FAECAL DNA TESTING FOR DETECTION OF COLORECTAL CARCINOMA: A PROSPECTIVE CASE CONTROL STUDY

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ABSTRACT Background: Using Faecal DNA test to detect these aberrant methylations and mutations in DNA shed from the colonic cells could help in the detection of a pre-cancerous or early stage of colorectal cancer which would improve the mortality rate. Guaiac based chemical detection of faecal occult blood (gFOBT) is one of the non-invasive screening methods but with lower sensitivity as not every colorectal cancerous lesion bleeds. Aims: To compare the sensitivity and specificity of stool DNA test against Faecal Occult Blood Test. Method: This was a prospective case-control study to compare the sensitivity and specificity of Faecal DNA test with gFOBT as to which test would make a better screening tool for colorectal cancer. Result: The sensitivity of Faecal DNA Test was found to be 86% whereas the sensitivity of gFOBT was found to be 66% and specificity for gFOBT was 86% compared to 72% for Faecal DNA test. Conclusion: Based on several studies including the present study it could be concluded that there is sufficient data to include Faecal DNA test as a viable option for CRC screening although testing stool for molecular markers is an evolving technology.

KEYWORDS Colorectal cancer (CRC), screening, Faecal DNA test/ Stool DNA test (sDNA), Guaiac based faecal occult blood (gFOBT), Sensitivity, Specificity

Introduction

Accumulation of variety genetic and epigenetic changes in the colonic epithelium is one of the main processes involved in the initiation of CRC and transformation of benign polyps to malignant tumours [1, 2]. The time period for the transformation of adenoma to high-grade dysplasia to colorectal cancer takes about ten years, and it’s a slow process. But since the patient hardly has any symptoms in the early stages, it goes unnoticed, and by the time the patient presents with symptoms, the disease has usually progressed to advanced stages. Hence the mortality can be significantly reduced by early surveillance and screening. Though colonoscopy remains the gold standard for screening CRC, this procedure is invasive, and patients show poor compliance [3]. Guaiac based chemical detection of faecal occult blood is one of the non-invasive screening methods, however, the sensitivity of faecal occult blood test for CRC and especially for colorectal adenomas is low because neoplasms may not bleed and thus cannot be detected this way [4, 5]. Eighty-five percent of colorectal cancers result from chromosomal instability, with mutations progressively accumulating in the adenomatous polyposis coli (APC) gene, the p53 tumour suppressor gene, and the K-ras oncogene [6]. The other 15% arises from a loss of genes involved in DNA-mismatch repair, manifested by microsatellite instability. [7]. Colorectal cancer may also be detected by DNA markers that are associated with disordered apoptosis [8]. Stool DNA test detects this aberrant methylation and mutation in DNA released from cells that are shed continuously from cancerous or precancerous lesions [9]. Using this principle, Faecal DNA test is being exploited as a viable non-invasive screening tool for colorectal carcinoma.
Aim & Objectives:

Primary Objective
To compare the sensitivity and specificity of stool DNA test against the Faecal Occult Blood Test.

Secondary Objectives
To establish the advantages and disadvantages of Faecal DNA testing.
To investigate if stool DNA Test (sDNA) can be used as a standard non-invasive screening test for colorectal carcinoma.
To ascertain the clinical relevance of a positive Faecal DNA test in a patient with no evidence of lesions on colonoscopy.

Material & Methods
This was a prospective case-control study to compare the sensitivity and specificity of Faecal DNA test with fFOBT in individuals at average risk for colorectal carcinoma. A total of 116 individuals who came with lower gastrointestinal symptoms attending the General Surgery Out-patient or Gastro-medicine Out-patient of Hamidia Hospital, Bhopal were subjected to colonoscopy and on the basis of colonoscopy and histopathological result the individuals were divided into case and control. For the ease of the study 50 individuals were chosen as case who had colonscopic and histopathological findings of either colorectal cancer or advanced precancerous lesions and 50 were taken as control who had either negative colonscopic findings or non-advanced adenomas of size < 0.5cm. All the patients had to undergo a guaiac-based faecal occult blood test and faecal DNA test in order to compare the sensitivity and specificity of sDNA test to fFOBT.

Inclusion criteria
• Patients who had Stage I to III colorectal cancer or advanced precancerous lesions on colonoscopy and histopathological examination.
• Patients who had known family history of colorectal cancer or a personal history of inflammatory bowel disease.
• Patients who were willing to be enrolled in the study after signing the consent form.

Exclusion criteria
• Patients with Stage IV colorectal cancer, in the advanced inoperable stage.
• Those who had already undergone surgical intervention for colon and rectal malignancy.
• Patients with iron deficiency anemia or anal fissures or haemorrhoids or diverticulosis.
• Patients unwilling or unfit to undergo colonoscopy
• Those individuals who were not willing to participate in the study.

DNA analysis was performed on all the stool samples from all subjects of the case as well as a control group. The faecal DNA panel consisted of 21 mutations: 3 in the k-ras gene, 10 in the APC gene, 8 in the p53 gene; the microsatellite instability marker BAT-26; and a marker of long DNA which is thought to reflect the disordered apoptosis of cancer cells sloughed off into the colonic lumen.

The laboratory handling of all samples was fully automated, quantitative analysis and measure of signal intensity of the labelled nucleotides were compared with that of the control DNA fragments with a known mutation. Based on the logistic regression algorithm, the values of 183 or more was considered to be positive for Faecal DNA test.

A post hoc McNemar’s test was performed using software XLSTAT version 2019.1.2 to compare the ability of the faecal DNA panel and gFOBT to identify subjects with fully specified advanced neoplasia. Statistical significance was defined as P < 0.05.

Observation & Results
Out of the 50 case subjects, 32 (64%) were diagnosed cases of CRC and remaining 18 (36%) cases were diagnosed with advanced precancerous lesions out of which 11 (61.1%) patients were diagnosed as high-grade dysplasia through histopathological examination and 7 (38.8%) patients had sessile serrated polyps of 1cm or more. Out of these 50 control subjects, 38 candidates had negative colonoscopic findings, and 12 individuals had non-advanced adenomas lesser than 0.5cm.

The sensitivity of gFOBT for CRC patients was 75% in comparison to 87.5% sensitivity of faecal DNA test for CRC patients. (Fig. 1&2). The sensitivity for proximally located tumours was 40% and for distally located tumours sensitivity was 83.3% as compared to sDNA for which the sensitivity for proximal tumours was 70% and for distally based tumours was 95.4%. (Fig. 3)

The sensitivity for advanced precancerous lesion patients was 50% as compared to the sensitivity of sDNA for these patients, which was 83.3%. (Fig. 4) The sensitivity for gFOBT for proximal lesions was 37.5% compared to sDNA which had a sensitivity
of 75% & sensitivity for gFOBT was 60% for distal lesions as compared to sDNA which showed a sensitivity of 90% for distal lesions. (Fig. 5)

The sensitivity of gFOBT for polyps 1cm to 1.9cm was 20%, for polyps of size 2.0cm to 2.9cm was 60% and for polyps of size >3cm was 62.5% in comparison to sDNA which showed a sensitivity of 60%, 80% and 100% respectively. (Fig. 6)

The sensitivity of sDNA for the 50 case-patients was found to be 86%, whereas the sensitivity of gFOBT was found to be 66%. (Fig. 7)

The overall specificity for gFOBT for the 50 control subjects was 86% compared to 72% for Faecal DNA test. (Fig. 8)

Summary of Faecal DNA v/s gFOBT Sensitivity in Case and Specificity in Control is depicted in Table 1.

**Discussion**

The overall sensitivity for a faecal DNA test or the true positives for CRC patients was found to be higher as compared to patients with advanced precancerous lesions. So the sensitivity of faecal DNA test for CRC patients was found to be 87.5% as compared to 83.3% sensitivity for patients with advanced precancerous lesions. But the difference was not significant. The value of p was >0.05, which was not significant. Thus it could be concluded that the faecal DNA test did not vary much concerning sensitivity for both CRC patients and patients with advanced precancerous lesions. Using a similar DNA mutation marker panel Ahlquist and colleagues also reported a sensitivity of 90% for cancer and 82% for advanced adenomatous polyps [10]. Tagore et al. also used an identical DNA mutation marker panel to analyse stool specimens from 52 patients with colorectal cancer and 28 patients with advanced adenomas and reported sensitivities of 64% and 57% respectively [11]. Having a non-invasive screening tool that has a high sensitivity for not only the detection of CRC but also the advanced precancerous lesions is of great benefit for the early detection of advanced lesions. Treating a precancerous lesion would increase the survival rate. In our study, the detection rates were comparable for CRC and advanced adenomas.

In the present study, a faecal DNA test was found to be more sensitive than gFOBT for detection of CRC patients. In a study by Imperiale et al. [12], they compared a panel of faecal DNA markers and gFOBT called Hemoccult II as a screening test for colorectal cancer in average-risk, asymptomatic population. It was found that DNA panel was four times more sensitive than gFOBT for invasive cancer and more than twice as sensitive for
Table 1 Summary of Faecal DNA vs gFOBT Sensitivity in Case and Specificity in Control.

<table>
<thead>
<tr>
<th>Case (N=50)</th>
<th>sDNA</th>
<th>% Sensitivity of sDNA</th>
<th>False Negatives</th>
<th>gFOBT</th>
<th>% Sensitivity of gFOBT</th>
<th>False Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC (N=32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I CRC (N=3)</td>
<td>2</td>
<td>66.6%</td>
<td>1 case</td>
<td>1 case</td>
<td>33.3%</td>
<td>2 cases</td>
</tr>
<tr>
<td>Stage II CRC (N=11)</td>
<td>11</td>
<td>100%</td>
<td>0 case</td>
<td>9 cases</td>
<td>81.8%</td>
<td>2 cases</td>
</tr>
<tr>
<td>Stage III CRC (N=18)</td>
<td>15</td>
<td>83.3%</td>
<td>3 cases</td>
<td>14 cases</td>
<td>77.7%</td>
<td>4 cases</td>
</tr>
<tr>
<td>Acc. to Location of Tumour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal Tumour (N=10)</td>
<td>7 cases</td>
<td>70%</td>
<td>3 cases</td>
<td>5 cases</td>
<td>40%</td>
<td>5 cases</td>
</tr>
<tr>
<td>Distal Tumour (N=22)</td>
<td>21</td>
<td>95.4%</td>
<td>1 case</td>
<td>19 cases</td>
<td>83.3%</td>
<td>2 cases</td>
</tr>
<tr>
<td>Advanced Precancerous Lesions (N=18)</td>
<td>15 cases</td>
<td>83.3%</td>
<td>3 cases</td>
<td>9 cases</td>
<td>50%</td>
<td>9 cases</td>
</tr>
<tr>
<td>High Grade Dysplasia (N=11)</td>
<td>10 cases</td>
<td>90.9%</td>
<td>1 case</td>
<td>6 cases</td>
<td>54.5%</td>
<td>5 cases</td>
</tr>
<tr>
<td>Sessile Serrated Polyps &gt;1cm (N=7)</td>
<td>5 cases</td>
<td>71.4%</td>
<td>2 cases</td>
<td>3 cases</td>
<td>42.8%</td>
<td>4 cases</td>
</tr>
<tr>
<td>Acc. to Location of Polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal Polyps (N=8)</td>
<td>6 cases</td>
<td>75%</td>
<td>2 cases</td>
<td>3 cases</td>
<td>37.5%</td>
<td>4 cases</td>
</tr>
<tr>
<td>Distal Polyps (N=10)</td>
<td>9 cases</td>
<td>90%</td>
<td>1 case</td>
<td>6 cases</td>
<td>60%</td>
<td>4 cases</td>
</tr>
<tr>
<td>Acc. to Size of Polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1cm-1.9cm (N=5)</td>
<td>3</td>
<td>60%</td>
<td>2 cases</td>
<td>1 case</td>
<td>20%</td>
<td>2 cases</td>
</tr>
<tr>
<td>2.0-2.9cm (N=5)</td>
<td>4</td>
<td>80%</td>
<td>1 case</td>
<td>3 cases</td>
<td>60%</td>
<td>1 case</td>
</tr>
<tr>
<td>&gt;3.0cm (N=8)</td>
<td>8</td>
<td>100%</td>
<td>0 case</td>
<td>5 case</td>
<td>62.8%</td>
<td>3 cases</td>
</tr>
<tr>
<td>Total Case (N=50)</td>
<td>43</td>
<td>86%</td>
<td>7 cases</td>
<td>33 cases</td>
<td>66%</td>
<td>17 cases</td>
</tr>
<tr>
<td>Control (N=50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sDNA True Negative</td>
<td></td>
<td>Specificity%</td>
<td>False Positive</td>
<td>gFOBT True Negative</td>
<td>Specificity%</td>
<td>False Positive</td>
</tr>
<tr>
<td>Negative Colonoscopy Finding (N=38)</td>
<td>29 cases</td>
<td>76.3%</td>
<td>9 cases</td>
<td>34 cases</td>
<td>89.4%</td>
<td>4 cases</td>
</tr>
<tr>
<td>Non-advanced Adenomas (N=12)</td>
<td>7 cases</td>
<td>58.3%</td>
<td>5 cases</td>
<td>9 cases</td>
<td>75%</td>
<td>3 cases</td>
</tr>
<tr>
<td>Total Control (N=50)</td>
<td>36</td>
<td>72%</td>
<td>14 cases</td>
<td>43 cases</td>
<td>86%</td>
<td>7 cases</td>
</tr>
</tbody>
</table>
adennomas containing high-grade dysplasia.

The sensitivity of gFOBT was much less as compared to sDNA test for advanced precancerous lesions (50% vs 83.3%). The statistical difference was significant (<0.05). A faecal DNA test showed much higher detection rates for advanced precancerous lesions. Detection at this stage would help in tackling the disease at a curable stage before it manifests as cancer. Therefore Faecal DNA test would prove to be a useful screening tool to detect patients at a much earlier stage of the disease, especially knowing that the adenoma-carcinoma sequence takes several years to progress. Nipping the disease at its precancerous stage would lessen the morbidity and mortality to a very great extent.

For the proximal polyps, the detection rate was better for faecal DNA test than gFOBT (75% vs 37.5%), and for distal polyps also stool DNA tested better than gFOBT (90% vs 60%). In both cases, the statistical difference was significant. Also, the stool DNA test could identify both the proximal as well as distal lesions and the statistical difference was not significant (>0.05). Thus multtarget stool DNA test again proved to be an essential armamentarium among the screening tests that can detect polyps even located proximally. A study performed by Ahlquist and colleagues [9] showed that faecal DNA test could detect 55% of adenomas >1cm in proximal vs 53% in the distal colorectum. The detection rates were comparable between proximal and distal adenomas.

For polyps of size 1.0cm to 1.9 cm the sensitivity of stool DNA test was far more as compared to gFOBT (60% vs 20%) and the statistical difference was significant (<0.05). Ahlquist and his colleagues found that neoplasm size had a significant effect on detection rates [9].

The specificity of gFOBT in this study was found to be greater than the stool DNA test (72% vs 86%). This can probably be explained by the assumption that using a multtarget faecal DNA test which uses at least 21 point mutation markers tends to detect mutation markers in benign polyposis too that may not necessarily be associated with its transformation into carcinoma. Therefore using multiple mutation markers does increase the sensitivity but may tend to compromise the specificity to some extent and to improve the specificity of the stool DNA test the mutation marker panel used should be refined further to be specific to denote the dysplastic changes rather than the benign changes. Also, these hyperplastic polyps don’t usually bleed, so detection of trace haemoglobin by gFOBT is not possible and therefore gFOBT test more negative for these polyps giving it more specificity over faecal DNA test. A similar finding was seen in a study by Imperiale et al. [12] where the DNA tests had twice as many abnormal results as FIT, with a higher rate of false-positive results which would mean that more colonoscopies would be required to further evaluate the patient which would lead to increase in costs and risk of invasive screening.

One of our objectives was to understand the advantages and limitations of the faecal DNA test. From what we have come to understand from all this endeavor is that faecal DNA test as compared to gFOBT is not affected by the proximal location of tumours and its superior sensitivity over gFOBT for detection of advanced adenomas with the most considerable risk of progression it may be a good candidate for interval testing after initial colonoscopy. Another advantage was the lack of need for purging or dietary changes.

Another advantage of using DNA as the analyte is that a marker panel can be expanded or refined as knowledge about tumour biology evolves.

The technical difficulties regarding faecal DNA test may involve the burden of large volume stool collection and shipping for the patients undergoing screening. [13]

A combined use of sDNA test along with FOBT would improve the sensitivity as was seen in a retrospective analysis of stool samples from patients with CRC and donor controls combined results from a standard gFOBT and a panel DNA markers (APC, BAT-26 and L-DNA) which resulted in combined sensitivity for cancer of 93% and specificity of 89% [14].

One of the objectives which merit further evaluation is the clinical significance of faecal DNA positive results in patients with negative colonoscopy results. In this study, these results were taken as false positive. But it is still debatable whether it is truly false positive or does it indicate the risk of development of adenomas in the future. To find out what it means we would require longitudinal follow up of such patients and these patients would have to undergo interval colonoscopic evaluation.

**Limitations of the study**

The study was designed to compare two non-invasive screening methods, and there were too few cancers and advanced adenomas with high-grade dysplasia to provide a narrow confidence interval for the estimated sensitivity of either test. No inference could be drawn about the appropriate interval for screening with Faecal DNA test mainly if the patient tested negative.

The significance of positive faecal DNA test in patients with negative colonoscopic findings remains uncertain. If it should be considered as false positive or should it be considered as a forewarning that the patient may develop CRC at a later stage of life and that what should be the time interval for the screening of such patients. This was beyond the scope of this study as it requires a larger sample size and a long term follow up of such patients. The DNA mutation markers were not analysed individually. The sensitivities of each DNA marker could not be correlated to the cancer lesions. Neither the frequency of which mutation marker appeared the most in the lesion and at what

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>DNA</td>
<td>Deoxyribo Nucleic Acid</td>
</tr>
<tr>
<td>gFOBT</td>
<td>Guiac based Faecal Occult Blood Test</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal Cancer</td>
</tr>
<tr>
<td>sDNA</td>
<td>Stool DNA</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous Polyposis Coli</td>
</tr>
<tr>
<td>K-ras</td>
<td>Kirsten Rat Sarcoma</td>
</tr>
</tbody>
</table>

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stage of the disease could not be analysed as the results for faecal DNA test were considered positive based on logistic regression algorithm where a result of 183 or more was considered positive.

**Conclusion**

Based on the several studies including the present study it can be concluded that there is sufficient data to include Faecal DNA test as a viable option for CRC screening although testing stool for molecular markers is an evolving technology. Despite the current challenges, faecal DNA testing offers a very promising future in the detection of colorectal cancer. Although much work remains to be done in molecular testing, the fact remains that it presents a strong possibility of its worthiness which may lead to the identification of new diagnostic markers, prognostic indicators and chemotherapeutic agents. The idea that the mutations that cause cancer can be used to identify early lesions is an attractive idea from many standpoints. Molecular testing is still in its infancy stage but carries a promise. Ongoing scientific work in this area may lead to the identification of new diagnostic markers, prognostic indicators and chemotherapeutic agents.

**Funding**

There was no financial support from any organization.

**Ethics committee approval**

I hereby declare that this article has full compliance with consent committee. The approval was taken before submission of the manuscript. Patient informed consent Details relating to individual participants included in the manuscript have received all participant’s consent.

**Conflict of interest**

There are no conflicts of interest to declare by any of the authors of this study.

**References**


