Can Noninvasive Tests Substitute Endoscopy for diagnosis of colonic diseases?

Abstract

Background and aims: Fecal calprotectin was approved as a non-invasive screening tool for organic colonic diseases. The aim of this study was to evaluate fecal calprotectin and inflammatory indices as non-invasive markers for diagnosing and differentiating inflammatory bowel diseases (IBD) and colorectal cancer (CRC) in comparison to irritable bowel syndrome (IBS) in a cohort of Egyptian patients.

Methods: In this cross-sectional study, fecal calprotectin, neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR) and systemic inflammatory index (SII) were assessed in 40 IBD patients, 40 CRC patients, and 20 IBS patients.

Results: Fecal calprotectin was significantly higher in IBD and CRC groups compared to IBS control group (P= 0.018 and 0.022 respectively), with no significant difference between IBD and CRC groups. PLR, NLR and SII showed no significant differences between the 3 studied groups (P= 0.469, 0.101 and 0.84 respectively). At a cut-off value 113, fecal calprotectin had the ability to differentiate CRC patients from IBS patients with 75% sensitivity and 60% specificity, while At cut-off value of 116, fecal calprotectin had the ability to differentiate IBD patients from IBS patients with 67.5% sensitivity and 65% specificity.

Conclusion: Inflammatory indices tested in our study (PLR, NLR, SII) showed no role for the differentiation between IBD, CRC, and IBS. Fecal calprotectin should be used with caution as a primary step for screening of colonic diseases as it cannot differentiate IBD from CRC. It has only moderate sensitivity in differentiating IBD and CRC from IBS. Unfortunately, neither fecal calprotectin nor inflammatory indices can substitute endoscopy in the screening or diagnosis of IBD and CRC.

Keywords: Colorectal cancer, inflammatory bowel disease, irritable bowel syndrome, fecal calprotectin, systemic inflammatory index.

Introduction

Colonoscopy screening is associated with reduced colorectal cancer (CRC) incidence and mortality **(1).** It is considered as a corner stone for diagnosing colonic diseases. However, endoscopic examination has some limitations; as it is not tolerable in many patients leading to a considerable miss rate of early diagnosis of different colonic disorders **(2,3**). Alongside the need for special equipment, trained operators and the possibility of complications are still a matter of consideration **(4)**. These limitations propose the need for noninvasive diagnostic tests for colonic diseases.

Several biomarkers have been tested for their role in the diagnosis of gastrointestinal tract (GIT) disorders based on the rational of inflammation. The disturbance of interaction between systemic inflammation and the local immune response in GIT results in loss of the local immune system homeostasis and trigger inflammation of intestinal mucosa. Added to its role in pathology of inflammatory bowel disease (IBD), this process was claimed to play a role in colorectal cancer (CRC) initiation, development, and progression **(5,6).**

One of these markers is fecal calprotectin. It is considered as an important marker in evaluating the inflammation of the intestinal mucosa **(7).** It was recommended as a diagnostic marker for organic colonic diseases including inflammatory bowel disease (IBD) and CRC **(8**,**9).**

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are good indicators of the systemic inflammatory conditions. In 2014, Hu et al. introduced the systemic immune-inflammation index (SII) as a prognostic marker after curative resection of hepatocellular carcinoma. Since then, these indicators were tested in many malignancies

including colorectal cancer **(10).** Also, it was postulated to have a role in confirming ulcerative colitis (UC) diagnosis and identifying its activity **(11).** Recently, it was considered as a reliable indicator of the inflammatory process in irritable bowel syndrome (IBS) patients **(12).**

The aim of this study was to evaluate fecal calprotectin and inflammatory indices as non-invasive diagnostic markers for differentiating IBD, CRC and IBS versus colonoscopy as gold standard.

Patients and Methods

This cross-sectional study was performed at the department of Tropical medicine and infectious diseases , Tanta University in the period from April 2021 to March 2022. It was designed complying with the declaration of Helsinki and gained approval from the ethical committee of Tanta University Faculty of Medicine (approval number 34324/12/2020).

Patients (male or female) 18 years and older with various indications of colonoscopy were recruited. According to the results of colonoscopy, we asked the patients to join the study if they have IBD, CRC or IBS. The patients were enrolled consecutively after they sign a written informed consent. They were allocated into 3 groups: group I included 40 IBD patients (either ulcerative colitis or Crohn's disease), group II included 40 CRC patients and group III included 20 IBS patients.

Patients were excluded if unwilling to participate, or had any of the following: coexisting malignancy, history of immunosuppressive drug, use of non-steroidal anti-inflammatory drug or proton pump inhibitors during the last four weeks.

After enrolment, patients were subjected to the following: Full history taking and complete physical examination; colonoscopy and biopsy taking for histopathological examination; CT on abdomen and pelvis for staging of CRC patients; laboratory testing including complete blood

count, and stool samples for fecal calprotectin testing. All patients were informed by the instructions for gathering of the stool sample (**13**). Stool samples were sent to the lab within 24 hours and examined using commercially available fecal calprotectin ELISA kits (EDI™ Quantitative Fecal Calprotectin ELISA Kit for the determination of human calprotectin (neutrophil cytoplasmic protein S100A8/A9). Fecal calprotectin level under 50 µg /g was considered normal (**14**).

- Calculation of indices

  a- SII = (PxN)/L

  Where P = peripheral platelet x 10^9/L, N = neutrophil x 10^9/L, and L =lymphocyte counts x 10^9/L (**15**).

  b- PLR = P/L

  Where P = peripheral platelet x 10^9/L, and L =lymphocyte counts x 10^9/L

  c- NLR= N/L.

  Where N = neutrophil x 10^9/L, and L =lymphocyte counts x 10^9/L.

Analysis of collected data

SPSS software package version 20.0. (Armonk, NY: IBM Corp) was used for the analysis of collected data. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using mean, and standard deviation, or median and interquartile range (IQR). To test differences between included groups we used Chi-square test for categorical variables, ANOVA (Tukey`s test was used as post hoc tests), Kruskal Wallis test (Dunn's multiple comparisons test was used as post hoc in significant tests).

For assessment of diagnostic power of fecal calprotectin and tested indices, we used Receiver Operator Characteristic (ROC) curve with

computer calculated optimum cut off value (point with highest sum of sensitivity and specificity). For all used tests, P value < 0.05 was considered significant.

Results

One hundred patients were included in this study 43 males and 57 females, with age ranging between 19 years to 78 years. Age was significantly higher in CRC group than both IBD and IBS groups (P value <0.001), while IBS patients were significantly older than patients in IBD group (P value=0.003). The prevalence of urban residency was higher in CRC group compared to IBS and IBD groups (P value = 0.008, 0.001 respectively)**(Table 1).**

Hemoglobin level was significantly decreased in CRC group when compared to IBS group (P=0.002), while IBD patients had higher lymphocytic count than CRC group (P= 0.013). Fecal calprotectin was significantly elevated in IBD and CRC patients compared to IBS patients (P=0.018 and 0.022 respectively), while there was no significant difference between IBD and CRC patients (P=0.937) **(Table 1).**

Clinically, abdominal pain, weakness, fatigue, constipation, weight loss and anorexia were the main presentations in CRC group (70%, 35%, 42.5%, 40 %, 75 % and 20 % of patients respectively), while 80% of IBD patients presented with diarrhea and 40 % with bloody stool. Whereas abdominal pain, bloating and constipation were the main presenting symptoms in IBS patients (70%, 55% and 70% respectively) **(Table 2).**

As regard histopathological examination; 34 (85%) of CRC patients had adenocarcinoma, 3 (7.5%) patients with Atypical tubulovillous adenoma with high grade dysplasia, 1 (2.5 %) patient with tubular adenoma with moderate dysplasia, 1 (2.5%) patient with tubulovillous adenoma with mild degenerative dysplasia, and 1 (2.5%) patient with tubulovillous

adenoma with mild to moderate degenerative dysplasia. whereas 35 patients were diagnosed with ulcerative colitis, and5 patients diagnosed with Chron's disease. While 20 patients in IBS group had normal endoscopic findings **(Table 2).**

ROC curve revealed optimal cutoff value > 113µg/g fecal calprotectin to differentiate between CRC and IBS with 75 % sensitivity and 60 % specificity, and at cutoff > 116µg/g to distinguish IBD patients from IBS patients with67.50% sensitivity and 65.0% Specificity, but it couldn't discriminate between IBD and CRC **(Table 3).**

Inflammatory indices (PLR, NLR, and SII) showed insignificant difference between studied groups (P=0.469, 0.101and 0.084 respectively) **(Table 1).** ROC curve analysis of collected data showed that SII at cutoff >556 x 10^9/L could discriminate CRC patients from IBS patients With only 62.50% sensitivity, and 60.0% Specificity, while it couldn't differentiate either IBD from IBS nor IBD from CRC **(Table 3).**

Discussion

Older age had been assumed as one of the risk factors for CRC development **(16-17),** similarly our patients in CRC group were significantly older than other groups. On the other hand, IBS patients in this study were significantly older than patients in IBD group, which disagree with the results obtained by **Costa et al., 2003 (18).** Despite the IBD traditional main peak at 15-25 years old which was postulated in1980s was revised and showed tendency to shift for older age (19-29 years old) particularly in men with ulcerative colitis **(19),** it is still below the peak prevalence of IBS, which ranges from 20 to 39 years of age **(20).** However, the mean age of our patients is higher than assumed ages in both groups. This can be explained by lag between onset of the disease and its diagnosis. The delayed diagnosis was previously reported in IBD **(19).** While Rome criteria facilitate the early clinical diagnosis of IBS, endoscopic diagnosis is usually delayed as the natural history of the disease is lacking red flag signs that mandate colonoscopy. As the age included in the study is age at endoscopic diagnosis rather than age of onset, we think this difference in age is mostly reflecting more delayed endoscopic diagnosis in IBS patients compared to IBD group.

In 2008, Lasson et al., **(21)** matched the colonoscopic diagnosis to clinical manifestations in patients suffering GIT symptoms in a trial to detect relation between colonoscopic findings and presenting symptoms. They concluded that apart from bleeding and diarrhea colonoscopic examination is unlikely to discover serious colonic disease in patients with GIT symptoms. These results support ours, as diarrhea was the main symptom (80%) of our IBD patients with nearby prevalence previously reported **(22,23).** Also, 70% of our IBS patients were presented with abdominal pain and similar prevalence was previously reported **(24,25).**

On the other hand, it seems against our findings that 70 % of CRC patients had abdominal pain as main symptom. This difference is explained as most of our CRC patients who lack other GIT symptoms had associated systemic manifestations including weight loss, anorexia, and pallor which were previously reported as red flags in CRC patients (26). Also, abdominal pain was reported to be nonspecific symptom (22), and similar prevalence of abdominal pain in CRC patients was reported **(17,27,28)**.

CRC patients had significantly lower hemoglobin level than IBS group. This was in accordance with **Väyrynen et al** who stated that CRC patients frequently have anemia at the time of the diagnosis **(26)**, and **Jellema et al., 2010** who stated that anemia was documented as the main cause of primary care entry of CRC patients **(28).**

There was a significant difference of lymphocytic count between IBD and CRC groups. However, the values of lymphocytic count in all patients of both groups were within normal range which render the difference of no clinical impact. Our results disagreed with study done by **Abraham and Sellin** in **2016** and concluded that peripheral blood of patients with IBD characterized by lymphopenia **(29).** This difference may be attributed to the difference in study population, as they included IBD patients taking either azathioprine or 6-mercaptopurine for a minimum of 6 months of therapy.

The role of inflammatory indices in the diagnosis of different GIT disorders is still controversial. Similar to our results, NLR and PLR were found to be invaluable in the diagnosis of IBS **(30)**, or in prediction of active disease in patients with UC **(31)**.

SII index was suggested to be more predictive of inflammation than NLR and PLR (32). However, SII showed no significant difference between

studied groups in current study. This was in accordance with previous studies advised the cautious use of SII as a screening biomarker for early detection of CRC or IBD because it is a non-specific general inflammatory marker **(33,34**).

Xie et al., 2021 found that SII levels were significantly higher in UC patients than healthy controls (**34**). In our cohort, despite SII index had higher values in CRC and IBD groups compared to IBS group, the difference was not statistically significant. This discrepancy may reflect the previously reported elevated SII index in patients with IBS compared to healthy controls **(12).** Longley, IBS was thought for as a pure functional disorder without underlying organic pathology. In 2018, NG et al., **(35)** in their review concluded that: despite no definite reproducible pattern of immune response in IBS, several studies revealed increased activity of mast cells, chronic, low-grade subclinical inflammation which can disturb microbiome, neuroinflammatory cytokines altering neuroendocrine pathways and glucocorticoid receptor genes, and assumed this proinflammatory phenotype to account for the symptoms of IBS at least in part **(35).** The context of mild inflammatory state in IBS versus evident inflammation in CRC and IBD can explain why the ROC curve revealed that SII was able to discriminate between CRC and IBS with moderate sensitivity and specificity, while it did not have this ability between IBD and IBS patients.

Several studies had investigated the role of fecal calprotectin for the diagnosis of IBD and CRC. It was even included in practical guidelines as a part of the diagnostic work up of IBD (**36**). In addition, its concentration was found to increase in CRC and adenomatous polyps when compared to healthy tissues (**37**). In accordance with previous

studies **(18,38),** fecal calprotectin was significantly higher in both CRC and IBD groups compared to IBS group.

Fecal calprotectin at cut off value >116 µg/g had the ability to discriminate IBD patients from IBS patients with moderate sensitivities and Specificities. **Sharbatdaran et al., 2018 (39)** set a slightly higher cutoff **(**>127.65µg/g) with higher sensitivity and specificity for the discrimination of IBD from non IBD including IBS and healthy individuals. This difference may be related to higher cut off value and inclusion of healthy control with no symptoms. Absence of symptoms is prone to overestimate the specificity of fecal calprotectin as discussed in a diagnostic meta-analysis (**40**).

At cut off >113 µg/g fecal calprotectin could differentiate CRC from IBS patients with moderate sensitivity and specificity, but hadn't the ability to differentiate between IBD and CRC. Similar results were obtained by Ross et al., 2022 who included 35 studies for systematic review and meta-analysis and concluded that despite CRC patients are more prone to have an elevated fecal calprotectin than controls, yet the low specificity of fecal calprotectin render it unsatisfactorily for diagnosis or screening of CRC **(41).**

Conclusion: Despite the ability of fecal calprotectin for the discrimination of IBD and CRC from IBS, still its sensitivity and specificity is problematic to depend on it as the sole factor for diagnosis. SII had a limited role for the differentiation between CRC and IBS.

One of the limitations of this study is limited number of patients included, in addition the cross sectional design of this study did not allow for follow up and evaluation of the prognostic value of these markers.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the authors (approval number 34324/12/2020).

Consent

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

Table (1): Comparison of demographic and laboratory data among studied groups.

	GroupI IBD (n = 40)		**GroupII CRC (n = 40)**		**GroupIII IBS (n = 20)**		**P**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Age (years) Mean ± SD.	31.28 ± 10.09		57.05 ± 14.58		43.15 ± 14.46		<0.001*
	p_1<0.001*, p_2=0.003*, p_3<0.001*						
Residency Urban	22	55.0	33	82.5	8	40.0	0.002*
	p_1=0.008*, p_2=0.273, p_3=0.001*						
Sex	M:12 F: 28	30.0 70.0	M: 20 F: 20	50.0 50.0	M: 11 F: 9	55.0 45.0	0.094
Smokers	7	17.5	8	20.0	5	25.0	0.791
Hb (g/dL) Mean ± SD.	11.33 ± 1.82		10.44 ± 2.22		12.40 ± 2.01		0.002*
p_1=0.126,p_2=0.133,p_3=0.002*							
PLT ($\times 10^9$) Median (IQR)	292.0 (225.5 – 377.5)		241.0 (203.0 – 288.5)		277.5 (238.0 – 308.5)		0.095
TLC ($\times 10^9$) Median (IQR)	8.35 (5.8 – 10.9)		7.70 (6.1 – 10.7)		7.20 (5.8 – 8.2)		0.199
Lymphocyte ($\times 10^9$)	2.10 (1.6 – 2.8)		1.70 (1.3 – 2.2)		1.90 (1.4 – 2.0)		0.034*

Median (IQR)				
$p_1=0.013^*, p_2=0.082, p_3=0.778$				
Neutrophils ($\times 10^9$) Median (IQR)	5.10 (3.3 – 7.7)	5.0 (4.0 – 6.9)	5.15 (3.8 – 6.6)	0.898
Monocyte ($\times 10^9$) Median (IQR)	0.35 (0.2 – 0.5)	0.40 (0.2 – 0.7)	0.40 (0.3 – 0.4)	0.504
PLR Median (IQR)	120.2 (84.5 – 186.2)	134.3 (106.1 – 184.7)	149.6 (112.3 – 205.3)	0.469
NLR Median (IQR)	2.22	2.78	2.78	0.101
Fecal calprotectin (µg/g)Median (IQR)	235.5(70.8 – 700.0)	201.0(117.5 – 324.5)	99.5(50.5 – 230.0)	0.038^*
$p_1=0.937$, $p_2=0.018^*$, $p_3=0.022^*$				
SII ($10\times^{11}$) Median (IQR)	6.29 (4.0 – 9.9)	6.46 (4.6 – 12.6)	4.68(3.50 – 6.52)	0.084

HTN: hypertension, DM: diabetes mellitus, Hb: Hemoglobin, PLT: Platelet, TLC: total leucocytic count, PLR: Platelet lymphocyte ration, NLR: Neutrophil lymphocyte ratio, SII: Systemic inflammatory index, IQR: Inter quartile range, IBD: Inflammatory bowel disease, CRC: Colorectal cancer, IBS: irritable bowel syndrome.

p_1: p value for comparing between Group I and Group II, p_2: p value for comparing between Group I and Group III, p_3: p value for comparing between Group II and Group III

*: Statistically significant at $p \leq 0.05$

Table (2): Clinical, endoscopic, and histopathological data of studied groups.

	Group I IBD (n = 40)	Group II CRC (n = 40)	Group III IBS (n = 20)
	No. (%)	No. (%)	No. (%)
Abdominal pain	16 (40)	28 (70)	14 (70)
Bloating	1 (2.5)	3 (7.5)	11 (55)
Diarrhea	32 (80)	4 (10)	5 (25)
Weakness	2 (5)	14 (35)	1 (5)
Fatigue	7 (17.5)	17 (42.5)	4 (20)
Constipation	2 (5)	16 (40)	14 (70)
Weight loss	4 (10)	30 (75)	1 (5)
Bloody stool	16 (40)	8 (20)	0 (0)
Anorexia	0 (0)	8 (20)	0 (0)
Pallor	6 (15)	12 (30)	0 (0)
Toxic look	0 (0)	2 (5)	0 (0)
Abdominal distention	1 (2.5)	4 (10)	4 (20)
Colonoscopic and histopathological findings	Chronic moderate active colitis (Crypt abscess and lymphocytic infiltration) 30 (75%)	Adenocarcinoma 34 (85%)	Normal mucosa with preserved vascular pattern biopsies revealed normal muosal structure 15 (75%)

	Mild colitis 5 (12.5%)	Atypical tubulovillous adenoma with high grade dysplasia 3 (7.5%)	Erythema ous mucosa 5 (25%) biopsies revealed Normal mucosa without pathologi cal abormaliy
	Chronic ileitis with focal atrophy 3 (7.5%)	Tubular adenoma with moderate dysplasia 1(2.5%)	
	Chronic follicular proctitis with nonspecific ileitis 1 (2.5%)	Tubulovillous adenoma with mild degenerative dysplasia 1(2.5%)	
	Hyperplastic mass at ileocecal valve 1 (2.5%)	Tubulovillous adenoma with m moderate degenerative dysplasia 1 (2.5%)	

Table (3):Validity of fecal calprotectin and SII in differentiating colonic diseases

Test	To discriminate	AUC	P	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
Fecal calprotectin	**CRC from IBS**	0.707	0.009*	0.560 – 0.854	>113	75	60	78.9	54.5
	IBD from IBS	0.664	0.04	0.528 – 0.799	>116	67.5	65	79.4	50
	IBD from CRC	0.518	0.788	0.386 – 0.649	>194	55	47.5	51.2	51.4
SII ($x10^{11}$) cells/L	**CRC from IBS**	0.673	0.030*	0.534 – 0.811	>5.56	62.5	60	75.8	44.4
	IBD from IBS	0.636	0.087	0.493 – 0.780	>5.3	67.5	60	77.1	48
	IBD from CRC	0.544	0.494	0.417 – 0.672	≥6.45	52.5	50	51.2	51.3

AUC: Area under curve, CI: Confidence interval, PPV= positive predictive value, NPV= negative predictive value, SII systemic inflammatory index, *= significant

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