The Role of Fecal M2-Pyruvate Kinase (M2-PK) in Colorectal Cancer Screening

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ABSTRACT

Colorectal cancer is a malignancy with high incidence and mortality rate. The long carsinogenesis sequence from adenoma unto carsinoma enable early detection and screening as part of the management but until recently the commercialy available test has low sensitivity and specificity. M2-pyruvate kinase (M2-PK), an isomer of glycolisis enzyme involved in aerobic metabolism, is found in high amount in cancerous cell and is shed unto luminal mucosa in colorectal cancer. Quantification of this protein in feces is a promising method to detect malignant lesion of colon. Several studies until recently demonstrate moderate sensitivity and specificity but clearly with better performance than commonly-used guaiac based faecal occult blood test (gFOBT).

Keywords: *M2-pyruvate kinase (M2-PK), fecal, colorectal cancer, screening*

ABSTRAK

Kanker kolorektal merupakan keganasan dengan insiden dan tingkat mortalitas tinggi. Panjangnya perjalanan karsinogenesis adenoma menjadi karsinoma memungkinkan peran deteksi dini dan skrining dalam manajemennya namun hingga saat ini pemeriksaan yang tersedia masih memiliki sensitivitas dan spesifisitas yang rendah. M2-Pyruvate Kinase (M2-PK), suatu isomer enzim glikolisis metabolisme aerob, dijumpai dalam kadar yang tinggi pada sel kanker dan pada kanker kolorektal dilepaskan melalui mukosa lumen. Kuantifikasi protein ini pada feses merupakan metode yang menjanjikan untuk mendeteksi lesi maligna kolon. Beberapa studi hingga saat ini mendapatkan sensitivitas dan spesifisitas moderate namun jauh lebih baik dibandingkan dengan guaiac based faecal occult blood test gFOBT yang selama ini digunakan

Kata kunci: *M2-piruvat kinase, M2-PK, feses, kanker kolorektal, skrining*

INTRODUCTION

Colorectal cancer is still contributing for its high incidence and cancer-related mortality worldwide. This malignancy ranked the second most after breast cancer in woman and the third most after lung cancer and prostate cancer in man.[1] Globoscan data revealed 1.3 million new case with 700,000 mortality in 2012.[2] Majority of cases are diagnosed at late stage albeit the long process of adenoma-carsinoma transformation. Finding this disease in early stage could lead to a better prognosis. Hence, screening in the population is highly encouraged.[3,4] Some hindrance are found in

the community. Colonoscopy as a gold standard is invasive, high cost, and has low acceptability.[5,6] The most frequent used in vitro diagnostic test, fecal occult blood test, is limited by its low sensitivity, specificity, and several number of confounding factor.[2,3]

In the past decade, several fecal biomarker with more sensitive and specific profile has been introduced. One of which is M2-pyruvate kinase (M2-PK). Pyruvate kinase is an enzyme found in cancer cell used mainly in glycolisis process. This enzyme catalyze the conversion of phosphoenolpyruvate (PEP) unto pyruvate and lactate which is latter used for cellular metabolism. During tumorigenesis, exposure with proto-oncogen will replace the tissue specific isoenzyme with non-specific M2-PK. This enzyme can be found in other malignancy such as kidney, oesophagus, pancreas, gaster, colorectal, lung, ovarium, and breast.[7-9] Shedding of this protein in fecal material make it a promising alternative as a screening tool in diagnosing colorectal cancer.[7,10]

COLORECTAL CANCER AND SCREENING

Colorectal cancer is still a commonly encountered neoplasm. In Europe, this disease ranked number one in incidence. In 2008, there were 436,000 patient diagnosed with colorectal cancer followed by breast, lung, and prostate cancer. The number of mortality at that year made colorectal cancer number two in rank after lung cancer.[3] In UK, this malignancy was the second most commonly found cancer after breast cancer for women and third most commonly cancer after lung and prostate cancer for men.[1]

Colorectal cancer posses good prognosis if it was found at early stage. Given the long carsinogenesis process from adenoma unto carsinoma (adenoma-carsinoma sequence), the earlier found means there is more place for curative option.[4] Figure 1 showed the adenoma-carsinoma sequence. However, in reality, most patient come at late stage (either from patient's side or from late diagnosis). To combat this issue, several countries has tried to encourage colorectal cancer screening and early detection program.[2,4]

Until recently, colonoscopy is still considered the gold standard for early detection. The main advantage of colonoscopy is its diagnostic and therapeutic ability especially for adenoma as precursor lesion. Some limitation mentioned are the invasiveness of the procedure and the high cost for mass screening purpose. Adding to that is the reception among community that is still low. For example, in Germany only 2.7% insured patients used their colonoscopy screening right eventhough it was covered by the company.[3,11]

The most commonly used in vitro method for colorectal cancer screening is the fecal occult blood test/FOBT (formerly guaiac-based and recently immunochemical-based). The main principle for guaiac-FOBT based on haemoglobin peroxidase activity which will induce oxidation and change in the blue coloration of guaiac in the feces. However, some limitation encountered regarding g-FOBT such as the false positive result from some red meat and vegetable; positive result for any kind of gastrointestinal bleeding including the non-neoplastic origin (hemorrhoid, collitis, erosive gastritis, or diverticulosis); and false negative result from patient taking vitamin C. Hence, one should put dietary restriction unto consideration three days prior until three days after the examination.[2,3] The latest immunochemical-based FOBT (i-FOBT) specifically quantify hemoglobin with antibodies and can differentiate heme peroxidase activity from those from diet.[5,6]

The main critic for this test is its low and varied sensitivity for adenoma and carsinoma (13-50%) and even lower for single adenoma (10-20%). This is probably because small tumour doesn't cause significant bleeding due to the absence of tumour necrosis or angiogenesis. Nevertheless, g-FOBT has been shown to reduce colorectal cancer related

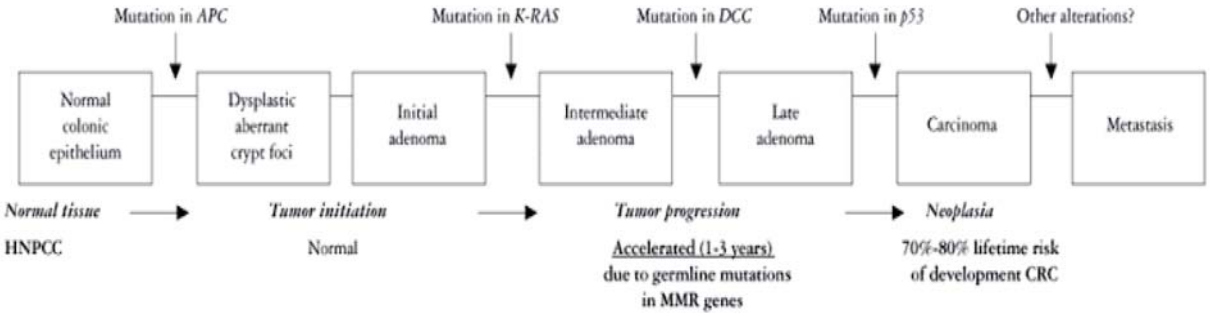

Figure 1. Adenoma-carsinoma sequence[4]

mortality. Meta analysis from 4 big RCT from USA and Europe showed 16% mortality reduction in the grup which early detection with g-FOBT was done.[2,3] The i-FOBT also has high variability in sensitivity. A meta-analysis regarding its sensitivity revealed 67% for colorectal cancer detection.[12] The low sensitivity of g-FOBT and i-FOBT plus the increasing incidence of de novo carsinoma (colorectal cancer which occured without going through the adenoma sequence) make the more sensitive early detection and screening method prudent.[13]

M2-PYRUVATE KINASE (M2-PK)

M2-pyruvate kinase (M2-PK), an iso-enzyme of pyruvate kinase, is a key enzyme in aerobic glycolisis which catalyze the conversion of phosphoenolpyruvate (PEP). This unique feature of aerobic glycolisis in cancer cell is known as Warburg effect because pyruvate is converted to lactate instead of acetyl co-A. Pyruvate-kinase can be found in several isoform (L-Liver, R-Red blood cell, M1-Adult, and M2-Embryonic/Tumour) with organ spesific characteristic. In normal proliferating cell, this M2 iso-enzyme is usually found in active tetrameric form with high affinity to PEP. In tumour tissue, after exposure with proto-oncogen, M2-PK will be changed unto inactive dimeric form with low affinity toward PEP. This will lead to the accumulation of building block substances needed for cancerous cell metabolism (nucleic acids, phospholipids, and amino acids). M2-PK is not specific and can be found in several cancers like kidney, oesophagus, stomach, pancreas, colorectal, lung, ovarium, and breast cancer. Given its low affinity toward PEP, M2-PK is easily released from tumorous cell and can be quantified from the body fluid. In colorectal cancer, M2-PK is shed unto colonic lumen thus can be detected from the stool.[10,13,14]

The use of fecal M2-PK test was firstly introduced by Hardt et al in 2003 when they reported the quantification of fecal M2-PK and recommended the use for colorectal cancer early detection. Until now, several validation studies has been done with fair sensitivity and specificity. There are two type of fecal M2-PK test widely known: the quantitative test (ELISA-based) and the qualitative test (immunochromatographic-based/point of care/POC). The quantitative test used sandwich ELISA and need a certified laboratory. The POC M2-PK test is an immunochromatographic test. This test detect M2-PK in feces with a monoclonal antibody. Bounding of monoclonal antibody with the enzyme will form gold-labelled complex (antibody-M2PK complex) which will migrates along membrane and reach the Testline (T). This T line has second monoclonal antibody which will develop pink colour line after mix with the antibody-M2PK complex. This test is faster and easier than ELISA but fecal point of care test is not as practical as the blood point of care test. To note, the latest M2-PK point of care test also incorporate fecal hemoglobin test in its device.[10]

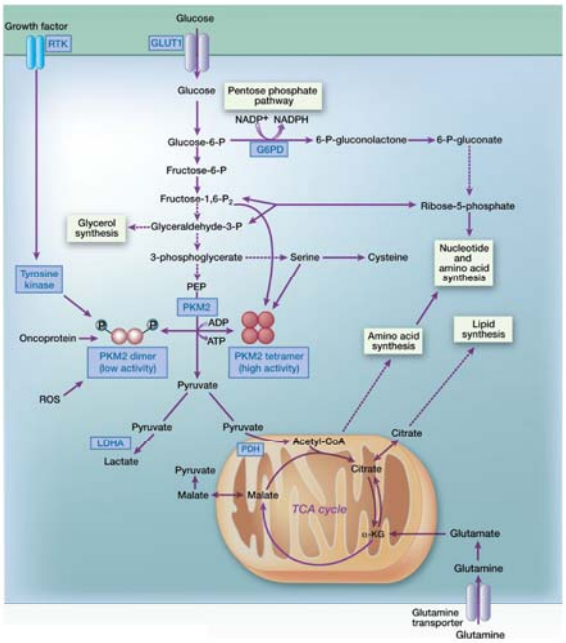

Figure 2. Metabolic pathway regulated by M2-PK in cancerous cell[14]

FECAL M2-PK IN COLORECTAL CANCER SCREENING: CURRENT EVIDENCE

Several studies have been published regarding the use of fecal M2-PK as a screening tool in colorectal cancer. Three meta-analysis showed varied result, which are meta-anaylsis from Tonus et al (2012), Li et al (2012), and the latest from Uppara et al (2015).[3,15,16]

Tonus et al performed a meta analysis from seventeen studies from 2004-2010 covering 704 colorectal cancer patients and 11.412 healthy subjects. They found sensitivities for colorectal cancer detection ranging from 68-97% (mean sensitivity 80.3%±7.1%) with specificities ranging from 89.5-100% (mean specificity 95.2% ± 3.9%). The sensitivities are also corresponding with the TNM and dukes staging. In other word, the higher the T staging and dukes staging the higher sensitivities we can get. The sensitivities for detecting adenoma are also counted and they found number around 51% ± 24% (adenoma without

Table 1. Evidence based for fecal M2-pyruvate kinase (M2-PK) in colorectal cancer screening

Study	Sensitivity	Specificity	PPV	NPV	Accuracy
Tonus et al[3]	80.3% ± 7.1%	95.2% ± 3.9%	50.5% (calculated)	98.8% (calculated)	-
Li et al[15]	79% (95% CI = 75-83%)	81% (95% CI = 73-87%)	74% (95% CI = 56-87%)	86% (95%CI = 79-91%	-
Uppara et al[16]	Adjusted: 0.73 (0.66-0.79)	Adjusted: 0.76 (0.72-0.79)	-	-	Adjusted: 0.80 (0.77-0.84)

PPV: positive predictive values; NPV: negative predictive values

diameter), 25% ± 4% (adenoma diameter < 1cm), and 44% ± 21% (adenoma diameter >1cm). The author also compared head to head the sensitivity for fecal M2-PK and gFOBT in detecting carsinoma and adenoma and found mean sensitivity for detecting carsinoma respectively 81.1% ± 3.3% and 36.9% ± 18.5% adenoma < 1cm diameter respectively 25% ± 5% and 9% ± 9%; for adenoma > 1cm diameter respectively 47% ± 24% and 27% ± 6%. From the description we can conclude that fecal M2-PK is more superior than g-FOBT in detecting colorectal carsinoma or adenoma. This is probably due to the advantage in M2-PK as a metabolic biomarker which are not restricted to bleeding tumour and adenoma. The high specificities from healthy subject recruited also showed that fecal M2-PK does not give false positive result from the non cancerous source of bleeding. One of the limitation is not all study has colonoscopy performed as a gold standard. From eight studies counted for spesificities, only half which colonoscopy has been done. The rest of the study calculated the estimated specificity based on the prevalence of CRC.[3]

The second meta analysis by Li et al was done from 10 studies covering 1999 samples (2004-2008). They found the sensitivity and specificity respectively 79% (95% CI = 75-83%) and 81% (95% CI = 73-87%) with positive predictive value 74% (95% CI = 56-87%) and negative predictive value 86% (95% CI = 79-91%). All of the included study used colonoscopy as reference standard but it was unclear whether the interpretator were blinded or not. The heterogeneity for sensitivity result was low (Q test 11.37 [p = 0.25]; I^2 20.82%) but with significant heterogeneity for specificity (Q test 36.1 [p = 0.00]; I^2 75.07%). Head to head comparison of fecal M2-PK and gFOBT in diagnosing colorectal carsinoma found higher sensitivity (77-85.2% vs. 27-62.9%) but lower specificity (65.4-72% vs. 86.7-94%). One consideration should be put in the PPV of 74% which means 26% patient will be false positive (positive with M2-PK test but no cancer detected with colonoscopy). This big false positive rate will lead to unnecessary colonoscopy which will make M2-PK still difficult to be used as a mass screening tool. The author didn't recommend the use of fecal M2-PK as a single screening tool but to combine it with gFOBT or iFOBT. Several limitations attributed to this meta analysis are the quality of the studies (more than half are case-control), the presence of publication bias, and the probability of test review bias.[15]

The lattest meta analysis by Uppara et al was done from eight studies (2008-2012) with total of 2,654 patients. They found sensitivity for fecal M2-PK to be 79% (95% CI: 73-83%) with specificity 80% (95% CI: 73-86%) and accuracy 0.85 (0.82-0.88). There was moderate heterogeneity regarding sensitivity (Q 12.85 [p = 0.08]; I^2 = 45.51) and considerable heterogeneity regarding specificity (Q 48.8 [p < 0.001]; I^2=85.65). Removal of outlier resulted in adjusted accuracy of 0.80 (0.77-0.84) with adjusted sensitivity 0.73 (0.66-0.79) and adjusted specificity 0.76 (0.72-0.79). Heterogeneity regarding sensitivity was nullified after removal of outliers while significant heterogeneity still encountered regarding specificity. Some individual studies reported that fecal M2-PK level also increased in benign condition such as inflammatory bowel disease, infective collitis, and amoebic collitis. Several limitation encountered are lack of randomisation, selection bias, high false positive result, and not standardised cut off values between neoplastic and non-neoplastic condition.[16] The summary of the evidence is presented in Table 1.

CONCLUSION

Fecal M2-PK is a promising diagnostic tools for colorectal cancer diagnosis with moderate sensitivity and specificity. However, the use of fecal M2-PK as a single screening agent is not justified until recently. Combination of fecal M2-PK with g-FOBT/i-FOBT may be beneficial but more rigorous study are needed prior to implement it as a mass screening tool.

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