.61
Norovirus GI	1	0.61
Adenovirus F40/F41	1	0.61
Vibrio sp.	1	0.61
Yersinia enterocolitica	1	0.61
Total	164	100%

found (Table 2). EPEC and EAEC and EPEC and Plesiomonas *shigelloides* were commonest pathogens when two pathogens were detected (Table 3). Types of pathogens in co-infections with more than 2 pathogens are shown in Table 4.

The Mann-Whitney test showed that for the association between f-CP and intestinal pathogens a p=0.048 was found, showing a significant association between f-CP and detection of intestinal pathogens. On the other hand, for the association between M2-PK and intestinal pathogens, we found p=0.381, indicating that there was no significant association between M2-PK concentration and detection of intestinal pathogens.

Table 3: Types of pathogens in co-infections with 2 pathogens (n=31).

Pathogens (2 pathogens)	N	%
EPEC and EAEC	9	29.02
EPEC and Plesiomonas *shigelloides*	5	16.12
EPEC and ETEC (lt/st)	2	6.45
EAEC and *Plesiomonas shigelloides*	2	6.45
EAEC and *C. difficile* toxin A or B	2	6.45
EPEC and *C. difficile* toxin A or B	2	6.45
Plesiomonas shigelloides and *Campylobacter spp*.	2	6.45
EPEC and *Salmonella spp.*	1	3.23
EAEC and Norovirus GII	1	3.23
EPEC and *Giardia lamblia*	1	3.23
EPEC and *Campylobacter spp.*	1	3.23
ETEC (lt/st) and Norovirus GII	1	3.23
EIEC/*Shigella* and STEC (stx1 or stx)	1	3.23
STEC (stx1 or stx2) and *C. difficile* toxin A or B	1	3.23

DISCUSSION

In this study, the sex distribution showed that most subjects were women at 61%, as compared to men at 39%. The participants of this study had a median age of 52.5 years (range 18-84). A study from Cameroon by Ousenu et al, also found the proportion of gastroenteritis cases in women to be 67.3% compared to men.[13] Our previous study also found that the most frequent cases of gastroenteritis occurred in women (53.8%).[14] In a study from US, 62% of those having complaints of gastroenteritis were women with mean age of 44.3±19.6) years.[15] The study by Schmidt et al, also found a prevalence of 52% in women, somewhat higher than in men (48%).[16] Slightly differing results were found in a study from Shanghai, in which there were more cases of gastroenteritis in men at 52.1%, as compared to women at 47.9%.[17] Sinha et al, concluded that women show a greater diversity of intestinal microbiota as compared to men, but this diversity does not significantly affect the functional level of the intestinal microbiota.[18]

Table 4: Types of pathogens in co-infections with more than 2 pathogens (n=8).

Pathogens (>2 pathogens)	N	%
EAEC, EPEC and ETEC	1	12.5
EAEC, ETEC (lt/st) and Norovirus GI	1	12.5
EAEC, EPEC and EIEC/*Shigella*	1	12.5
EAEC, STEC (stx1 or stx2) and *Salmonella sp*.	1	12.5
ETEC (lt/st), Sapovirus and Adenovirus F40 or F41	1	12.5
EPEC, ETIC/*Shigella* and *Cyclospora cayetanensis*	1	12.5
Campylobacter spp., *Plesiomonas shigelloides* and Sapovirus	1	12.5
Vibrio sp., *Plesiomonas shigelloides*, and *Yersinia enterocolitica*	1	12.5

In our study, we found a detection rate of 41.5% for intestinal pathogens in fecal specimens, where the 5 most frequent pathogens were EPEC, EAEC, *Plesiomonas shigelloides*, *Clostridioides difficile* toxin A or B and ETEC. Our results are higher than those of Axelrad et al, with a positivity rate of 29.2% using GI-syndromic testing.[19] Nearly identical findings were obtained by Johansen et al, with a positivity rate of 37% using GI syndromic testing.[1] Likewise, a study by Sobczyk et al, found that the multiplex gastrointestinal panel can detect pathogens at a rate of 52.5%.[20]

According to Hu et al, the most prevalent pathogens were EPEC, ETEC, Norovirus, and EAEC. Similar results were found by Axelrad et al, where the 2 most frequent causative pathogens of gastroenteritis were EPEC and Norovirus.[19,21] The Indonesian goverment study conducted in 2018 by the Ministry of Health, found as the most frequent diarrhea-causing viral pathogens the Rotavirus, Adenovirus and Norovirus, whereas the most frequent bacteria were ETEC, but the study did not detect other pathogenic *E. coli*. Pathogenic *E. coli* are the main etiology of diarrhea in low and middle income countries.[22] A study conducted in several Indonesian cities found that more than 90% of food-borne diseases were caused by contaminating microorganisms and a study from East Java found that rolled noodles contained *E. coli*.[23]

Statistical testing determined that f-CP had a significant relationship with detection of intestinal pathogens, as had also been obtained previously by Lam et a.[8] However, the limitation of the this study is that the specificity and

sensitivity of f-CP is only 53-55%, with a cut off value of 133 µg/g according to the ROC curve (data not shown). Migration of the neutrophil and secretion of the calprotectin causes apoptosis or epithelial damage and calprotectin secretion by dead or damaged epithelial cells, such that the calprotectin level increases.[8]

Czub et al, found that M2-PK performed equally in differentiating both acute rotaviral and *Salmonella enteritidis* diarrhea and healthy persons but M2-PK is not proven to be clinically beneficial for the differential diagnosis of infectious acute diarrhea.[9] M2-PK is one of the isoforms of pyruvate kinase that catalyses glycolysis in rapidly dividing cells. Because M2-PK dimers may be expressed in cancer cells and may be detected in plasma, currently M2-PK is used to detect the presence of colorectal cancer. Some studies demonstrated that M2-PK may also be used to show the presence of intestinal inflammation.[24]

The limitation of our study is that there is no cut-off value for f-CP that has excellent sensitivity and specificity, as found by other investigators. We did not differentiate between bacterial pathogens, viruses, and parasites for separate analysis, because of the limited distribution of the viruses as well as the parasites. This study may be taken into consideration by clinicians for using the f-CP biomarker to support the diagnosis of gastrointestinal infections.

CONCLUSION

Fecal calprotectin was significantly associated with gastrointestinal infection and may therefore be considered for supporting the diagnosis of gastrointestinal infections in adult patients, particularly in healthcare facilities without resources for microbial culture and syndromic testing.

Author Contributions:
Conception and design: Ade Dharmawan, Pusparini.
Collection and assembly of data: Ade Dharmawan, Pusparini.
Analysis and interpretation of data: Ade Dharmawan
Drafting of the article: Ade Dharmawan.
Critical revision of article for important intellectual content: Pusparini.
Statistical Expertise: Pusparini.
Final approval and guarantor of the article: Pusparini.
Corresponding author email: Pusparini: pusparini@trisakti.ac.id
Conflict of Interest: None declared.
Source of Funding: None disclosed.
Rec. Date: Aug 13, 2024 Revision Rec. Date: Aug 22, 2024 Accept Date: Sept 1, 2024.

REFERENCES

1. Johansen RL, Schouw CH, Madsen TV, Nielsen XC, Engberg J. Epidemiology of gastrointestinal infections: lessons learned from syndromic testing, Region Zealand, Denmark. Eur J Clin Microbiol Infect Dis 2023;42:1091-1101.
2. Adedire O, Love NK, Hughes HE, Buchan I, Vivancos R, Elliot AJ. Early Detection and Monitoring of Gastrointestinal Infections Using Syndromic Surveillance: A Systematic Review. Int J Environ Res Public Health 2024;21:1-13.
3. Kementerian Kesehatan RI. Laporan Nasional Riskesdas 2018. Badan Penelit dan Pengemb Kesehat 2018;1:1. https://www.kemkes.go.id/article/view/19093000001/penyakit-jantung-penyebab-kematian-terbanyak-ke-2-di-indonesia.html
4. Sokic-Milutinovic A, Pavlovic-Markovic A, Tomasevic RS, Lukic S. Diarrhea as a Clinical Challenge: General Practitioner Approach. Dig Dis 2022;40:282-9.
5. Sýkora J, Siala K, Huml M, Varvaovská J, Schwarz J, Pomahaová R. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young children with acute gastroenteritis. Acta Paediatr Int J Paediatr 2010;99:1389-95.
6. Cassidy H, Van Genne M, Lizarazo-Forero E, Gard L, Niesters HGM. A discussion of syndromic molecular testing for clinical care. J Antimicrob Chemother 2021;76:58-66.
7. Bard JD, McElvania E. Panels and Syndromic Testing in Clinical Microbiology. Clin Lab Med 2020;40:393-420.
8. Lam YA, Warouw SM, Wahani AMI, Manoppo JIC, Salendu PM. Correlation between gut pathogens and fecal calprotectin levels in young children with acute diarrhea. Paediatr Indones 2014;54:193-7.
9. Czub E, Nowak JK, Moczko J, Lisowska A, Banaszkiewicz A, Banasiewicz T, et al. Comparison of fecal pyruvate kinase isoform M2 and calprotectin in acute diarrhea in hospitalized children. Sci Rep 2014;4:2-4.
10. Aboelsoud A, Elehleh A, Montser B, Talaat A. Study of the diagnostic role of fecal M2 - pyruvate kinase level in patients with colorectal cancer. Menoufia Med J 2021;34:1268-74.
11. Boers SA, Peters CJA, Wessels E, Melchers WJG, Claas ECJ. Performance of the QIAstat-Dx gastrointestinal panel for diagnosing infectious gastroenteritis. J Clin Microbiol 2020;581:e01737-19.
12. Qiagen. Instruction For Use (Handbook) QIAstat-Dx® Gastrointestinal Panel. 2020;1-91.
13. Ousenu K, Sama LF, Ali IM, Fonbah JL, Nadine OS, Dabou S, et al. Aetiology and risk factors of bacterial gastroenteritis among febrile outpatients at the Dschang District Hospital, West Region of Cameroon: A cross-sectional study. BMJ Open 2021;11:1-8.
14. Dharmawan A, Pusparini. Syndromic testing for increasing diagnostic accuracy in gastrointestinal infection. J Infect Dev Ctries 2023;17:1262-7.
15. Moon RC, Bleak TC, Rosenthal NA, Couturier B, Hemmert R, Timbrook TT, et al. Epidemiology and Economic Burden of Acute Infectious Gastroenteritis among Adults Treated in Outpatient Settings in US Health Systems. Am J Gastroenterol 2023;118:1069-79.
16. Schmidt MA, Groom HC, Rawlings AM, Mattison CP, Salas SB, Burke RM, et al. Incidence, Etiology, and Healthcare Utilization for Acute Gastroenteritis in the

Community, United States. Emerg Infect Dis 2022;28:2234-42.
17. Luo L, Gu Y, Wang X, Zhang Y, Zhan L, Liu J, et al. Epidemiological and clinical differences between sexes and pathogens in a three-year surveillance of acute infectious gastroenteritis in Shanghai. Sci Rep 2019;9:1-9.
18. Sinha T, Vich Vila A, Garmaeva S, Jankipersadsing SA, Imhann F, Collij V, et al. Analysis of 1135 gut metagenomes identifies sex-specific resistome profiles. Gut Microbes 2019;10:358-66.
19. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA. Impact of gastrointestinal panel implementation on health care utilization and outcomes. J Clin Microbiol 2019;57:1-8.
20. Sobczyk J, Jain S, Sun X, Karris M, Wooten D, Stagnaro J, et al. Comparison of multiplex gastrointestinal pathogen panel and conventional stool testing for evaluation of patients with HIV infection. Open Forum Infect Dis 2020;7:547.
21. Hu P, Liu C, Ruan J, Yuan M, Ju C, Ma Y, et al. FilmArray GI-panel performance for the rapid and multiple detection of gastrointestinal microorganisms in foodborne illness outbreaks in Shenzhen during 2018–2019. Infect Genet Evol 2020;86:1-6.
22. Puspandari N, Amalia N, Hartoyo Y, Nursofiah S, Sunarno S, Sariadji K, et al. Enteric pathogen among children under five years old with diarrheal diseases in Indonesia. IOP Conf Ser Earth Environ Sci 2021;913:1-6.
23. Syahrul F, Wahyuni CU, Notobroto HB, Wasito EB, Adi AC, Dwirahmadi F. Transmission media of foodborne diseases as an index prediction of diarrheagenic Escherichia coli: Study at elementary school, Surabaya, Indonesia. Int J Environ Res Public Health 2020;17:1-13.
24. Alhadi SC, Zain WZW, Zahari Z, Hashim MNM, Aziz SHSA, Zakaria Z, et al. The use of M2-pyruvate kinase as a stool biomarker for detection of colorectal cancer in tertiary teaching hospital: A comparative study. Ann Coloproctol 2021;36:409-14.

Association of fecal calprotectin and M2 pyruvate kinase with fecal pathogenic microorganisms in gastrointestinal infection

By Pusparini Pusparini

WORD COUNT 3279
TIME SUBMITTED 12-NOV-2024 08:08AM
PAPER ID 112875658

Original Article

Association of fecal calprotectin and M2 pyruvate kinase with fecal pathogenic microorganisms in gastrointestinal infection

Ade Dharmawan,[1] Pusparini[2]

[1]Department of Microbiology, Faculty of Medicine and Health Science, Krida Wacana Christian University,
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Trisakti, Jakarta Indonesia.

Objective: To determine the association of Fecal Calprotectin (f-CP) and M2 Pyruvate Kinase (M2-PK) with intestinal pathogens by means of the gastrointestinal syndromic panel.

Methodology: This retrospective analytic study was conducted in the laboratory department a private hospital in Jakarta from October 2021 to September 2022. The inclusion criteria comprised patients aged ≥18 years with gastrointestinal complaints who had undergone f-CP and M2-PK examinations and gastrointestinal syndromic testing. The exclusion criteria were incomplete data on laboratory examinations, follow-up examinations or treatment evaluations. We used the Mann-Whitney test with $p<0.05$ indicating significantly different.

Results: Out of 282 subjects, 69% were women and 31% men, aged 18 to 84 years (mean 50.52±15.20). In 117 subjects, single pathogen was found in 78 specimens and ≥2 pathogens in 39. Median f-CP was 77.1 μg/g (3.68-2100) and median M2-PK 3.55 U/mL (1-72.6). Mann-Whitney test found a significant association between f-CP and intestinal pathogens (p=0.048), whereas for the M2-PK, no significant association was found with intestinal pathogens a(p=0.381).

Conclusion: f-CP increases in gastrointestinal infection with intestinal pathogens, but M2-PK does not increase. Therefore, f-CP can be considered a biomarker for gastrointestinal infections caused by microorganisms.

Keywords: Fecal calprotectin, GI-syndromic testing, gastrointestinal infection, M2-PK.

INTRODUCTION

Gastrointestinal diseases contribute significantly to the infectious disease burden throughout the world.[1] Gastrointestinal infections lead to symptoms such as diarrhea and result morbidity and mortality in certain populations.[2] The national prevalence of diarrhea is 6.8% based on the Indonesian goverment data for 2018.[3] It is of utmost importance that empirical treatment of diarrhea and preventive measures be guided by the impact and distribution of infectious agents on diarrheal disease.[4]

Clinicians frequently have difficulty in differentiating infectious from non-infectious diarrhea.[5] Faecal culture, microscopy, tests for occult blood in feces, and fecal lactoferrin assay are frequently not sensitive enough. Currently, multiplex polymerase chain reaction (PCR) based examinations are preferred, because they can detect the presence of infection in a short time.[6,7] Syndromic testing is a molecular assay that can detect several targets simultaneously, including parasites, viruses and bacteria.[6,7] Gastrointestinal infections assays for biomarkers are needed that are simple, rapid, and accurate, for the diagnosis because faeces flows along the whole intestinal mucosa where molecular markers of damage or inflammation accumulate.[5,8]

Faecal calprotectin (f-CP) and M2 pyruvate kinase (M2-PK) are gastrointestinal mucosal inflammatory biomarkers. M2-PK is the enzyme responsible for the transfer of phosphate groups in glycolysis. Both dimer M2-PK and tetramers M2-PK are found in proliferating cells of various tissues.[9] As M2-PK is also present in leukocytes, M2-PK may be found in the feces mass that is formed while in contact with the inflamed mucosa of the digestive tract. Because of the high stability of M2-PK, its faecal concentration may reflect the presence of gastrointestinal tract inflammation. The two important uses of M2-PK are the evaluation of cancer screening and the degree of inflammatory bowel disease (IBD).[9,10]

Faecal calprotectin is a Ca-binding protein, that accounts for 60% of the cytosolic proteins in neutrophils. The immunomodulatory and antiproliferative properties of calprotectin serve an important function in antibacterial neutrophil defence.[8] Neutrophil infiltration of the mucosa and migration into the intestinal lumen is indicated by f-CP concentration that correlates positively with inflammatory symptoms and epithelial damage. One study showed that f-CP concentration was higher in diarrhea caused by intestinal bacterial pathogens as compared with diarrhoea from other causes.[8] Lam et al,[8] found that a mean of 100.77 μg/g indicated a significant correlation between f-CP and intestinal pathogens, while

Sýkora et al, determined the cut-off value for differentiating acute bacterial gastroenteritis to be 103.9 μg/g.[5] Not all healthcare facilities have resources for bacterial culture, particularly syndromic testing, which is a rather expensive examination. The aim of this study was to determine the association of f-CP and M2-PK with intestinal pathogens

METHODOLOGY

This retrospective analytical study used the data of 282 subjects and was carried out from October 2021 until September 2022 in Laboratory department a private hospital in Jakarta. The study obtained ethical clearance from the Ethics Committee, Medicine and Health Sciences Faculty, University of Christian Krida Wacana, Jakarta, (Ref No. 1405/SLKE-IM/UKKW/FKIK/KE/XI/2022, dated November 30, 2022).

The criteria for inclusion were age more than 18 years, having diarrhea, and having undergone simultaneous examinations for f-CP, M2-PK, and GI-syndromic testing. The exclusion criteria were incomplete follow-up, treatment evaluation, and laboratory data.

The fecal specimens were examined for f-CP, M2-PK and GI-syndromic testing. f-CP was examined by means of ELISA (Enzyme Linked Immunosorbent Assay), using IDK® Calprotectin reagents (Immundiagnostik AG, Germany) and an Euroimmune analyzer. M2-PK was also examined by means of ELISA, using the ScheBo® M2-PK™ Stool Test reagents (ScheBoBioTech AG, Germany). GI-syndromic testing was done using Gastrointestinal panel cartridge reagents from QIAstat Dx (QIAGEN, Germany) and QIAstat Dx analyzer. With GI-syndromic testing, in the timespan of 70 minutes, 4 parasites, 6 viruses and 14 bacteria can be determined. The assay is designed to be used with the QIAstat-Dx Analyzer 1.0 for integrated nucleic acid extraction and multiplex real-time RT-PCR detection. The Gastrointestinal panel assay followed the manufacturer's protocol. The integrated software automatically interpreted the real-time amplification signals and generated a report with results for 24 viral, bacterial, and parasite targets, along with the internal control (IC). The IC verifies the entire analysis process. A positive IC signal ensures a valid Gastrointestinal panel assay result, while a negative IC signal invalidates all results, except for identified Gastrointestinal panel assay targets.[11,12]

Statistical Analysis: Data were organized in SPSS version 26. We used the Mann-Whitney test to analyze the relationship between f-CP and GI-syndromic testing and between M2-PK and GI-syndromic testing, with $p<0.05$, as a significant difference.

RESULTS

Out of the 546 faecal specimens collected between October 2021 and September 2022, 282 specimens met the inclusion and exclusion criteria after selection. The subject characteristics and laboratory results are detailed in Table 1. EPEC and EAEC were commonest pathogen

Table 1: Characteristics of study subjects and laboratory results (n=282).

Variable	N (%)
Sex	
Male	110 (39)
Female	172 (61)
Age (years)	
Age range	**18 – 84**
Mean (SD)	**50.52±15.20**
< 60	190 (67.4)
≥ 60	92 (32.6)
GI-syndromic testing	
Pathogens found	117(41.5)
No pathogens found	165 (58.5)
Fecal calprotectin	
Median (min-max)	**77.1 (3.68-2100)**
<50 μg/g	103(36.5)
≥50 µg/g	179 (63.5)
M2-PK (U/mL)	
Median (min-max)	**3.55 (1-72.6)**
< 4 U/mL	157 (55.7)
≥ 4 U/mL	125 (44.3)

Table 2: Types of gastrointestinal pathogens (n=117).

Pathogens	N	%
EPEC	56	34.15
EAEC	30	18.29
Plesiomonas shigelloides	15	9.15
Clostridioides difficile toxin *A or B*	14	8.54
ETEC *(lt/st)*	10	6.10
EIEC/*Shigella*	8	4.88
STEC *(stx1/stx2)*	6	3.66
Campylobacter spp.	5	3.05
Salmonella spp.	4	2.43
Norovirus GII	4	2.43
Adenovirus	3	1.83
Cryptosporidium spp.	2	1.22
Entamoeba histolytica	1	0.61
Giardia lamblia	1	0.61
Cyclospora cayetanensis	1	0.61
Norovirus GI	1	0.61
Adenovirus F40/F41	1	0.61
Vibrio sp.	1	0.61
Yersinia enterocolitica	1	0.61
Total	164	100%

found (Table 2). EPEC and EAEC and EPEC and Plesiomonas *shigelloides* were commonest pathogens when two pathogens were detected (Table 3). Types of pathogens in co-infections with more than 2 pathogens are shown in Table 4.

The Mann-Whitney test showed that for the association between f-CP and intestinal pathogens a p=0.048 was found, showing a significant association between f-CP and detection of intestinal pathogens. On the other hand, for the association between M2-PK and intestinal pathogens, we found p=0.381, indicating that there was no significant association between M2-PK concentration and detection of intestinal pathogens.

Table 3: Types of pathogens in co-infections with 2 pathogens (n=31).

Pathogens (2 pathogens)	N	%
EPEC and EAEC	9	29.02
EPEC and Plesiomonas *shigelloides*	5	16.12
EPEC and ETEC (lt/st)	2	6.45
EAEC and *Plesiomonas shigelloides*	2	6.45
EAEC and *C. difficile* toxin A or B	2	6.45
EPEC and *C. difficile* toxin A or B	2	6.45
Plesiomonas shigelloides and *Campylobacter spp.*	2	6.45
EPEC and *Salmonella spp.*	1	3.23
EAEC and Norovirus GII	1	3.23
EPEC and *Giardia lamblia*	1	3.23
EPEC and *Campylobacter spp.*	1	3.23
ETEC (lt/st) and Norovirus GII	1	3.23
EIEC/*Shigella* and STEC (stx1 or stx)	1	3.23
STEC (stx1 or stx2) and *C. difficile* toxin A or B	1	3.23

DISCUSSION

In this study, the sex distribution showed that most subjects were women at 61%, as compared to men at 39%. The participants of this study had a median age of 52.5 years (range 18-84). A study from Cameroon by Ousenu et al, also found the proportion of gastroenteritis cases in women to be 67.3% compared to men.[13] Our previous study also found that the most frequent cases of gastroenteritis occurred in women (53.8%).[14] In a study from US, 62% of those having complaints of gastroenteritis were women with mean age of 44.3±19.6) years.[15] The study by Schmidt et al, also found a prevalence of 52% in women, somewhat higher than in men (48%).[16] Slightly differing results were found in a study from Shanghai, in which there were more cases of gastroenteritis in men at 52.1%, as compared to women at 47.9%.[17] Sinha et al, concluded that women show a greater diversity of intestinal microbiota as compared to men, but this diversity does not significantly affect the functional level of the intestinal microbiota.[18]

Table 4: Types of pathogens in co-infections with more than 2 pathogens (n=8).

Pathogens (>2 pathogens)	N	%
EAEC, EPEC and ETEC	1	12.5
EAEC, ETEC (lt/st) and Norovirus GI	1	12.5
EAEC, EPEC and EIEC/*Shigella*	1	12.5
EAEC, STEC (stx1 or stx2) and *Salmonella sp.*	1	12.5
ETEC (lt/st), Sapovirus and Adenovirus F40 or F41	1	12.5
EPEC, ETIC/*Shigella* and *Cyclospora cayetanensis*	1	12.5
Campylobacter spp., *Plesiomonas shigelloides* and Sapovirus	1	12.5
Vibrio sp., *Plesiomonas shigelloides*, and *Yersinia enterocolitica*	1	12.5

In our study, we found a detection rate of 41.5% for intestinal pathogens in fecal specimens, where the 5 most frequent pathogens were EPEC, EAEC, *Plesiomonas shigelloides*, *Clostridioides difficile* toxin A or B and ETEC. Our results are higher than those of Axelrad et al, with a positivity rate of 29.2% using GI-syndromic testing.[19] Nearly identical findings were obtained by Johansen et al, with a positivity rate of 37% using GI syndromic testing.[1] Likewise, a study by Sobczyk et al, found that the multiplex gastrointestinal panel can detect pathogens at a rate of 52.5%.[20]

According to Hu et al, the most prevalent pathogens were EPEC, ETEC, Norovirus, and EAEC. Similar results were found by Axelrad et al, where the 2 most frequent causative pathogens of gastroenteritis were EPEC and Norovirus.[19,21] The Indonesian goverment study conducted in 2018 by the Ministry of Health, found as the most frequent diarrhea-causing viral pathogens the Rotavirus, Adenovirus and Norovirus, whereas the most frequent bacteria were ETEC, but the study did not detect other pathogenic *E. coli*. Pathogenic *E. coli* are the main etiology of diarrhea in low and middle income countries.[22] A study conducted in several Indonesian cities found that more than 90% of food-borne diseases were caused by contaminating microorganisms and a study from East Java found that rolled noodles contained *E. coli*.[23]

Statistical testing determined that f-CP had a significant relationship with detection of intestinal pathogens, as had also been obtained previously by Lam et a.[8] However, the limitation of the this study is that the specificity and

sensitivity of f-CP is only 53-55%, with a cut off value of 133 μg/g according to the ROC curve (data not shown). Migration of the neutrophil and secretion of the calprotectin causes apoptosis or epithelial damage and calprotectin secretion by dead or damaged epithelial cells, such that the calprotectin level increases.[8]

Czub et al, found that M2-PK performed equally in differentiating both acute rotaviral and *Salmonella enteritidis* diarrhea and healthy persons but M2-PK is not proven to be clinically beneficial for the differential diagnosis of infectious acute diarrhea.[9] M2-PK is one of the isoforms of pyruvate kinase that catalyses glycolysis in rapidly dividing cells. Because M2-PK dimers may be expressed in cancer cells and may be detected in plasma, currently M2-PK is used to detect the presence of colorectal cancer. Some studies demonstrated that M2-PK may also be used to show the presence of intestinal inflammation.[24]

The limitation of our study is that there is no cut-off value for f-CP that has excellent sensitivity and specificity, as found by other investigators. We did not differentiate between bacterial pathogens, viruses, and parasites for separate analysis, because of the limited distribution of the viruses as well as the parasites. This study may be taken into consideration by clinicians for using the f-CP biomarker to support the diagnosis of gastrointestinal infections.

CONCLUSION

Fecal calprotectin was significantly associated with gastrointestinal infection and may therefore be considered for supporting the diagnosis of gastrointestinal infections in adult patients, particularly in healthcare facilities without resources for microbial culture and syndromic testing.

Author Contributions:
Conception and design: Ade Dharmawan, Pusparini.
Collection and assembly of data: Ade Dharmawan, Pusparini.
Analysis and interpretation of data: Ade Dharmawan
Drafting of the article: Ade Dharmawan.
Critical revision of article for important intellectual content: Pusparini.
Statistical Expertise: Pusparini.
Final approval and guarantor of the article: Pusparini.
Corresponding author email: Pusparini: pusparini@trisakti.ac.id
Conflict of Interest: None declared.
Source of Funding: None disclosed.
Rec. Date: Aug 13, 2024 Revision Rec. Date: Aug 22, 2024 Accept Date: Sept 1, 2024.

REFERENCES

1. Johansen RL, Schouw CH, Madsen TV, Nielsen XC, Engberg J. Epidemiology of gastrointestinal infections: lessons learned from syndromic testing, Region Zealand, Denmark. Eur J Clin Microbiol Infect Dis 2023;42:1091-1101.
2. Adedire O, Love NK, Hughes HE, Buchan I, Vivancos R, Elliot AJ. Early Detection and Monitoring of Gastrointestinal Infections Using Syndromic Surveillance: A Systematic Review. Int J Environ Res Public Health 2024;21:1-13.
3. Kementerian Kesehatan RI. Laporan Nasional Riskesdas 2018. Badan Penelit dan Pengemb Kesehat 2018;1:1. https://www.kemkes.go.id/article/view/19093000001/penyakit-jantung-penyebab-kematian-terbanyak-ke-2-di-indonesia.html
4. Sokic-Milutinovic A, Pavlovic-Markovic A, Tomasevic RS, Lukic S. Diarrhea as a Clinical Challenge: General Practitioner Approach. Dig Dis 2022;40:282-9.
5. Sýkora J, Siala K, Huml M, Varvaovská J, Schwarz J, Pomahaová R. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young children with acute gastroenteritis. Acta Paediatr Int J Paediatr 2010;99:1389-95.
6. Cassidy H, Van Genne M, Lizarazo-Forero E, Gard L, Niesters HGM. A discussion of syndromic molecular testing for clinical care. J Antimicrob Chemother 2021;76:58-66.
7. Bard JD, McElvania E. Panels and Syndromic Testing in Clinical Microbiology. Clin Lab Med 2020;40:393-420.
8. Lam YA, Warouw SM, Wahani AMI, Manoppo JIC, Salendu PM. Correlation between gut pathogens and fecal calprotectin levels in young children with acute diarrhea. Paediatr Indones 2014;54:193-7.
9. Czub E, Nowak JK, Moczko J, Lisowska A, Banaszkiewicz A, Banasiewicz T, et al. Comparison of fecal pyruvate kinase isoform M2 and calprotectin in acute diarrhea in hospitalized children. Sci Rep 2014;4:2-4.
10. Aboelsoud A, Elehleh A, Montser B, Talaat A. Study of the diagnostic role of fecal M2 - pyruvate kinase level in patients with colorectal cancer. Menoufia Med J 2021;34:1268-74.
11. Boers SA, Peters CJA, Wessels E, Melchers WJG, Claas ECJ. Performance of the QIAstat-Dx gastrointestinal panel for diagnosing infectious gastroenteritis. J Clin Microbiol 2020;581:e01737-19.
12. Qiagen. Instruction For Use (Handbook) QIAstat-Dx® Gastrointestinal Panel. 2020;1-91.
13. Ousenu K, Sama LF, Ali IM, Fonbah JL, Nadine OS, Dabou S, et al. Aetiology and risk factors of bacterial gastroenteritis among febrile outpatients at the Dschang District Hospital, West Region of Cameroon: A cross-sectional study. BMJ Open 2021;11:1-8.
14. Dharmawan A, Pusparini. Syndromic testing for increasing diagnostic accuracy in gastrointestinal infection. J Infect Dev Ctries 2023;17:1262-7.
15. Moon RC, Bleak TC, Rosenthal NA, Couturier B, Hemmert R, Timbrook TT, et al. Epidemiology and Economic Burden of Acute Infectious Gastroenteritis among Adults Treated in Outpatient Settings in US Health Systems. Am J Gastroenterol 2023;118:1069-79.
16. Schmidt MA, Groom HC, Rawlings AM, Mattison CP, Salas SB, Burke RM, et al. Incidence, Etiology, and Healthcare Utilization for Acute Gastroenteritis in the

Community, United States. Emerg Infect Dis 2022;28:2234-42.
17. Luo L, Gu Y, Wang X, Zhang Y, Zhan L, Liu J, et al. Epidemiological and clinical differences between sexes and pathogens in a three-year surveillance of acute infectious gastroenteritis in Shanghai. Sci Rep 2019;9:1-9.
18. Sinha T, Vich Vila A, Garmaeva S, Jankipersadsing SA, Imhann F, Collij V, et al. Analysis of 1135 gut metagenomes identifies sex-specific resistome profiles. Gut Microbes 2019;10:358-66.
19. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA. Impact of gastrointestinal panel implementation on health care utilization and outcomes. J Clin Microbiol 2019;57:1-8.
20. Sobczyk J, Jain S, Sun X, Karris M, Wooten D, Stagnaro J, et al. Comparison of multiplex gastrointestinal pathogen panel and conventional stool testing for evaluation of patients with HIV infection. Open Forum Infect Dis 2020;7:547.
21. Hu P, Liu C, Ruan J, Yuan M, Ju C, Ma Y, et al. FilmArray GI-panel performance for the rapid and multiple detection of gastrointestinal microorganisms in foodborne illness outbreaks in Shenzhen during 2018–2019. Infect Genet Evol 2020;86:1-6.
22. Puspandari N, Amalia N, Hartoyo Y, Nursofiah S, Sunarno S, Sariadji K, et al. Enteric pathogen among children under five years old with diarrheal diseases in Indonesia. IOP Conf Ser Earth Environ Sci 2021;913:1-6.
23. Syahrul F, Wahyuni CU, Notobroto HB, Wasito EB, Adi AC, Dwirahmadi F. Transmission media of foodborne diseases as an index prediction of diarrheagenic Escherichia coli: Study at elementary school, Surabaya, Indonesia. Int J Environ Res Public Health 2020;17:1-13.
24. Alhadi SC, Zain WZW, Zahari Z, Hashim MNM, Aziz SHSA, Zakaria Z, et al. The use of M2-pyruvate kinase as a stool biomarker for detection of colorectal cancer in tertiary teaching hospital: A comparative study. Ann Coloproctol 2021;36:409-14.

Association of fecal calprotectin and M2 pyruvate kinase with fecal pathogenic microorganisms in gastrointestinal infection

ORIGINALITY REPORT

5%
SIMILARITY INDEX

PRIMARY SOURCES

1	docksci.com Internet	37 words — 1%
2	www.qiagen.com Internet	24 words — 1%
3	www.ncbi.nlm.nih.gov Internet	19 words — 1%
4	sites.kowsarpub.com Internet	15 words — 1%
5	academic.oup.com Internet	9 words — < 1%
6	www.journalijar.com Internet	9 words — < 1%
7	bmchealthservres.biomedcentral.com Internet	8 words — < 1%
8	www.medrxiv.org Internet	8 words — < 1%
9	www.wjgnet.com Internet	8 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 8 WORDS