RESEARCH ARTICLE

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Detection of Chitinase 3-Like 1 in Symptomatic Primary Care Patient Faecal Samples is Not a Reliable Biomarker of Colonic Lesions

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Abstract

Background: The current gold standard non-invasive test for detecting pre-cancerous changes is the faecal immunochemical test (FIT). However, this test can lack sensitivity and specificity and testing for another biomarker may address these limitations. Chitinase 3-like 1 (CHI3L1) is emerging as a potential biomarker of inflammation-associated carcinogenic changes in epithelial cells. In this study CHI3L1 levels were analysed in patients and controls to determine their ability to improve detection of early CRC either alone or in combination with a FIT. **Methods:** CHI3L1 levels were measured by ELISA in serum and stool samples from cohorts of CRC and healthy donors as well as stool samples from a cohort of symptomatic primary care patients. Faecal haemoglobin was also analysed in the same primary care samples using FIT. **Results:** CHI3L1 levels were a good discriminatory marker of CRC, with no significant difference between levels detected in the stool and serum samples. ROC curves that determined the optimal cut-point however identified that stool samples gave higher sensitivity (83% versus 69%) and specificity (89% versus 74%) than matched serum samples. Faecal CHI3L1 levels in the primary care patients were not significantly different (p=0.193) from those detected in the healthy controls. ROC curve analysis confirmed that faecal CHI3L1 levels had limited ability to discriminate between patients who did or didn't have evidence of lesions (AUC=0.52, p=0.74). Similarly, CHI3L1 levels did not reliably identify those symptomatic primary care patients who subsequently presented with early-stage disease (polyps and adenomas) or CRC. The discriminatory power of FIT was not increased by incorporating the CHI3L1 results in this setting. **Conclusion:** There was no evidence that measurement of faecal CHI3L1 has the potential to increase diagnostic accuracy, either alone or in combination with a FIT, in symptomatic primary care patients.

Keywords: Biomarkers- Colonic lesions- Chitinase 3-like 1- Faecal haemoglobin

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Introduction

Early diagnosis of CRC improves survival. The gold standard for detection of pre-cancerous changes in the bowel is colonoscopy where adenomas can be detected and removed. Colonoscopy however is resource limited, expensive and invasive, and non-invasive tests that identify patients for clinical investigation are needed (Keenan and Frizelle, 2022). The most widely used test is the faecal immunochemical test (FIT) that quantifies faecal haemoglobin in stool samples using antibodies. Differing thresholds are used to define positivity. For example. setting the threshold at 50 µg of haemoglobin (Hb) per gram of stool provides the FIT with limited ability to detect small adenomas (Keenan et al., 2019) whereas lowering the FIT threshold to 10 µg Hb per gram of stool reportedly allows detection of nearly all CRC (Westwood et al., 2017). This increased sensitivity however comes at the expense of specificity (Fraser, 2017) and the FIT lacks the discriminatory power to be used in isolation as a diagnostic test to identify all patients with early-stage disease(MacDonald et al., 2022). Chitinase 3-like 1 (CHI3L1) is a glycoprotein that is emerging as a potential biomarker of colorectal cancer (Eldaly et al., 2020; Johansen et al., 2015). This study investigated whether measuring faecal levels of CHI3L1 in conjunction with f-Hb might improve the identification of primary care patients with early-stage colorectal disease.

Materials and Methods

Patient cohorts

A total of three cohorts were investigated in this study. These included patients with CRC, self-reporting healthy controls and primary care patients presenting to their general practitioners with bowel problems.

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CRC patients (n=23) and age-matched healthy controls (n=19) provided blood and stool samples. Primary care patients (n=77) were asked to provide a stool sample. All gave written informed consent for their samples to be screened for biomarkers (Keenan et al., 2019). Serum was separated from blood and stored at -80°C prior to testing. Stool samples were stored at −20°C after collection and then at −80°C pending analysis. Stool samples from the primary care patients were also tested for the presence of faecal haemoglobin (Keenan et al., 2019). Findings at colonoscopy were recorded for each primary care patient.

Measurement of CHI3L1 levels

A commercially-available sandwich ELISA (Human Chitinase-3-like-1 DuoSet ELISA from R&D Systems, Minneapolis, MN) was used, as per the manufacturer's instructions. The range of the assay was 31.2–2000 pg/mL. Ten percent normal goat serum was added to the recommended diluent (1% BSA in phosphate buffered saline to optimise the serum sample signals (Permain et al., 2022). Faecal extracts were prepared from weighed aliquots of thawed stool samples. Levels of CHI3L1 in serum and stool were reported as ng/ml and ng/g, respectively.

Faecal immunochemical test (FIT)

A qualitative (one-step membrane cassette) immunoassay was used for detecting f-Hb in each stool sample (Ngaio Diagnostics Ltd, Nelson, New Zealand). This assay detects human haemoglobin above 50 µg of f-Hb per g of faeces, and is shown to be specific for human haemoglobin.

Statistical analysis

CHI3L1 levels in both patients and healthy controls had non-normal distributions and therefore raw data was log transformed prior to comparisons using unpaired or paired t tests. Where multiple comparisons were performed using a patient data set, a Bonferroni adjustment was performed in which the initial p value was multiplied by the number of tests prior to evaluation of significance at the 0.05 level. Differences between categorical variables were evaluated using a chi-square test. All analysis and graphing was performed using GraphPad Prism version 9.4.0 for Windows, GraphPad Software, La Jolla, California USA.

Results

Faecal and serum levels of CHI3L1 in CRC patients and healthy controls

CHI3L1 levels were measured in matched serum and stool samples from 23 CRC patients (age 40-70) and 19 age-matched healthy individuals. Both serum and stool levels of CHI3L1 were significantly elevated ($p<0.001$) in the CRC patients relative to those of the controls. There was no significant difference between the CHI3L1 levels detected in the stool and serum samples obtained from the CRC patient cohort ($p=0.26$). ROC curves were utilised to determine the optimal CHI3L1 level for discriminating between CRC and controls. At the optimal cut-point in serum and stool (69 ng/ml and 9.04 ng/gram, respectively), stool gave higher sensitivity (83% versus 69%) and specificity (89% versus 74%) than matched serum samples (Figures 1B &1C). At this threshold, faecal CHI3L1 levels were significantly elevated in 83% (19/23) of the CRC patients.

Table 1. Stool-Associated CHI3L1 levels do not Associate with Clinical Lesions Detected in Primary Care Patients Progressed for Clinical Investigation.

Diagnosis (n)	CHI3L1 positive	CHI3L1 negative
Any Lesion (30)	5	25
CRC (2)	1	1
TA (12)	0	12
TVA (4)	2	2
SSA (1)	0	1
Polyp (11)	2	9
No Lesions (47)	12	35

CRC, colorectal cancer; TA, tubular adenoma; TVA, tubulovillous adenoma; SSA, sessile serrated adenoma

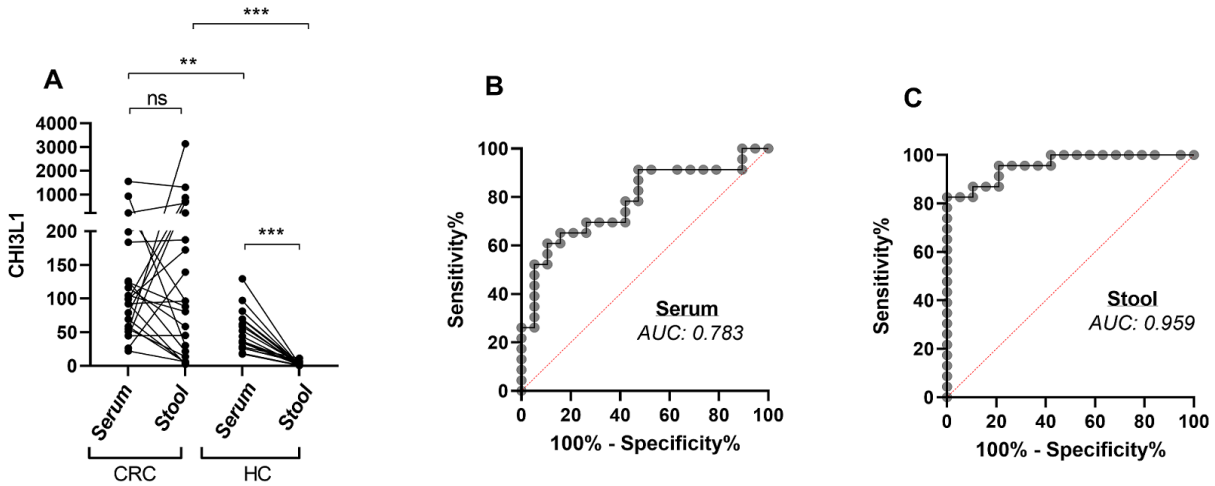

Figure 1. CHI3L1 in Serum and Faecal Samples from Age-Matched Colorectal Cancer Patients and Healthy Controls, as Determined by ELISA. (A) The levels of CHI3L1 in matched serum and stool samples. Data are shown as scatter plots and lines linked paired serum and stool samples. Asterisks indicate significance following application of a Bonferroni correction for multiple comparisons. * $p<0.05$, ** $p<0.01$, ***, $p<0.001$ respectively. (B, C) ROC curves generated using CHI3L1 levels measured in (B) serum and (C) stool samples as a discriminator of CRC. The AUC (95% CI) is indicated.

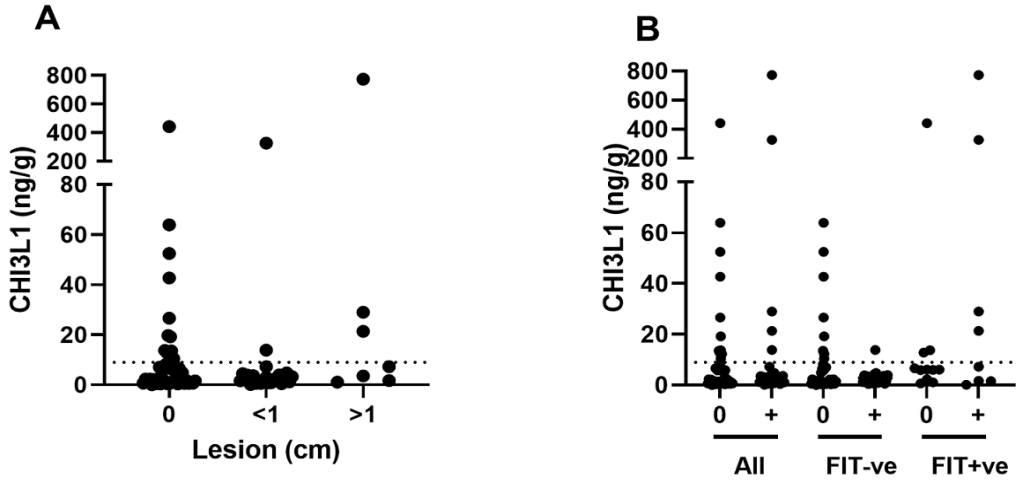

Figure 2. CHI3L1 Values in Primary Care Patients Subdivided on the Basis of Their Colonoscopy-Determined Lesion Size and FIT Results. (A). Faecal CHI3L1 levels in patients with either no lesions, lesions < than 1cm or lesions >1 cm size, respectively. (B) Faecal CHI3L1 levels in patients with no (0) or any (+) lesion, in combination with FIT negative (-ve) or FIT positive (+ve) status as determined using a faecal haemoglobin threshold of 50 µg f-Hb per g of stool.

Table 2. Stool-Associated CHI3L1 Levels Fail to Identify Primary Care Patients who would Benefit from Clinical Investigation

Diagnosis	Any lesion at colonoscopy (n=30)	FIT positive (n=8)	FIT or CHI3L1 positive (n=9)
CRC	2	2	2
TA	12	1	1
TVA	4	2	2
SSA	1	1	1
Polyp	11	2	3

CRC, colorectal cancer; TA, tubular adenoma; TVA, tubulovillous adenoma; SSA, sessile serrated adenoma

Clinical investigation of primary care patients

Samples from the cohort of primary care patients (n=77) were then analysed. At colonoscopy 30 (39%) of these patients were found to have evidence of lesions that included CRC (n=2), adenomas (17) and polyps (n=11) (Table 1). Of these 30 patients, the majority (n=23) presented with lesions that were less than 1 cm in size. Those with lesions > 1 cm in size included two patients with CRC, four with tubular adenomas (all reported as having low-grade dysplasia), and one with multiple sessile serrated adenomas. The remaining 47 patients has no evidence of lesions. Patients with diverticulosis were considered normal if no evidence of lesions was found.

Faecal CHI3L1 in primary care patients

Faecal CHI3L1 levels in the primary care patients (median=2.36, range 0.01-774.2), were not significantly different (p=0.193) from those detected in the healthy controls (median=1.88, range 0.31-11.2). ROC curve analysis confirmed that faecal CHI3L1 levels had limited ability to discriminate between patients who did or didn't have evidence of lesions (AUC=0.52, p=0.74, data not shown). Similarly, there was no clear association between CHI3L1 levels and lesion size (Figure 2A). The data was further analysed using the threshold identified in the ROC analysis of CRC and control samples. The proportion of patients with CHI3L1 levels above that threshold was higher in those who had lesions >1cm (43%, 3/7) compared to those with lesions < 1cm (9%, 2/23). However, 26% (12/47) of patients with no evidence of lesions at colonoscopy had CHI3L1 levels that were greater than the threshold (Figure 2A).

Faecal haemoglobin and CHI3L1 in primary care patients

Positivity for FIT was reported in 23% (11/47) of patients without lesions, 13% (3/23) of patients with lesions <1 cm and in 71% (5/7) of patients with lesions >1 cm, indicating that assessing f-Hb levels at this threshold lacked sensitivity and specificity. CHI3L1 levels were significantly higher in FIT positive versus FIT negative patients (p=0.0053). However, there was no significant difference (p=0.109) in the proportions of FIT negative and FIT positive patients who had CHI3L1 levels above the 9.04 ng/g threshold of the stool test (17% and 37%, respectively). Individually, a positive faecal CHI3L1 test (Table 1) and/or a positive FIT (Table 2) failed to identify colonic disease in many primary care patients. Moreover, when FIT negative patients were stratified by normal versus abnormal findings at colonoscopy, it was clear that the majority of faecal CHI3L1 positive samples (nine out of 10) were found in patients with normal colonoscopy results (Figure 2B). In contrast in FIT positive patients the majority of samples with faecal CHI3L1 above the cut-off (4/7) were found in patients with abnormal colonoscopy (Figure 2B).

Discussion

This study found elevated levels of CHI3L1 in serum samples from patients presenting with CRC, as reported elsewhere (Eldaly et al., 2020; Johansen et al., 2015). Ours is the first study however to report measurement of

CHI3L1 levels in CRC patient stool samples, where levels were found to be significantly higher than those in age-matched healthy controls. This was reflected in CHI3L1 levels being above the threshold of the test in 35% of the stool samples from patients with CRC, in marked contrast to matched serum samples from the same patients where only 17% were found to be positive. Our finding that levels of CHI3L1 in stool samples were associated with greater sensitivity and specificity with regards identifying patients with CRC when compared to age-matched serum samples supports this.

CHI3L1 is increasingly considered a potential biomarker of inflammatory bowel disease and recent studies report levels of this biomarker in patient stool have the potential to predict endoscopic activity in patients with this disease (Aomatsu et al., 2011; Buisson et al., 2016). Faecal CHI3L1 levels in 77 primary care patients progressed for colonoscopy however failed to identify those patients subsequently found to have evidence of precancerous lesions (with or without dysplasia), and only identified one patient with histological evidence of CRC. We acknowledge that our findings may, in part, reflect the relatively small sample size. However, the Johansen study likewise found no association between serum CHI3L1 levels and evidence of pre-cancerous disease in 854 of 4,469 patients (19%) subsequently found to have histological evidence of an adenoma at colonoscopy (Johansen et al., 2009). Collectively these studies suggest that measurement of CHI3L1 levels (faecal or serum) may have little diagnostic utility to identify symptomatic patients with precancerous lesions who should be referred for clinical investigation.

Assaying for the presence of faecal haemoglobin in stool samples is widely considered the gold standard test to identify symptomatic patients who should be progressed to clinical investigation. However, even at a lower threshold than the one used here, the FIT test can still fail to identify all symptomatic patients presenting with early-stage disease (D'Souza et al., 2021). Combining a second biomarker with the FIT is increasingly seen as a way to increase diagnostic accuracy when screening symptomatic primary care patients (Keenan and Frizelle, 2022), leading us to hypothesise that measuring faecal CHI3L1 in combination with detecting faecal haemoglobin would provide additional diagnostic information. This hypothesis was based on the hypothesis that increased CHI3L1 levels might identify a subset of patients presenting with colitis-associated neoplasia (Chen et al., 2011). The *CHI3L1* gene is a marker of macrophage differentiation (Rehli et al., 2003) and, in a setting of human colorectal cancer, CHI3L1 expression is significantly associated with the number of infiltrating CD68+ macrophages (Kawada et al., 2012). Instead we found that faecal CHI3L1 levels were more likely to be increased in patients with a positive FIT.

In summary, these findings suggest that faecal CHI3L1 levels would provide little (if any) gain, either alone or in combination with FIT, for preliminary identification of primary care patients presenting with early stage colorectal disease. Instead, lowering the threshold of the FIT from the 50 µg to 10 µg Hb/g faeces and using this as a stand-alone test would be more likely to have greater utility for identifying those patients who should be progressed for clinical investigation (Westwood et al., 2017).

Author Contribution Statement

Conceptualization: Keenan JI, Frizelle FA. Funding acquisition: Keenan JI, Hock BD. Methodology: Keenan JI, Hock BD. Patient recruitment: Keenan JI, Aitchison A. Data curation and lab experiments: Keenan JI, Aitchison A. Data analysis: Hock BD. Data interpretation: Keenan JI, Hock, BD. Writing - original draft: Keenan, JI. Writing - review & editing: Keenan JI, Aitchison A, Frizelle, FA, Hock BD. Approval of final manuscript: all authors..

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Ethical approval

Samples (blood and stool) were collected from a total of 23 patients diagnosed with CRC between 2012 and 2014 using standard endoscopic, histological or radiological criteria. Blood and stool samples were also collected from 19 age-matched healthy controls. All gave written informed consent for their samples to be screened for biomarkers (as approved by the Southern Health and Disability Ethics Committee (URA/12/02/005/AM03)). The primary care cohort consisted of 77 patients presenting to their general practitioners (between 2014 and 2017) with bowel problems who were referred for FIT and subsequently underwent follow up colonoscopy. Again, all gave written informed consent for their stool samples to be screened for potential biomarkers, and for possible clinical follow-up (as approved by the University of Otago Human Ethics Committee (H14/019)).

Availability of data

The data that support the findings of this study are available on request from the corresponding author.

Conflict of interest

No potential conflict of interest was reported by the authors.

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