RESEARCH ARTICLE

Diagnostic Value of Fecal Calprotectin as a Screening Biomarker for Gastrointestinal Malignancies

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Abstract

Background: Calprotectin in feces seems to be a more sensitive marker for gastrointestinal (GI) cancers than fecal occult blood, but its specificity may be too low for screening average risk populations. This study aims at evaluating the diagnostic value of fecal calprotectin as a screening biomarker for GI malignancies. Materials and Methods: In a case-control study, 100 patients with GI malignancies (50 patients with colorectal cancer and 50 patients with gastric cancer) and 50 controls were recruited in Tabriz Imam Reza and Sina hospitals during a 24-month period. One to two weeks after the last endoscopy/colonoscopy, fecal specimens were collected by the patients and examined by ELISA method for quantitative measurement of calprotectin content. The results were compared between the three groups. Results: The mean fecal calprotectin level was 109.1±105.3 (2.3-454.3, median:74), 241.1±205.2 (3.4-610.0, median:19.3) and 45.9±55.1μg/g (1.3-257.1, median:19.3) in gastric cancer, colorectal cancer and control group, respectively, the differences being significant (p<0.001) and remaining after adjustment for age. The optimal cut-off point for fecal calprotectin was ≥75.8μg/g for distinguishing colorectal cancer from normal cases (sensitivity and specificity of 80% and 84%, respectively). This value was ≥41.9μg/g for distinguishing gastric cancer from normal cases (sensitivity and specificity of 62%). Conclusions: Our results revealed that fecal calprotectin might be a useful and non-invasive biomarker for distinguishing colorectal cancer from non-malignant GI conditions. However, due to low sensitivity and specificity, this biomarker may not help physicians distinguishing gastric cancer cases from healthy subjects.

Keywords: Colorectal cancer - gastric cancer - calprotectin - screening tool

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Introduction

Colorectal cancer is the second commonest cause of death from malignancy in the Western world (Mohri et al., 2013). In Iran especially in northwestern provinces, the prevalence of gastrointestinal (GI) cancer is markedly high (Somi et al., 2008). According to some studies, gastric cancer was the most commonly detected malignancy (Norouzinia et al., 2012).

Survival rates are closely related to the stage of cancer at the time of diagnosis and the most promising approach to reduce mortality rates is early detection of precancerous or cancerous lesions (Ouyang et al., 2005). There is now overwhelming epidemiological evidence and molecular biological data to substantiate previous suggestions of the colonic adenocarcinoma progression. Collectively, such data have increased the pressure to develop novel approaches for colon cancer detection, critical for secondary prevention through mass population screening whereby early diagnosis of colorectal cancer will detect tumors with the best prognosis and result in improved survival rates (Stryker and Trebichavsky, 1987; Tibble et al., 2001).

Calprotectin (S100A8/A9), a heterodimer of the two calcium-binding proteins S100A8, S100A accounts for about 60% of cytosol proteins of neutrophil granulocytes, macrophage and epithelial cells, and was originally discovered as immunogenic protein expressed and secreted by neutrophils (Steinbakk et al., 1990). Subsequently, it has emerged as important pro-inflammatory mediator in acute and chronic inflammation. However recently, increased S100A8 and S100A9 levels were also detected in various human cancers, presenting abundant expression in neoplastic tumor cells as well as infiltrating immune cells. Although, many possible functions have been proposed for S100A8/A9, its biological role still remains to be defined. Altogether, its expression and potential cytokine-like function in inflammation and in

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cancer suggests that S100A8/A9 may play a key role in inflammation-associated cancer (Striz et al., 2004; Gebhardt et al., 2008).

Calprotectin is resistant to enzymatic degradation and can be easily measured in stools. Moreover, increased levels of fecal calprotectin have been reported in patients with several other inflammatory conditions of the lower gastrointestinal tract and even in patients affected by neoplastic disease of both upper and lower gastrointestinal tract (Brydon et al., 2001; Poullis et al., 2003). Therefore, measuring fecal calprotectin has been proposed as useful noninvasive diagnostic tool for differentiating patients with organic disease of the intestinal tract from those with functional diseases. The present study was conducted to determine the diagnostic efficacy of calprotectin as a biomarker for GI-associated cancers among a group of Iranian patients.

Materials and Methods

This case-control study was carried out in 150 men and women, without any age limitation, during a 2-year period between May 2010 and May 2012. Individuals were referred to Imam Reza and Sina hospitals, affiliated to Tabriz University of Medical Sciences, in Tabriz, a city in North West of Iran that welcome patients from all cities located in northwestern part of the country.

Totally, 50 individuals had CRC (colorectal cancer) and 50 had gastric cancer for whom the definite diagnosis was verified according to endoscopy, colonoscopy and biopsy achieved by an expert gastroenterologist. The remaining 50 subjects were normal controls. The following exclusion criteria were applied at baseline for control group: receiving NSAIDs in the past two weeks, IBD (inflammatory bowel disease), diarrhea, PU (peptic ulcer), esophagus cancer and gastric ulcer which was not associated with gastric cancer. The following initial data were gathered by a well-prepared questionnaire: age, gender, educational status, history of cancer in family, past medical history, occupation, and findings of prior studies and physical exams.

All participants were asked to bring their single 5-gram fresh stool specimen, 1-2 weeks after the last endoscopy or colonoscopy which was collected according to a well-described protocol. Then, fecal calprotectin level was measured in all subjects with gastric cancer, CRC and controls. The stool samples were extracted and analyzed by immunoassay, ELISA, with BUHLMAM kite (Germany), in a single laboratory whose staff was blind towards the studied groups. Finally, associated ROC (Receiver Operating Characteristic) curves of fecal calprotectin were drawn in different subgroups.

Having our goal explained, patients were asked to complete an informed consent prepared by the Ethical Committee of Tabriz University of Medical Sciences. There was the possibility of interrupting the patient's cooperation, as he/she desired.

Collected data were recorded in an electronic database and analyses by mean of SPSS statistical software (version 13.0, SPSS Inc., USA). Statistical analysis was carried out by means of chi-square, Fisher's exact test, Kruskal-Wallis test, one-way ANOVA and multivariate logistic regression, as appropriate. The p values lower than 0.05 were considered as statistically significant.

Results

The study population included 36 males and 14 females with the mean age of 63.7±11.6 years (a range, 34-85, median:65 years) in the gastric cancer; 32 males and 18 females with the mean age of 55.6±12.6 years (a range, 25-77, median:57 years) in CRC and 33 males and 17 females with the mean age of 40.4±14.1 years among healthy controls (a range, 20-73, median:37 years). There was no statistically significant difference in gender distribution among groups, however, CRC patients were significantly older while healthy controls were significantly younger ($p<0.001$).

Previous history of cancer was positive in 8 (16%), 16 (32%), and 3 (6%) subjects of gastric cancer, CRC, and healthy controls, respectively ($p<0.003$). When inquiring past medical history in subjects of gastric cancer group, the following were noted: hypertension (7 cases), cholecystectomy (2 cases), hemorrhoidectomy, congestive heart failure (CHF) plus hypertension, and hyperlipidemia (each in one case). Nevertheless, among CRC cases, 11 cases (22%) had positive past medical history including hypertension (8 cases), CHF, anemia, and osteoarthritis (each in one case). Finally, only one healthy control had hypertension. The differences did reach a statistically significant level among groups ($p<0.006$). Table 1 represents laboratory findings of subjects at baseline.

The mean fecal calprotectin level was 109.1±105.3 μg/g (a range, 2.3-454.3, median:74 μg/g), 241.1±205.2 μg/g (a range, 3.4-610.0, median:19.3 μg/g) and 45.9±55.1 μg/g (a range, 1.3-257.1, median:19.3 μg/g) in gastric cancer, colorectal cancer and healthy controls, respectively. Hence, fecal calprotectin was significantly highest among CRC patients and lowest among healthy controls. Meanwhile, patients with malignancy, either gastric or colorectal, had significantly higher calprotectin level ($p<0.001$).

Having adjusted for age, the mean calprotectin level was significantly higher among CRC patients when compared with gastric cancer ($p<0.002$) and healthy controls ($p<0.001$).

Figure 1 demonstrate the associated ROCs for distinguishing gastric and/or colorectal cancer from healthy controls. As shown in figure 1, the area under the ROC curve for colorectal cancer when compared with healthy controls is equal 0.84, therefore, on average, a patient with colorectal cancer will have a more abnormal test result than 84% of the controls.

The optimal cut-off point for fecal calprotectin was ≥75.8 μg/g for distinguishing colorectal malignancy from normal cases (sensitivity and specificity of 80% and 84%, respectively; $p<0.001$), however, this value was ≥41.9 μg/g for distinguishing gastric malignancy from normal cases (sensitivity and specificity of 62%, $p<0.001$). Meanwhile, with respect to an area under the ROC curve of 0.71 for gastric cancer, one can expect 71% of gastric cancer patients to have abnormal test results than controls.

Table 1. Laboratory Findings of Subjects with Gastric and Colorectal Cancer and Healthy Controls at Baseline

	Gastric cancer (n=50)	Colorectal cancer (n=50)	Healthy controls (n=50)	p
WBC (mm3)	5962±2046	5750±1234	5937±1298	0.84
Hemoglobin (g/dl)	11.0±1.1	11.6±1.3	12.4±1.2	0.001
Hematocrit (%)	33.4±3.2	35.0±3.9	36.8±3.4	0.001
Platelet count (mm3)	3.7×10^5	2.6×10^5	2.1×10^5	0.07
MCV (fl)	77.3±11.3	80.4±3.5	81.6±4.6	0.38
Total bilirubin (mg/dl)	1.0±0.2	1.0±0.1	1.0±0.1	0.96
Direct bilirubin (mg/dl)	0.2±0.1	0.3±0.1	0.2±0.1	0.52
AST (U/L)	24.4±11.2	20.3±6.4	18.4±6.3	0.3
ALT (U/L)	25.7±5.9	24.4±5.8	18.4±0.7	0.04
ALP (U/L)	142.4±16.3	43.3±10.0	25.5±17.7	0.25
Albumin (gr)	3.6±0.5	3.8±0.2	4.0±0.2	0.08
PT (s)	13.0±0.0	13.0±0.0	13.0±0.0	-
PTT (s)	35.5±3.7	35.2±1.7	33.9±2.7	0.33
BUN (mg/dl)	16.1±4.3	18.0±6.3	14.5±3.6	0.06
Creatinine (mg/dl)	1.0±0.3	0.9±0.2	0.8±0.2	0.18
FBS (mg/dl)	111.1±20.4	105.9±18.7	103.7±19.3	0.56
Cholesterol (mg/dl)	250.0±42.4	200.0±13.1	220.0±10.0	0.02
Triglyceride (mg/dl)	267.5±36.9	230.0±32.2	246.7±47.3	0.33
HDL-cholesterol (mg/dl)	43.8±7.9	38.3±2.6	55.0±14.1	0.04
LDL-cholesterol (mg/dl)	176.3±11.1	150.0±23.7	157.5±10.6	0.15
Iron (μg/dl)	62.5±15.0	73.3±22.5	69.5±29.0	0.78
TIBC (μg/dl)	327.5±29.9	335.0±17.3	335.0±41.4	0.93
Ferritin (ng/dl)	67.0±15.6	80.0±11.1	74.3±26.8	0.91

*WBC: White blood cell; MCV: Mean corpuscular volume; AST: Aspartate aminotransferase, ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; PT: Prothrombin time; BUN: Blood urea nitrogen; FBS: Fasting blood sugar; HDL: High density lipoprotein; LDL: Low-density lipoprotein; TIBC: Total iron binding capacity

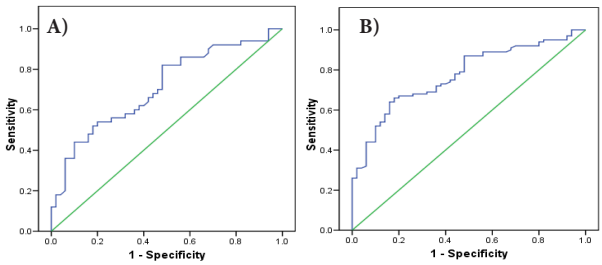

Figure 1. ROC Curve of Calprotectin for Distinguishing Patients with A) Gastric Cancer and B) Gastric or Colorectal Cancer, from Healthy Controls

Finally, when gastric and colorectal cancer patients were compared together against normal controls, the following values were calculated: area under ROC curve: 0.77, cut-off point: ≥63.1 μg/g, sensitivity: 68%, specificity: 74% and p<0.001.

Discussion

In this research, we have focused on the diagnostic value of fecal calprotectin level in recognition of digestive system cancer, including CRC and gastric cancer, in comparison with normal cases. The mean of fecal calprotectin level in patients suffering from stomach cancer and CRC was 109.1±105.3, and 241.1±205.2, respectively, as compared to a level of 45.9±55.1 μg/gin controls. Hence, the level of fecal calprotectin in CRC was the highest and in the control group, normal cases, the lowest.

Summerton et al. (2002) compared the level of stool calprotectin among 8 cases of CRC, 2 cases of stomach cancer and 28 normal cases. According to their findings, the median of fecal calprotectin level in the three mentioned groups were respectively 53.4, 30.0, and 4.5 milligram in litre (p<0.05). Similarly, Kronborg et al. (2000) surveyed 814 candidates of colonoscopy, among whom, the median of fecal calprotectin level in CRC and normal group were 17.5 and 6.6 milligrams per litre, respectively (p<0.05).

Tibble et al. (2001) studied in the same area on 62 patients with CRC and 96 individuals as normal group. The reported medians of fecal calprotectin level in these two groups were respectively 101 (57-133) and 2.3 (1.5-5) milligrams per liter (p<0.05). In contrast, Karl et al. (2008) evaluated six biomarkers including hemoglobin (iFOBT), hemoglobin-haptoglobin, calprotectin, carcinoembryogenic antigen, markers tissue inhibitor of metalloproteinase-1 (TIMP-1) and S100A12 to improve the sensitivity of detection of CRC in stool samples. They found the best diagnostic performance for S100A12 (0.95), followed by TIMP-1 (0.92), hemoglobin-haptoglobin (0.92), hemoglobin (0.91), calprotectin (0.90), and carcinoembryogenic antigen (0.66).

Apparently, due to the different units used in different studies, the direct comparison of fecal calprotectin level is not possible. Nevertheless, the difference in the level of this marker has been also confirmed by other studies.

In our study, the cut-off point of fecal calprotectin level in CRC was ≥75.8 μg/g in comparison with normal cases (associated sensitivity and specificity were 80 and 84%), however, among gastric cancer patients the aforementioned cut-off point was ≥41.9 μg/g (sensitivity and specificity were both 62%).

For investigating the diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy, quantitative meta-analysis on thirty studies including 5,983 patients was performed by van Roon et al. (2007). Fecal calprotectin levels in patients with CRC were higher but not significantly compared with noncancer controls and the sensitivity and specificity of fecal calprotectin for the diagnosis of CRC were 36% and 71%, respectively. As result they did not recommend fecal calprotectin as a screening test for CRC in the general population.

In a study conducted by Hoff et al, 16 cases of CRC, 195 cases with high risk adenoma of digestive system, 592 cases with low risk adenoma of digestive system, and 1518 normal individuals were assessed. The positive level of fecal calprotectin was considered greater than 50μg/g. As a result, the sensitivity of this marker to distinguish CRC from other conditions was reported 27%, however, when all suspected malignant conditions were considered together the sensitivity reached 76% (Hoff et al., 2004). Other studies reported different sensitivity and specificity values. In Johne et al. (2001) study over 453 symptomatic and asymptomatic CRC cases and healthy controls, a cut-off point of 50 μg/g was calculated with the associated sensitivity and specificity of 89 and 68%, respectively. In another study conducted by Kronborg et al. (2000) a cut-off point of 10 μg/g was reported, however, the sensitivity of fecal calprotectin to distinguish CRC from

other non-malignancy cases was ranged between 74% and 91% while the specificity was nearly 64%. Finally, Tibble et al. (2001) found a sensitivity and specificity of 79% and 72%, respectively, for fecal calprotectin to distinguish CRC or adenomatous polyps from non-malignant lesions. Therefore, the reported values for sensitivity and specificity of fecal calprotectin to distinguish GI-related malignancies from other benign lesions lies within the upper range, as calculated in our study, therefore, this marker can serve as a non-invasive tool for detecting GI-related malignancies, especially for colorectal cancers.

However, its use as a screening tool needs further studies, particularly with respect to its cost-effectiveness.

Our study has some potential weaknesses. Like previous studies of the same design, we did not include patients with GI-associated inflammation or non-malignant tumors since fecal calprotectin level may increase in these conditions (Summerton et al., 2002; Hoff et al., 2004; Fagerberg et al., 2005). Meanwhile, our findings demonstrated an abnormal distribution of fecal calprotectin level in CRC and gastric cancer patients. Indeed, other elements may influence the level of calprotectin, for which future studies are mandatory (Roseth et al., 1993; Johne et al., 2001).

In conclusion, the mean level of fecal calprotectin was higher among patients with CRC and gastric cancer. The optimal cut-off point for fecal calprotectin was ≥75.8 μg/g for distinguishing colorectal malignancy from normal cases (sensitivity and specificity of 80% and 84%, respectively). This value was ≥41.9μg/g for distinguishing gastric malignancy from normal cases (sensitivity and specificity of 62%). However, due to low sensitivity and specificity, this biomarker may not help physicians distinguishing gastric cancer from healthy subjects.

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