The Role of M2 Pyruvate Kinase in the Screening of Bowel Inflammation

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ABSTRACT

Background: This is a study to determine if M2 Pyruvate Kinase (M2-PK) can be used to screen the presence of bowel inflammation.

Method: The study design being used was diagnostic test. In this study, we recruited 76 participants and performed colonoscopy examination as a gold standard and faecal M2-PK as the examined procedure.

Results: From the result of this study, we identified the important role of M2-PK to screen the presence of bowel inflammation with the cut-off point of 1.05 U/mL compared to colonoscopy as gold standard with the sensitivity of 86.2%, specificity 81.8%, positive predictive value 96.6%, and negative predictive value 50%.

Conclusion: From the result of this study, we suggested M2-PK examination to screen bowel inflammation in patients with lower gastrointestinal tract problems.

Keywords: M2 Pyruvate kinase, colonoscopy, screening, intestinal inflammation.

ABSTRAK

Latar belakang: Penelitian ini adalah suatu studi untuk menentukan apakah M2 Piruvat kinase (M2-PK) dapat dipakai dalam menapis radang usus.

Metode: Penelitian yang digunakan adalah uji diagnostik. Pada penelitian ini direkrut sampel sebanyak 76 orang dan dilakukan pemeriksaan kolonoskopi sebagai baku emas dan pemeriksan M2-PK feses sebagai pemeriksaan yang diuji.

Hasil: Dari hasil penelitian ini didapatkan peran penting M2-PK untuk menapis radang usus dengan nilai titik potong 1,05 U/mL dibandingkan dengan kolonoskopi sebagai baku emas dengan sensitivitas 86,2 %, spesifisitas 81,8%, nilai duga positif 96,6% dan nilai duga negatif 50%.

Simpulan: Pemeriksaan M2 Piruvat Kinase disarankan dari hasil studi ini untuk menapis radang usus pada pasien dengan masalah saluran cerna bagian bawah.

Kata kunci: M2 Piruvat kinase, kolonoskopi, penapisan, radang usus.

INTRODUCTION

Clinical difference between inflammatory bowel diseases with non-inflammatory bowel diseases, such as irritable bowel syndrome (IBS) is a common problem found in daily practice. This problem becomes difficult because both frequently appear with similar clinical manifestations, including bloating, stomachache, and diarrhea. These conditions sometimes need clinical evaluation, laboratory examination, and invasive examinations that are exhausting. The prototypes of inflammatory bowel disease (IBD) consist of Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis (IC). Diagnosis of IBD is highly dependent on the clinical findings that are found after performing radiological, endoscopy, and anatomy pathological examinations. Although the difference between UC and CD is quite clear clinically, 10-20% of cases cannot be classified into either of them, and is classified as Indeterminate Colitis (IC).[1,2,3]

In Indonesia, some other inflammatory diseases, such as: colitis, ileitis, proctitis, and intestinal tuberculosis frequently happen. These conditions also need thorough examinations to confirm the diagnosis and administer prompt treatment. Therefore, there is a necessity to have an examination which is easy to perform and able to detect the presence of abnormalities in the form of inflammation in the bowel. This examination is also expected to have good sensitivity and specificity to the suspected disease. One of the examinations, which is currently studied widely, is faecal examination to identify marker of bowel inflammation. This marker is not invasive and relatively easy to be performed. The inflammatory marker is M2-PK. This marker is one of the isoforms of a pyruvate kinase enzyme that catalyse aerobic (glycolysis) examination in rapidly dividing cells. The dimeric form of M2-PK can express cancer cells, which can be detected in the plasma. Currently, this marker is used as a tool to detect the presence of colon cancer.[1,2,3,4]

However, in several other studies, M2-PK is proved to be able to reveal the presence of inflammation in the bowel, such as ileal pouch inflammation.[5,6,7] Other studies comparing the level of M2-PK in patients with inflammatory bowel disease, such as IBD to patients with non-inflammatory bowel disease showed that there was significant difference in the results of M2-PK.[8] If examination using protein marker from the faeces can be used to present inflammation in the bowel, then invasive examinations, such as endoscopy, which require high cost and cause inconvenience can be conducted in more selective manner. This is beneficial for both doctors and patients. Doctor can diagnose disease properly and patient becomes more comfortable and does not need to spend high cost for examinations. Besides, this study can also be a reference to studies on determinants to differentiate inflammatory bowel diseases and non-inflammatory bowel diseases.[9]

METHOD

The design of this study was a diagnostic test study with numerical data on the level of M2-PK in inflammatory bowel disease and non-inflammatory bowel disease patients' faecal specimen. This study was conducted to outpatient and hospitalized patients in the Gastroenterology Division, Internal Medicine Department, Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital and several other hospitals in 2012. The population of the study were patients with lower gastrointestinal tract symptoms, including inflammatory bowel disease or non-inflammatory bowel disease, such as functional bowel disorder and functional abdominal pain syndrome according to Rome III criteria. Accessible population was all patients with lower gastrointestinal tract complaints in Cipto Mangunkusumo Hospital and several other hospitals nearby. Study samples were patients who came to the hospital after undergoing colonoscopy procedure. Samples were recruited according to the inclusion and exclusion criteria. Study samples were included consecutively.

Inclusion criteria were patients with lower gastrointestinal tract problem aged > 18 years old; patients who came in the January-June 2012 period; patients who agreed to participate in the study and had signed the informed consent. Exclusion criteria were colon cancer, polyp adenoma, and hyperplastic. Sample size in this study was 76. Data was evaluated to obtain the sensitivity, specificity, positive predictive value, and negative predictive value. Appraisal of association and correlation between variables were evaluated by Chi-square test, using statistical program for social sciences (SPSS) for Windows version 17.

To all potential candidates who fulfil inclusion criteria, explanation on the procedure, study objectives, and purpose of examination were given. Later they were asked for consent to participate in the study in written from patient or patient's family who were responsible for them. They were then submitted to the Ethical Committee Faculty of Medicine Universitas Indonesia for ethical clearance.

RESULTS

In this study, there were 76 patients with varied background. Study subjects were recruited from six hospitals in Jakarta. There were more male patients compared to female patients, with the ratio of 57.9%: 42.9%. In regard to race, it was seen that most of them were Chinese and Javanese. Possible reasons of most patients came from these two races was the location of sample collection in several private hospitals. In this study, it was unintentional that there were no recruited patients were in the age group of 18-24 years old. The productive age group was the most recruited in this study.

Table 1. Patients' Characteristics

Variables	n (%)
Gender	
Male	44 (57.9)
Female	32 (42.1)
Race	
Javanese	16 (21.2)
Sundanese	7 (9.2)
Padang	6 (7.9)
Batak	7 (9.2)
Chinese	33 (43.4)
Others	7 (9.2)
Age groups (years old)	
18-24	0 (0)
25-31	6 (7.9)
32-38	9 (11.8)
39-45	10 (13.2)
46-52	19 (25.0)
53-59	15 (19.7)
60-66	10 (13.2)
> 66	7 (9.2)

Table 2. Indication of Colonoscopy Examination

Indication	n (%)
Haematochezia	7 (9.2)
Stomachache	47 (61.7)
Constipation	2 (2.6)
Chronic diarrhea	34 (44.7)
IBS	8 (10.5)
Screening	3 (3.9)

The most common indications for colonoscopy were stomachache 61.7 %, followed by chronic diarrhea in 34% subjects and IBS in 10.5%. Other noted indications of examination include haematochezia, screening, and constipation.

Table 3. Results of Colonoscopy Findings

Colonoscopy findings	n (%)
Normal	11 (14.5)
Infective Colitis	21 (27.6)
Tumour	2 (2.6)
Inflammatory polyp	24 (31.6)
Ulcerative colitis	3 (3.9)
Crohn's Disease	2 (2.6)
Ileitis	7 (9.2)
Diverticulosis	7 (9.2)
IBD	1 (1.3)
Haemorrhoid	55 (72.4)

Table 4. Cross tabulations between results of colonoscopy findings with M2-PK level

	Colonoscopy findings		Total
	Inflammatory	Non- inflammatory	
Inflammatory (M2PK ≥ 1,05)	56	2	58
Non-inflammatory (M2PK < 1,05)	9	9	18
Total	65	11	76

From table 5.4, the obtained sensitivity value was: 56/ (56+9) x 100% = 86.2%; specificity value: 9/ (2+9) x 100% = 81.8%; positive predictive value= 56/ (56+2) x 100% = 96.6%; negative predictive value: 9/ (9+9) x 100 %= 50%.

From ROC curve, it is seen that this M2-PK examination revealed good sensitivity and specificity value.

Table 5. Area Under the Curve (AUC)

Area	Std. Error^a	P	Asymptomatic 95% CI	
			Lower level	Upper level
.834	.079	.000	.679	.990

From the calculation results of SPSS 17, it was obtained that the area under the curve (AUC) value was 83.4 % in 95% CI (67.9%-99%). This AUC value showed that this examination had good benefit to confirm diagnosis.

DISCUSSION

According to the study performed by Joshi S, the value of M2-PK in healthy individuals were < 1 U/mL regardless of their age.[10] From this study, we obtained the value of M2-PK 1.05 U/mL, as a cut-off value to determine if someone suffered from bowel inflammation or not. The results of this study supported the study by Joshi et al that M2-PK value could be used to determine whether someone suffered from bowel inflammation or not. From the results of this study, according to the performed analysis with the cut-off of 1.05 U/mL, other values, such as sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Jeffrey J et al in their study obtained the sensitivity value to be 64%, specificity 88%, positive predictive likelihood ratio 5.6, and negative predictive likelihood ratio 0.18.[8] This data was slightly different to the data obtained in this study, which could possibly be caused by the different study design being used. From this data, we could evaluate that the sensitivity value was 86.2% which showed that this examination was quite sensitive to detect the presence of intestinal abnormality. The specificity value of 81.8% revealed

that this examination was quite good to determine if a patient was healthy if the examination result was negative.

With the positive predictive value of 96.6%, it also showed that if the examination result was positive, the possibility the patient was sick was very high; thus, it would be best if this examination was used as a screening tool in cases of lower gastrointestinal tract problems, which would enable general screening to patients. The lower value of negative predictive value, only 50% revealed that if the examination result was negative, the possibility of the patient not suffering from colonoscopy abnormalities was only half, therefore other examination was needed to support this examination as a supporting examination to eliminate differential diagnosis.

At the end of this study, we reviewed on the inference of these study results on the possibility to be applied in larger population. According to the principles of sample representation to population and sample collecting technique, generalization appraisal was performed to the internal and first and second external validities. Internal validity appraisal was performed by examining if subjects who completed the study (actual study subjects) could represent the samples that fulfilled the subject recruitment criteria (intended subjects). In this study, all subjects who fulfilled the subject recruitment criteria could be included as study sample. Therefore, the internal validity of this study was good.

For first external validity, appraisal was performed towards subject representation that was recruited according to the sample recruitment criteria (intended sample) towards to accessible population. Accessible population in this study was patients who visited the gastroentero-hepatology outpatient clinic with the complaint of lower gastrointestinal tract. Sampling technique in patients who fulfilled the inclusion criteria was consecutive sampling. This sampling technique was the best non-random sampling to represent the accessible population. Based on this, the first external validity of this study was good.

For second external validity, appraisal was performed by common sense and based on general knowledge if available. In this matter, it was evaluated if the accessible population of this study was the best representation of the target population. By calculating that the accessible population was population of patients with lower gastrointestinal tract complaints in big hospitals that had tertiary care facility, such as gastrointestinal tract endoscopy, then the patients' characteristics were relatively similar to other patients' characteristics in other hospitals that had endoscopy facility. From this result, we concluded that the second external validity was good.

CONCLUSION

M2-PK can be used as an aid to screen the presence of inflammatory bowel with the cut-off value of $\geq$ 1.05U/mL. This M2-PK examination can be used selectively in patients with lower gastrointestinal tract complaints.

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