Result Comparison of Fecal Occult Blood Test between FOBT Hb and FOBT Hb + Transferrin in Detecting Upper Gastrointestinal Tract Bleeding

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ABSTRACT

Background: *Immunological fecal occult blood test (FOBT) using anti human hemoglobin (Hb) has a low sensitivity in detecting upper gastrointestinal (GI) bleeding, due to Hb degradation. Transferrin (Tf) is more stable in stool when compared to Hb, provides an alternatives on diagnosing upper GI bleeding. This study aim to determine the advantage of FOBT Hb + Tf in detecting upper GI bleeding compared with FOBT Hb alone.*

Method: *This study was conducted by comparing the diagnostic value of rapid immunochromatographic FOBT that use anti-human Hb alone, with the one using combination of anti human Hb & Tf simultaneously in detecting upper GI bleeding. Stool sample from 48 patients with upper GI bleeding and 29 controls (without any upper GI bleeding) were collected then tested with both FOBT methods. Endoscopy study was used as gold standard endoscopy to determine test's diagnostic value.*

Results: *In detecting upper GI bleeding, the sensitivity of FOBT Hb + Tf (85.42%) was higher than FOBT Hb (29.17%). The specifity of both methods were accurate at 89.66% and 93.10% respectively. Positive predictive values (PPV) for both methods were also good at 93.18% and 87.50% while negative predictive value (NPV) FOBT Hb + Tf (78.79%) were higher than FOBT Hb (44.26%).*

Conclusion: *Between these two test methods on detecting upper GI bleeding, FOBT Hb + Tf has higher sensitivity, PPV and NPV value compared to FOBT Hb. It is advisable to use FOBT Hb + Tf for upper GI bleeding screening.*

Keywords*: FOBT, immunochromatography, hemoglobin, transferrin, upper GI bleeding*

INTRODUCTION

Upper gastrointestinal (GI) bleeding is blood loss of gastrointestinal tract proximal to Treitz ligament. In the Western country, the cause of upper GI bleeding is peptic ulcer (50%), while in Indonesia it was leakage of esophageal varices (70-75%). Incidence of upper GI bleeding is 4 times higher than lower GI bleeding.[1,2] Clinical manifestation of upper GI bleeding varied from occult bleeding to a life threatening condition. Obvious clinical manifestations are hematemesis and melena. The occult bleeding in gastrointestinal has prevalence of 1 from 20 adults.[1,3]

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Endoscopy was the gold standard for diagnostic test in upper GI bleeding. However, endoscopy is a semi-invasive test and gives uncomfortable feeling for patients. Hence, screening tests that are non-invasive, simple, and easy to applicate is needed to detect upper GI bleeding. One of them is by performing fecal occult blood test (FOBT).[2]

FOBT were used in patients with high suspicion of gastrointestinal bleeding. The FOBT has several variations in methods and diagnostic value.[2,4,5,6] Conventional FOBT uses a chemical methods based on peroxidase enzyme activity. This method has the weakness that is showing a false positive result on a diet of food containing hematin. Several chemicals can also influence its reactions and causing false negative results. In order to overcome those disadvantages, an immunology method was developed by use of hemoglobin antibody (Hb). This method has

no dietary limitation and gives high diagnostic accuracy. But, in spite of it all, the variation of stool hydration, degradation of existing Hb by protease, absorption of mucin, loss of Hb antigenicity due to *Enterobacter* activity, have made no specific and sensitive FOBT methods in screening and detecting upper GI bleeding.[4,6,7]

The development of FOBT examination conducted by using anti-human Hb and anti-transferrin (Tf) to detect fecal Hb and Tf simultaneously.[4,7,8,9,10] Detection of fecal Tf is an alternative method for screening of upper GI bleeding, because Tf is more stable in stool compared with Hb. Recently, FOBT Hb + Tf were commonly performed to screen upper GI bleeding in colon cancer or colon polyps. Miyoshi et al, suggested that FOBT Hb + Tf to high sensitivity in lower GI bleeding (sensitivity 90%).[9,10] Typical studies to detect upper GI bleeding is very limited.[4, 5] Chiang et al, suggested that FOBT Hb + Tf would increase FOBT diagnostic value when compared to conventional methods.[4] The diagnostic value of FOBT Hb + Tf in upper GI bleeding detection were never conducted or reported in Indonesia.

Based on this, the study will be conducted diagnostic value of FOBT Hb + Tf compared with FOBT Hb alone. This is important to the selection of kits to be used in laboratory, because it has wide variation of diagnostic value in each method. We need to understand whether the diagnostic value [sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)] of FOBT Hb + Tf are better than FOBT Hb alone in detecting upper GI bleeding. The objectives of this study is to determine advantages of FOBT Hb + Tf in detecting upper GI bleeding compared with FOBT Hb alone. From this study, we hope to be able to decide a screening test that is non-invasive, simple, easy to do with good diagnostic value, thus increasing the health service in general.

METHOD

The study used analytical observational cross sectional design. The study population was all dyspeptic patients who underwent endoscopy procedure at the Division of Gastroenterology, Department of Internal Medicine, Saiful Anwar hospital.

The minimum sample size were 55 patients. However, to obtain control sample is a lot more difficult than endoscopic cases, therefore we use 2 : 1 ratio between case and control, after the statistical calculation were obtained at least 42 the number of cases and 21 controls.

Case samples inclusion criteria are: (1) patient willing to undertake the study (approved informed consent); (2) the presence of ulcer, erosion, or etc that could lead to upper GI bleeding from endoscopic examination. Inclusion criteria for control samples are: (1) patient willing to undertake the study (approved informed consent); (2) normal result in endoscopic examination or normal upper GI; (3) endoscopic result showed only upper GI edema without erosion, ulcer, polyps, or malignancy. Samples exclusion criteria are: (1) patient are not willing to undertake the study; (2) there was melena, hematochezia, or hemorrhoid bleeding; (3) female patients who were menstrual period

Materials of study were stools taken from patients samples before undergoing endoscopic. Samples then tested for FOBT Hb + Tf (Oncoprobe®), and FOBT Hb alone (delta) in accordance to the kit procedure. Cut off value to detect Hb on FOBT Hb + Tf are 200 ng/mL or 10 mg Hb/g stool, and 40 ng/mL transferrin. Positive results were defined if one or two red stripes are shown on T line or one red strip on the C line. If the strip is present on Hb line, it proved Hb content in the stool.

The same condition were applied to Tf. If there was pink strip on the Tf line, it suggested the presence of Tf in the stool. If there were two pink strips, each on Hb and Tf line, it suggested the presence of Hb and Tf in stool simultaneously. On the contrary, results will be defined as negative if there were only one pink strip appeared in control line. Presence or absence of upper GI bleeding was approved by the Gastroenterologist in Saiful Anwar General Hospital. The sensitivity, specificity, PPV, and NPV were counted using McNemar formula.

RESULTS

There were 77 samples that comprises to 48 cases and 29 control population. Male patients were 36 individuals (46.75%) with age ranges from 28 – 80 years old with 48.06 ± 15.61 (mean ± SD) years old. Female patients were 41 individual (53.25%), with age ranges from 18 – 74 years old with 47.37 ± 13.64 (mean ± SD) years old. From 48 cases population, they were comprised of various clinical condition as seen in table 1 with test result listed in table 2 and compared as negative and positive result in table 3.

From 29 control samples, they were comprised of clinical conditions as seen in table 4, with test result listed in table 5 and compared as negative and positive result in table 6. Diagnostic value of FOBT Hb + Tf and FOBT Hb alone were depicted in table 7.

Table 1. The result of FOBT test from upper GI bleeding cases

Case group	n (%)	FOBT result*			
		Hb + Tf		Hb	
		Positive	Negative	Positive	Negative
Erosive upper GI	17 (35.42)	16	1	-	17
Erosive upper GI + ulcer	10 (20.83)	8	2	8	2
Erosive upper GI + polyp	2 (4.17)	1	1	-	2
Erythematous upper GI + mild erosive	11 (22.92)	10	1	1	10
Erythematous upper GI + polyp	1 (2.08)	-	1	-	1
Upper GI ulcer	7 (14.58)	6	1	5	2
Total	48 (100)	41	7	14	34

* FOBT Hb + Tf defined as positive if Tf positive

Table 2. The FOBT result from the case group

Case group	FOBT Hb + Tf positive*			FOBT
	Tf	Hb	Tf + Hb	Hb positive
Erosive upper GI	16	7	6	-
Erosive upper GI + ulcer	8	10	8	8
Erosive upper GI + polyp	1	2	1	-
Erythematous upper GI + mild erosive	10	10	9	1
Erythematous upper GI + polyp	-	-	-	-
Upper GI ulcer	6	7	6	5
Total	41	36	30	14

* FOBT Hb + Tf defined as positive if Tf must be positive

Table 3. The comparison of FOBT Hb + Tf with FOBT Hb on the case group

Case group	n (%)	FOBT results*			
		Hb + Tf		Hb	
		Positive	Negative	Positive	Negative
Erosive upper GI	17 (35.42)	17	-	-	17
Erosive upper GI + ulcer	10 (20.83)	10	-	8	2
Erosive upper GI + polyp	2 (4.17)	2	-	-	2
Erythematous upper GI + mild erosive	11 (22.92)	11	-	1	10
Erythematous upper GI + polyp	1 (2.08)	-	1	-	1
Upper GI ulcer	7 (14.58)	7	-	5	2
Total	48 (100)	47	1	14	34

* FOBT Hb + Tf defined as positive if both Tf/Hb/ are positive

Table 4. The FOBT result on the control group

Control group	n (%)	FOBT Results*			
		Hb + Tf		Hb	
		Positive	Negative	Positive	Negative
Erythematous gastritis	19 (65.51)	2	17	1	18
Normal	4 (13.79)	-	4	-	4
Gastropathy	2 (6.90)	-	2	-	2
Esophagitis	1 (3.45)	-	1	-	1
Pyloric lumen narrowing with unclear cause	1 (3.45)	-	1	1	-
Erythematous upper GI & bile reflux	2 (6.90)	1	1	-	2
Total	29 (100)	3	26	2	27

* FOBT Hb + Tf positive if Tf must be positive

Table 5. Comparison of FOBT Hb + Tf with FOBT Hb on control group

Control group	n (%)	FOBT Results*			
		Hb + Tf		Hb	
		Positive	Negative	Positive	Negative
Erythematous gastritis	19 (65.51)	2	17	1	18
Normal	4 (13.79)	-	4	-	4
Gastropathy	2 (6.90)	-	2	-	2
Esophagitis	1 (3.45)	-	1	-	1
Pyloric lumen narrowing with unclear cause	1 (3.45)	1	-	1	-
Erythematous upper GI & bile reflux	2 (6.90)	1	1	-	2
Total	29 (100)	4	25	2	27

* FOBT Hb + Tf positive if both Tf/Hb are positive

Table 6. The result of FOBT in control group

Cases group	FOBT Hb + Tf positive*			FOBT Hb positive
	Tf	Hb	Tf + Hb	
Erythematous gastritis	2	2	2	1
Normal	-	-	-	-
Gastropathy	-	-	-	-
Esophagitis	-	-	-	-
Pyloric lumen narrowing with unclear cause	-	1	-	1
Erythematous upper GI and bile reflux	1	-	-	-
Total	3	3	2	2

*****FOBT Hb + Tf positive, Tf must be positive

Table 7. The diagnostic on 2 types of FOBT

	FOBT Hb + Tf		FOBT Hb
	Tf positive	Both of Tf/Hb positive	
Sensitivity (%)	85.42	97.92	29.17
Specificity (%)	89.66	86.21	93.10
Positive predictive value (%)	93.18	92.16	87.50
Negative predictive value (%)	78.79	96.15	44.26

DISCUSSION

The result showed that FOBT Hb + Tf has better sensitivity, PPV, and NPV compared to FOBT Hb only, while specificity of FOBT Hb is better than FOBT Hb + Tf (table 7). This is appropriate with study conducted by Chiang et al, when they were comparing FOBT Hb + Tf with FOBT chemical method (*o*-toluidine) and immunochemical methods (OC-Hemodia test) on detecting upper GI bleeding. From their study, Chiang et al had proven that FOBT Hb + Tf has better diagnostic value.[4]

In this study, we evaluated FOBT Hb + Tf positive in two ways, which is defined as positive if Tf is positive because we did not perform colonoscopy to patients, therefore by using only exc lusion criteria, there were still a possibility of occult bleeding in lower GI. However from the procedures in our kit, it would be defined as positive if one or both Tf and Hb were proven positive by the second method. Results shows that sensitivity of FOBT Hb + Tf were increased if appropriate guidance kit, which was mentioned positive when Tf or Hb or both strips appear. Nevertheless, the specificity of the test were decreased.[11-13]

Diagnostic value of FOBT is varies widely, depending on the principle of the method , plants, and other conditions such hydration and stool sample. Bleeding of upper GI tract release haptoglobin, fibrinogen, and transferrin. Transferrin is a glyco-protein resistant to bacterial degradation, therefore its condition in stool is a lot more stable than Hb. FOBT that verify only Hb in stools has false negative due to stool hydration condition, Hb degradation by protease enzymes, vanished antigenicity of Hb resulted from *Enterobacter* and mucin absorption. FOBT Hb-only was better used to detect lower GI bleeding e.g. carcinoma and colonic polyps, while simultaneous Hb + Tf FOBT was better than Hb only or chemical tests to detect upper GI bleeding.[4,7-10,14-17]

In this study, FOBT Hb + Tf on control population showed 25 out of 29 control samples were negative both in Tf and Hb. While in FOBT Hb only, we found 27 negative sa mples out of 29 control population. In FOBT Hb + Tf, 2 controls were positive for both Tf and Hb in patients with erythematous gastritis. This was possible due to intermitten occult bleeding in stomach, therefore at time of endoscopy evaluation, we did not find any abnormalities which is one of them was positive in FOBT Hb only. We also found one patient with pylori lumen narrowing (control) whom in FOBT Hb + Tf test were positive Hb only, in which, the result of FOBT Hb only (delta) was also positive. Whether this because the Tf level is too low to detect or because other cause, could not be ascertained from this study.

In this study, 48 patients with upper GI bleeding that were examined by FOBT + Tf, there were 6 patients with negative Tf but positive Hb, in which 3 of them is also positive in FOBT Hb-only. The positive Hb and negative Tf result emerged due to Tf level in these patients is too low, thus made it undetectable in FOBT Hb +Tf. However, we can not validate it because we did not measure Tf level in the patient's blood. From 48 patients with upper GI bleeding, in FOBT Hb + Tf, we found 22 samples positive in Hb, but in FOBT Hb only the result were negative.

This was somewhat surprising, because the cut off point of FOBT Hb only is just 50 ng/mL, while in FOBT Hb + Tf the cut-off point 200 ng/mL. Therefore, it is supposed to be positive in FOBT Hb only if it is positive in FOBT Hb + Tf, but the truth was not like that. Whether this results from different use of anti-human Hb in each tests therefore provide different sensitivity, also could not be ascertained

CONCLUSION

The results of this study indicate that diagnostic value of FOBT Hb + Tf was better than FOBT Hb only in detecting upper GI bleeding. Therefore, FOBT Hb + Tf can be used for upper GI bleeding screening tests.

REFERENCES

1. Lau JYW, Chung SCS. Hematemesis and melena. In: Weinstein WM, eds. Clin Gastroenterol Hepatol. USA: Elsevier Mosby 2005.p.121-31.
2. Pangestu A. Pengelolaan perdarahan saluran cerna bagian atas. In: Sudoyo AW, Setyohadi B, Alwi I, et al, eds. Buku Ajar Ilmu Penyakit Dalam. 4th ed. Jakarta: Pusat Penerbitan Ilmu Penyakit Dalam 2006.p.291-4.
3. Murdani A. Perdarahan saluran cerna bagian bawah (hematokezia) dan perdarahan samar *(occult*). In: Sudoyo AW, Setyohadi B, Alwi I, et al eds. Buku Ajar Ilmu Penyakit Dalam. 4th ed. Jakarta: Pusat Penerbitan Ilmu Penyakit Dalam 2006.p.295-9.
4. Chiang CH, Jeng JE, Wang WM, Jheng BH, Hsu WT, Chen BH. A comparative study of three fecal occult blood test in upper gastrointestinal bleding. Kaohsiung J Med Sci 2006;22:223-8.
5. Harewood GC, McConnell JP, Harrington JJ, Mahoney DW, Ahlquist DA. Detection of occult upper gastrointestinal tract bleeding: performance differences in fecal occult blood tests. Mayo Clin Proc 2002;77:23-8.
6. Kao YS, Keng Liu FJ, Alexander DR. Laboratory diagnosis of gastrointestinal tract and exocrine pancreatic disorders. In: Henry JB, eds. Clinical Diagnosis and Management by Laboratory Method. 19th ed. USA: WB Saunders Co 1996.p.538-40.
7. Widijanti A, Dewi J. Nilai diagnostik rapid *chromatographic immunoassay* untuk mendeteksi darah samar dalam tinja. Medika 2007;32:142-5.
8. Miyoshi H, Uchida K, Matsuse R, Amatsu T, Shimamoto C, Hirata I, et al. Clinical Study of a new fecal occult blood test using a combination assay of hemoglobin and transferrin. Gastroenterol Jpn 1991;26:151-6.
9. Miyoshi H, Ohshiba S, Asada S, Hirata I, Uchida K. Immunological determination of fecal hemoglobin and transferrin levels: a comparison with other fecal occult blood tests. Am J Gastroenterol 1992;87:67-73.
10. Miyoshi H, Oka M, Sugi K, Saitoh O, Katsu K, Uchida K. Accuracy of detection of colorectal neoplasia using an immunochemical occult blood test in symptomatic referred patients: comparison of retrospective and prospective studies. Intern Med 2000;39:701-6.
11. Jutabha R, Jensen DM. Acute upper gastrointestinal bleeding. In: Friedman SL, McQuaid KR, Grendell JH, eds. Current Diagnosis & Treatment in Gastroenterology. 2nd ed. Mc Graw Hill Co 2003.p.53-69.
12. Rockey DC. Occult gastrointestinal bleeding. In: Friedman SL, McQuaid KR, Grendell JH, eds. Curr Diagn Treat Gastroenterol. 2nd ed. Mc Graw Hill Co 2003.p.83-95.
13. Rockey DC. Occult gastrointestinal bleeding. N Engl J Med 1999;341:38-46.
14. Beg M, Singh M, Saraswat MK, Rewari BB. Occult gastrointestinal bleeding: detection, interpretation, and evaluation. JIACM 2002;3:153-8.
15. Dulai GS, De Rosa VP. Gastrointestinal causes of anemia and occult bleeding. In: Weinstein WM, eds. Clin Gastroenterol Hepatol. USA: Elsevier Mosby 2005.p.75-9.
16. OIVD/DIHD. Guidance for industry and FDA staff, review criteria for assessment of qualitative fecal occult blood in vitro diagnostic devices. Center Dev Radiol Health 2007.p.1-15.
17. Harewood GC, Mc Connell JP, Harrington JJ, Mahoney DW, Ahlquist DA. Detection of occult upper gastro-intestinal tract bleeding: performance difference in fecal occult blood test. Mayo Clin Proc 2002;77:23-8.