RESEARCH ARTICLE

Editorial Process: Submission:10/03/2024 Acceptance:02/14/2025

Accuracy of Faecal Matrix-Metalloproteinase-9 (MMP-9) for Colorectal Cancer Detection in Makassar, Indonesia

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Abstract

Background: Colorectal cancer (CRC) is one of the most frequent malignancies. Insufficient comprehensive screening by health authorities raises colorectal cancer risk in developing countries. While colonoscopy is the gold standard for CRC diagnosis, it has limitations. Faecal immunochemical test (FIT) and guaiac faecal occult blood test (gFOBT) are famous for colorectal cancer screening tests. FIT is more sensitive and specific than gFOBT. However, both FIT and gFOBT have limited accuracy in diagnosing advanced adenoma and proximal colon cancer. Matrix-metalloproteinase-9 (MMP-9) remodels the extracellular matrix and is associated with CRC progression. Its levels can be detected in faecal samples. Studies specifically evaluating the accuracy of faecal MMP-9 as a diagnostic test are limited. Therefore, this study aims to evaluate the accuracy of faecal MMP-9 for detecting colorectal cancer in our centre. **Methods:** Ninety patients provided faecal samples for MMP-9 analysis. Faecal MMP-9 levels were measured by ELISA. Diagnostic accuracy was evaluated using the diagnostic test method. **Result:** Patients were divided into four groups: control: 10; hyperplastic and adenoma polyp: 9; colitis and proctitis: 37; and colorectal carcinoma: 34. Faecal MMP-9 levels significantly increased in colorectal carcinoma compared to the other group (P<0.001). ROC analysis indicated that the accuracy of faecal MMP-9 in differentiating CRC patients against controls was good, with the area under curve (AUC) 0.85 (sensitivity 82.35% and specificity 80%). The accuracy of faecal MMP-9 for detecting proximal colon cancer was excellent, with an AUC of 0.96 (sensitivity 80% and specificity 80%). The AUC for adenoma detection was 0.83, with a sensitivity of 77.78% and specificity of 80%. **Conclusion:** Faecal MMP-9 demonstrated significant accuracy in differentiating colorectal cancer from normal subjects, exhibiting satisfactory sensitivity and specificity. Compared to FIT, Faecal MMP-9 demonstrated superior detection of proximal colon cancer and adenoma.

Keywords: Faecal MMP-9- Colorectal Cancer- Detection- Diagnostic Test

Asian Pac J Cancer Prev, **26 (2)**,

Introduction

Colorectal cancer (CRC) is among the most frequent malignancies worldwide, with around 1.93 million new cases diagnosed in 2020 [1]. The cost of CRC treatment is significant, ranking second in cancer-related healthcare expenses worldwide, totaling $2.8 trillion [2, 3]. Early detection of CRC remains a challenge, with only 37.5% of cases identified at Stage I, where the 5-year survival rate for localized CRC is as high as 90.6% [4]. Screening for CRC is a proven preventive measure that reduces mortality [5]. In developing countries, the risk of colorectal cancer continues to rise, along with the lack of mass screening promoted by health authorities [6]. Colonoscopy is the gold standard for CRC diagnosis, but it has limitations, such as high costs, availability in rural regions, potential complications, and low patient acceptance. In Makassar, Indonesia, not all hospitals are available for colonoscopy, and many patients refuse to have it done because it is considered quite invasive and expensive. Due to the high costs of colonoscopy and the risks involved, non-invasive early screening is needed to reduce unnecessary colonoscopy.

Non-invasive screening methods have been proposed, such as serum, blood, and faecal tests [7]. Non-invasive screening with faeces is more reliable than others due to its high acceptance, non-invasiveness, and accessible collection. Guaiac faecal occult blood test (gFOBT) and Immunochemical FOBT (iFOBT), which is also referred to as immunochemical test (FIT), are famous for colorectal cancer screening. gFOBT is based on the pseudoperoxidase activity of free haemoglobin. gFOBT

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is not completely specific to human blood as it can be interfered with by animal blood in the diet and peroxidases derived from raw vegetables [8]. In addition, blood from upper gastrointestinal haemorrhages was still stable and can be detected [9]. Sensitivity values of gFOBT vary widely, 13-100%, specificity of 90-98%, with a summary sensitivity of 39% and specificity of 94%) [10]. FIT, a more specific type of FOBT, uses monoclonal or polyclonal antibodies specific for the globin part of human haemoglobin [11]. FIT is more sensitive and specific and also primarily replaced gFOBT in many guidelines; FIT is used as a reference in CRC screening by the European Guidelines for Quality Assurance (8). However, the FIT test has a low accuracy value for detecting advanced adenoma, which can be an early stage of CRC [12, 13]. FIT and gFOBT also showed low accuracy in detecting advanced neoplasia in the proximal colon [14, 15].

Efforts to find non-invasive, low-cost, and high-accuracy screening methods are still ongoing. One such method is assessing the role of Matrix Metalloproteinase-9 (MMP-9) protein in faeces. The Extracellular Matrix (ECM) plays a vital role in cancer development, including CRC, influencing processes like angiogenesis and metastasis. MMP-9 crucial in ECM remodeling, has been linked to poor prognosis in CRC, with higher levels of MMP-9 correlating with tumor progression and severity [16, 17]. Theoretically, MMP-9 secreted from tumour tissue may show up in stool examination. Currently, there are not many studies that focus on the evaluation of faecal MMP-9 as a non-invasive examination, to our knowledge, there are only 4 studies that specifically assessed the accuracy of faecal MMP-9. There is a pilot study specifically evaluating the role of faecal MMP-9 as a non-invasive diagnostic test, which showed the result faecal MMP-9 was suitable [18]. Those studies show that faecal MMP-9 is more accurate and reliable as a potential non-invasive diagnostic marker; nevertheless, more studies need to assess its clinical relevance. In light of this context, we conducted this research to evaluate the faecal MMP-9's accuracy for detecting colorectal cancer at our centre. This study examines data obtained from colonoscopic and histopathological findings, and we hope our findings can be an additional reference for further research.

Materials and Methods

Patient selection and sample collection

Based on the current validated formula for diagnostic studies we calculated by using G Power program 3.1.0 (G Power program version 3.1, Heinrich-heine-University, D'sseldorf, Germany) with power of 90% to detect an effect size of 0.9 assuming a type I error of 0.05 [19]. A total of 90 patients who fulfill inclusion and exclusion criteria and had symptoms of bloody, mucous stools, chronic constipation, and diarrhea (male/female: 46/44, age range: 18–84 years, mean age: 50.88 years) enrolled in this study after being referred to our digestive specialist unit at Wahidin Sudirohusodo General Hospital and Hasanuddin University Hospital, all individuals had a colonoscopy in April until May 2024. The inclusion criteria in this study were patients aged at least 18 years and complete patient medical record data, while the exclusion criteria were pregnant patients, having a history of malignancy and previously known infectious or inflammatory diseases of the gastrointestinal tract. The enrollment of subjects is shown in Figure 1.

We collected faecal samples from all patients one day to several hours before the colonoscopy and were frozen at -20 °C within one hour after collection. All Patients were on a lower fiber diet and received sodium phosphate for bowel clearance. Colonoscopies were conducted until the terminal ileum was reached. Biopsies were obtained from all suspicious lesions during colonoscopy, followed by histological analysis. Patients were categorized into four groups based on colonoscopy and histological findings: control, hyperplastic and adenoma polyp, colitis and proctitis, and colorectal carcinoma. The Ethical Committee of Hasanuddin University / Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia approved the study protocol (No. 89/UN4.6.4.5.31,1 PP36/2AZ4). All participants provided written and informed consent for participation.

Validation assay

This research used the Human Quantitative Enzyme-Linked Immunosorbent Assay Kit from Invitrogen (Vienna, Austria, Cat. No. BMS2016-2) to measure MMP-9 levels in faecal samples. For the purpose of determining the extent to which the feces matrix affects the measurement of MMP-9 and determining whether or not the R&D Systems kit is capable of measuring MMP-9 in human faecal extracts, we carried out a validation assay procedure.

Overall mean recovery rates of spike were 103.1 % and determined in 2 independent experiments with 4 replicates each. The calculated overall intra-assay coefficient of variation was 7.3% and overall inter-assay coefficient of variation was 10.2%. Aliquots of samples (spiked or un-spiked) were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human MMP9 level determined after 24 hours. There was no significant loss of human MMP-9 immunoreactivity detected under above conditions.

The full-length active human MMP-9 was incorporated into the extraction medium, maintaining a sample to extraction medium ratio of 1:4. The samples were homogenized for two intervals of 30 seconds and subsequently incubated for 10 minutes at room temperature. After centrifuging all of the homogenates at a speed of 1500 g for ten minutes, the supernatants from the first step were subjected to a second centrifugation at a speed of 10,000 g for ten minutes. The final supernatants underwent analysis for linearity testing. The MMP-9 levels in all of the samples were measured using the ELISA kit that was previously stated for human MMP-9. According to the manufacturer, the kit is capable of detecting both the 82 kDa active form of MMP-9 and the human 92 kDa Pro-MMP-9. The 92 kDa Pro-MMP-9 was the MMP-9 protein used in this analysis.

Samples were stored at -80 °C for a maximum duration of three months and subsequently thawed at 4 °C for one

day. A total of 1.0 g of each faecal sample was diluted, mixed, and homogenized in 4 ml of ice-cold Tris-buffer (0.15 M NaCl + 20 mM Tris-HCl, pH 8.3). Following centrifugation (10 min, 4500 RPM, 4 °C), the pellets were removed, and the supernatants underwent a second centrifugation (10 min, 10,000 g; 4 °C). After filtering the final supernatants via syringe filters with particle sizes of 0.8 mm, the aliquots were kept at -20 °C until analysis. The enzyme-linked immunosorbent assay, which has been previously reported, was used to quantify MMP-9.

Statistical Analysis

The results were presented in accordance with the STARD criteria, which stand for Standards for the Reporting of Diagnostic Accuracy Studies. We expressed continuous data as mean ± standard deviation (SD). To compare the two groups' faecal MMP-9 levels, the Mann-Whitney test was used. P<0.05 was used to define statistical significance. To evaluate the prediction potential of faecal MMP-9, the receiver operating characteristic (ROC) curve was used. In addition to determining the sensitivity and specificity, we computed the area under the ROC curve (AUC) and its confidence interval. Applying Youden's index (sensitivity + specifity -1) allowed us to determine the ideal cutoff values. The data was used to illustrate the 95% confidence intervals. Version 21.0 of SPSS was used for the statistical analysis.

Results

A total of 90 patients who underwent consecutive colonoscopy examinations were used as research subjects. The mean age of the subjects was 50.8 years (18-84 years). The gender distribution was 46 males (51.1%) and 44 females (48.9%). Neither age nor gender has no any significant association with faecal MMP-9. According to colonoscopy findings, the level of faecal MMP-9 distribution in CRC averaged 0.789 ng/ml with a range of 0.125-7.556. A mean of 0.201 ng/ml was found for inflammatory bowel disease (IBD) colitis and proctitis, with a range of 0.105-2.119. Hyperplastic polyp and adenoma findings averaged 0.209 ng/ml and a range of 0.106-0.831. MMP-9 distribution in control subjects (normal and diverticle without inflammation) with a mean of 0.112 ng/ml and a range of 0.101-0.146 ng/ml. In CRC, histopathological grading of the subjects of this study found the highest results level of faecal MMP-9 in moderately differentiated grade as much as 52.94%, mean 0.695 ng/ml and range 0.126-2.808 ng/ml. There is no significant relationship between faecal MMP-9 and histopathological grading. The most tumour location was in the rectum, with a total of 38.23%, mean faecal MMP-9 1.735 ng/ml, and range 0.136-7.556. A summary of the characteristics can be seen in Table 1.

There were eight subjects with normal examination results and two subjects with diverticles without any sign of inflammation, and both groups served as controls in this study. Premalignant or polyp adenoma was found in 9 subjects, and there was a significant relationship between faecal MMP-9 polyp adenoma and control with P=0.016. ROC analysis was performed to assess the accuracy of faecal MMP-9 in adenoma, with AUC = 0.83 and 95% CI: 0.633-1 (sensitivity 77.78%, specificity 80%, positive predictive value (PPV) 77.78%, and negative predictive value (NPV) 80%). Thirty-seven subjects were suffering from IBD, which had a significant relationship between faecal MMP-9 of IBD subjects and controls P<0.001. ROC analysis for IBD subjects was AUC = 0.87 and 95% CI: 0.714-1 (sensitivity 83.78%, specificity 80%, PPV 93.94%, and NPV 57.14%). The summary of these results can be seen in Tables 1 and 2.

There were 34 subjects with confirmed histopathological results with CRC, 10 subjects with proximal colon cancer and 24 subjects with distal colon and rectum cancer. In this

Table 1. Characteristics and Bivariate Analysis

Colonoscopy findings	n	(%)	Fecal MMP-9 Level (ng/ml)			
			Mean±SD	Minimum	Maximum	P value
Normal and Diverticulosis (Control)	10	-11.11	0.112±0.016	0.101	0.146	
Hyperplastic and Adenoma Polyp (Pre-cancer Lesion)	9	-10	0.209±0.234	0.106	0.831	0.013[a]*
Colitis and Proctitis (IBD)	37	-41.1	0.201±0.236	0.105	2.119	0.003 [a]*
Colorectal Carcinoma	34	-37.77	0.789±1.529	0.125	7.556	<0.001 [a]*
Histopathological Grading						0.713[b]
Well Differentiated	13	-38.23	1.005±2.220	0.125	7.556	
Moderately Differentiated	18	-52.94	0.695±0.969	0.126	2.808	
Poor Differentiated	3	-8.88	0.207±0.096	0.137	0.317	
Tumor Site						0.290[b]
Rectum	14	-41.17	1.625±2.158	0.136	7.556	
Sigmoid Colon	7	-20.58	0.233±0.063	0.126	0.285	
Decendens Colon	3	-8.82	0.235±0.088	0.141	0.317	
Transversum Colon	5	-14.7	0.162±0.041	0.125	0.23	
Ascendens Colon	4	-11.76	0.168±0.013	0.153	0.184	
Caecum	1	-2.94	-	0.279	0.279	

* P value <0,05 (Compared with control subject); [a], Mann-whitney U test; [b], Kruskal-wallis test

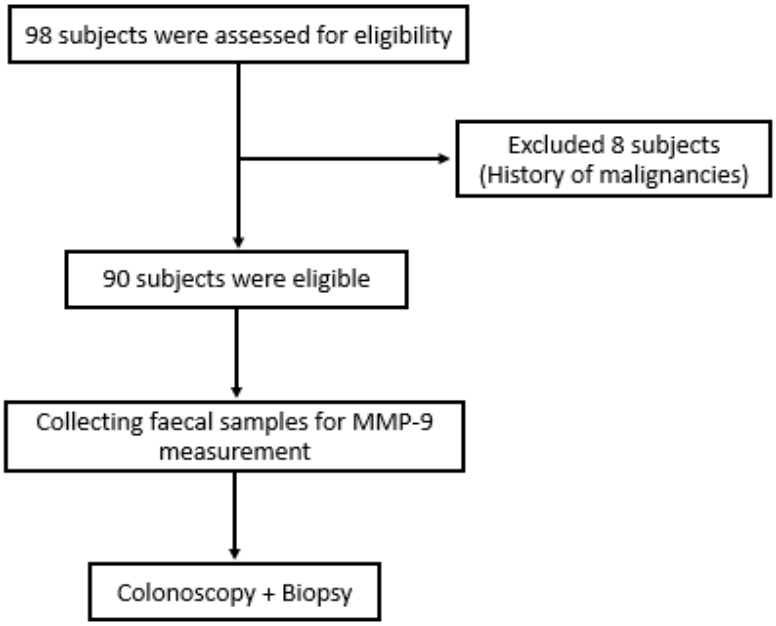

Figure 1. The Study Diagram

study, the proximal colon defined by caecum to the splenic flexure of colon transversum. There was a significant relationship between faecal MMP-9 in CRC patients and controls with P<0.001. ROC analysis showed the accuracy of faecal MMP-9 discrimination of CRC patients against controls was good, with AUC = 0.85 and 95% CI: 0.902-1 (sensitivity 82.35%, specificity 80%, PPV 93.33% and NPV 57.14%) with a cut off point of faecal MMP-9 was 0.1415 ng/ml. There were statistically significant results for the association between faecal MMP-9 in proximal

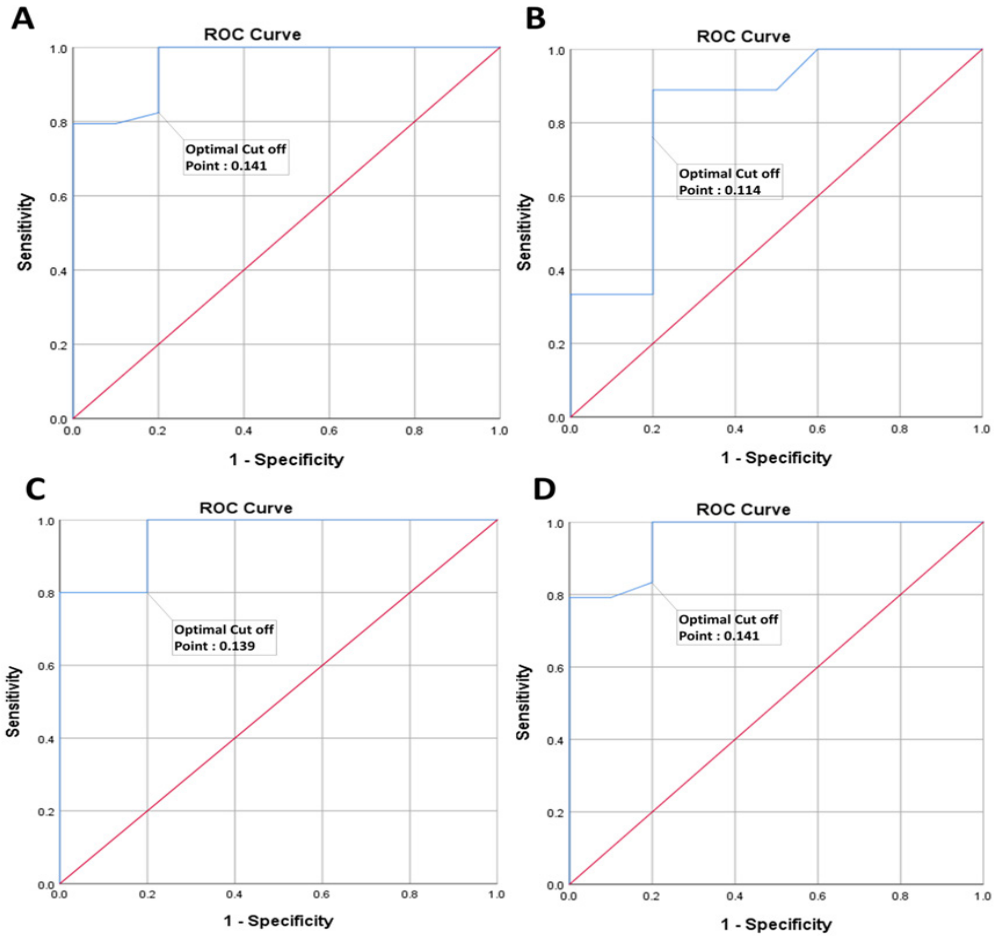

Figure 2. ROC Curve Fecal MMP-9 Level (A) CRC versus Control, (B) Adenoma versus Control. (C) Proximal Colon Cancer versus Control (D) Distal Colon and Rectal Cancer versus Control

Table 2. Diagnostic Test and Fecal MMP-9 Optimal Cut off

Variable	Cut off MMP-9	AUC (95% CI)	FP	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Colorectal Cancer	> 0.141	0.85 (0.902-1)	2	6	82.35	80	93.33	57.14
Proximal Colon Cancer	> 0.139	0.96 (0.883-1)	2	2	80.00	80	80.00	80.00
Distal Colon & Rectum cancer	> 0.141	0.96 (0.899-1)	2	4	83.33	80	90.91	66.66
Adenoma	> 0.114	0.83 (0.633-1)	2	2	77.78	80	88.78	80.00
Colitis & Proctitis	> 0.125	0.87 (0.714-1)	2	6	83.78	80	93.94	57.14

AUC, Area Under Curve; CI, Confidence Interval; PPV, Positive Predictive Value; NPV, Negative Predictive Value; FP, False Positive; FN, False Negative

and distal colon cancer including rectum against control, P = 0.001 and P = <0.001, respectively. ROC analysis to assess the accuracy of faecal MMP-9 in proximal colon cancer against controls resulted in AUC = 0.96 and 95% CI: 0.883-1 (sensitivity 80%, specificity 80%, PPV 80% and NPV 80%) cut-off faecal MMP-9 level 0.1395 ng/ml. ROC analysis to assess the accuracy of faecal MMP-9 in the distal colon and rectal cancer against controls obtained AUC = 0.96 and 95% CI: 0.899-1 (sensitivity 83.33%, specificity 80%, PPV 90.90% and NPV 66.67%) with a cut-off point 0.1415 ng/ml. Table 2 and Figure 2 show a summary of the ROC analysis or diagnostic test.

Discussion

In this study, we consecutively examined faecal MMP-9 from the study population and confirmed with gold standard colonoscopy and histopathology results. We found several diagnoses other than CRC and evaluated the discriminatory ability or accuracy of faecal MMP-9 for each. Regarding subject characteristics, gender and age were not significantly associated with faecal MMP-9. Histopathological results confirmed colonoscopy findings. It can be seen that the mean level of faecal MMP-9 in controls (normal and diverticulosis) was low compared to the others, and the highest level was in CRC. This finding suggests that faecal MMP-9 levels are strongly influenced by inflammation conditions caused by various abnormalities in the colon and rectum mucosa. This study found hyperplastic and adenoma polyps affect the faecal MMP-9 level. The study by Annahazi et al. also found low faecal MMP-9 levels in normal and diverticulosis patients and elevated levels in adenomas and CRC [18].

MMPs contribute to all stages of tumour progression. MMPs are not traditional oncogenes in the sense that gene mutations do not activate them, but rather, their expression is increased either as a direct effect of the activation of an oncogenic pathway or an indirect response to the tumour cells [20]. MMP-9 is integral to the carcinogenesis of colorectal cancer, contributing to extracellular matrix degradation, promoting angiogenesis, and enabling invasion and metastasis. MMP-9 facilitates the release of VEGF from ECM, thereby promoting the angiogenesis necessary for supplying blood to proliferating tumour cells or tissues [21]. ECM degradation, particularly type IV collagen, known to be affected by MMP-9. The ECM degradation represents a critical initial phase in tumour invasion and metastasis. The destruction of the physical barrier offered by the ECM promotes the spread of cancer cells0020to adjacent tissues and, ultimately, to remote sites.

MMP-9 was generated by cancer cells as well as stromal and inflammatory cells within the tumor microenvironment. These cells' interaction with the tumour microenvironment promotes cancer progression by remodelling the ECM and facilitating invasion and metastasis [22]. Cancer cells necessitate integrins for adhesion and MMPs for proteolysis. Protein levels of MMP-2 and MMP-9 are much higher in colorectal cancer patients as compared to healthy controls. This finding demonstrates superior diagnostic sensitivity compared to two other biomarkers currently utilized in clinical practice, CEA and CA19-9 [23]. The degradation of ECM in colonic and rectal mucosal tissues is influenced by MMP-9 activity, so in theory, MMP-9 should be detectable in faeces and assessable.

This study did not find a significant relationship between histopathological grading, tumour location and faecal MMP-9 levels. Although well differentiated, the faecal MMP-9 level was very high in one of our subjects, at 7.556 ng/ml, so it does not guarantee that a good grade will align with the faecal MMP-9 level. The rectum exhibited the highest level of faecal MMP-9 in relation to the location of the tumor. However, the proximal colon did not exhibit a significant difference in faecal MMP-9 levels from the distal colon. We found a significant association between the hyperplastic and adenoma polyp groups with faecal MMP-9, P = 0.013. The accuracy of Faecal MMP-9 in discriminating hyperplastic and adenoma polyps against control showed good results AUC = 0.83, sensitivity 77.78%, and specificity 80%. Annahazi et al. found favourable results for high-risk adenoma, AUC = 0.806, sensitivity 76% and specificity 85.3% [18]. In contrast to FIT, FIT showed poor accuracy in the discrimination of adenoma, with a sensitivity of 17-33.9% and specificity of 20.5-27.9% [12]. Another study showed weak strength of FIT in discriminating against adenoma precancerous lesions with an AUC of 0.58, sensitivity of 37.50 and specificity of 79.84 [24]. Early CRC and adenoma usually do not have much bleeding from the tumour, and haemoglobin is quickly degraded in the colon so that the amount of Hb detected in stool examination is less or even absent for FIT examination [24].

The results of our study showed that faecal MMP-9 has a good discrimination power against controls in the diagnosis of CRC, AUC 0.85 with high sensitivity and

specificity. The results of this study showed a very high PPV of 93.33%, and it can be said that if the faecal MMP-9 level is more than the cutoff >0.141 ng/ml, then the probability of finding CRC in colonoscopy examination is very high. Four studies worldwide specifically analyse the role of faecal MMP-9 in CRC diagnosis. Annahazi et al. reported a exellent AUC of 0.913, with a cutoff of 0.23 ng/ml, a sensitivity of 89.3% and a specificity of 91.2% [18]. Rutka et al. showed AUC = 0.77, sensitivity of 72.2% and specificity of 95% [25]. Beauty et al. also reported on unpublished research that faecal MMP-9 play a role in detecting colorectal carcinoma, achieving an AUC value of 0.855, with a sensitivity of 88.9% and a specificity of 76.7% [26]. However, Cruz et al. reported that faecal MMP-9 has no discrimination role in detecting colorectal carcinoma with an AUC value below 0.55 [27]. Three studies showed that faecal MMP-9 is suitable for distinguishing CRC from healthy subjects.

Compared with FIT according another study results, the accuracy or discrimination power of faecal MMP-9 against CRC is almost the same. In terms of colorectal cancer, FIT had an average sensitivity of 93% (95% CI, 53%-99%) and specificity of 91% (95% CI, 89%-92%) [28]. According to a research conducted by Leonie et al. there were considerable variations in the accuracy of FIT, with sensitivity ranging from 0 to 100% and specificity ranging from 71 to 99%. In overall, sensitivity was 76% and specificity was 94% [10]. However, the ability of FIT is less sensitive in discriminating CRC in the proximal colon against controls. For detecting CRC located in the proximal colon and distal colon/rectum, Ming Lu et al were observed in their review (0.67, 95% CI 0.62 - 0.72 and 0.72, 95% CI 0.68-0.75, respectively) [14]. The systematic review by Hirai et al. showed proximal versus distal colon sensitivity results of 71.1% (60.9-79.6%) and 79.0% (69.2-86.3%) [29]. Our results showed the opposite, faecal MMP-9 has a better discrimination ability against proximal colon cancer AUC 0.96, sensitivity and specificity 80%, respectively. These results are consistent with the characteristics of the proximal colon; lesions in the right-sided colon are typically non-polypoid or flat, which may be linked to less heemorrhage [29]. In addition, faeces' haemoglobin is quickly degraded, making its level low in FIT examination [24]. MMP-9 activity influences the destruction of ECM in the mucosal tissue of the colon and rectum during inflammation, regardless of blood present, and MMP-9 may be identified in feces.

Our study showed that faecal MMP-9 accurately discriminates against CRC and adenoma polyps. Faecal MMP-9 also has superior sensitivity and specificity and also has good accuracy in detecting proximal colon cancer. We are aware of the limitation of this study, and we assume that a study with a more significant number of samples can provide more convincing results to see the strength and limitation of faecal MMP-9 as a new potential non-invasive examination as early detection before colonoscopy. Care should be taken when collecting and storing faecal samples for better results. In terms of financing, in this study, the cost of examining one faecal MMP-9 sample was relatively affordable at around 8 USD per each, in line with the examination cost required in the study of Annahazi et al. < 10 USD [18]. The cost of FIT examination worldwide currently ranges from 8 - 30 USD per sample, and in Indonesia it is around 12 USD each. With its superiority to the widely used of FIT, faecal MMP-9 could be a new hope for future CRC screening methods. In developing countries, especially in Indonesia, low-cost and effective screening methods will benefit the early diagnosis of colorectal cancer.

In conclusion, a significant association existed between faecal MMP-9 and colorectal cancer as well as adenomas. This research demonstrated that faecal MMP-9 exhibited great accuracy in differentiating colorectal cancer from normal tissue and adenomas from normal tissue, along with strong sensitivity and specificity for each comparison. Faecal MMP-9 is more effective than FIT in detecting adenoma and proximal colon cancer.

Author Contribution Statement

AJ, WS, JAU, RBL, SS, ES were involved in concepting and planning the research. AJ and RBL performed the data acquisition/collection AJ and RBL calculated the experimental data and performed the analysis, AJ drafted the manuscript and designed the figures and AJ also aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

Acknowledgements

General

Thank you to Indah Khoirunn Nisa MD, all study subjects, the health service and health centers at the study location, the volunteers, and all colleagues who participated in the implementation of this study.

Funding Statement

This study was funded by Eka Tjipta Foundation.

Approval

Conflict of Interest: The Authors declare that there is no conflict of interest.

Ethical Declaration

The ethics approval was granted by the The Ethical Committee of Hasanuddin University / Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia (No. 89/UN4.6.4.5.31,1 PP36/ 2AZ4).

Data Availability

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

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