Editorial

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Methylated BCAT1/IKZF1 DNA: a breakthrough in colorectal cancer diagnosis?

Abhinav Bhattarai, BSc[a], Sangam Shah, MBBS[a,*], Priyatam Khadka, MSc[d], Hashem Abu Serhan, MD[h], Ranjit Sah, MD[b,e,f], Sanjit Sah, MD[c,g]

Dear Editor,

Colorectal cancer (CRC) is one of the most common cancers in the world and the second-leading cause of cancer-related deaths. There have been 52 580 deaths estimated in the United States in 2022, and almost 1 million deaths were recorded. Remarkably, CRC cases in developed nations have been decreasing in recent years as accomplished by large-scale screening, timely diagnosis, and treatment[1]. On the other hand, low- and low-middle-income countries (LLMICs) are reporting increased incidences of CRC and associated deaths, sadly due to lack of screening and late diagnosis. Developed nations have raised their standards and practices in CRC diagnosis with the advancement of sophisticated molecular genetic techniques; nevertheless, LLMICs have been relying on conventional carcinoembryonic antigen (CEA), histological and radiological findings for both diagnostic and prognostic markers. The pitfall of relying on plasma CEA levels in establishing CRC diagnosis is its aberrant false-positive elevation and inadequacy in detecting most potentially curable CRC recurrences. Furthermore, since the cut-offs of CEA vary according to laboratories, oncologists do face confusion in interpreting the results, which makes the need for a sensitive and reliable diagnostic tool evident.

Undeniably, molecular genetic testing has benefitted CRC management through precise screening, timely diagnosis, identifying risk, monitoring, and foreseeing treatment outcomes by means of analyzing the presence of specific genetic alterations related to CRC. Novel approaches to the detection of circulating tumor DNA (ctDNA) in CRC are being recently emphasized. ctDNAs are released from the tumor cells into the bloodstream and their detection has been linked to tumor diagnosis, progression, recurrence, and even recovery[2,3]. In the case of CRC, research has put forward impressive diagnostic performance using a PCR assay for the detection of epigenetic markers in CRC. Hypermethylated DNA of *BCAT1/IKZF1* genes has been found in over 95% of CRCs. A study has found that 95% of CRC cases have hypermethylated regions in both of these genes, and 99% of cases have that in any one of the two genes[4,5]. Detecting the hypermethylated *BCAT1/IKZF1* has shown higher sensitivity and specificity in detecting CRC recurrence than plasma CEA estimation. The mechanism underlies the fact that ctDNAs decline following tumor resection and elevate as metastases develop.

Symonds *et al.*[2] compared the sensitivity and specificity of CEA and methylated *BCAT1/IKZF1* genes in CRC recurrence. The investigators found a lower sensitivity and specificity of the conventional CEA in contrast to quantitative ctDNA analysis (sensitivity, specificity: 31.9%, 96.4% and 66.0%, 97.9%, respectively). Also, the odds of developing recurrence were significantly higher with a positive quantitative *BCAT1/IKZF1* methylated ctDNA (12.7 for CEA and 89.3 for ctDNA). *BCAT1/IKZF1* methylation testing has further been indicated as an independent prognostic indicator in CRC. Pedersen *et al.*[6] found significantly higher 3-year recurrence-free survival (RFS) in patients without detectable methylation.

Testing for *BCAT1/IKZF1* hypermethylated genes is a moderately easy-to-perform assay and is in compliance with the patient's comfort and well-being since it follows the least invasive procedure, phlebotomy, and blood samples are obtained. The procedure is often referred to as liquid biopsy in a bundle of literature[7]. DNAs are extracted and PCR assay is then employed, targeting the methylated regions near to the promoter sequence of these genes. A real-time PCR is carried out in a thermal cycler with 50 cycles, and the result is expressed as positive if methylated *BCAT1/IKZF1* is detected in any of the replicates[5].

This two-gene PCR assay is commercially available as COLVERA, which is supplied with validated primers and reagents[8]. The utilization of COLVERA testing for CRC diagnosis and prognostication has been rising in the United States. This molecular testing holds the potential to uplift the standards in CRC diagnosis and patient management via its highly sensitive and CRC-specific performance in detecting methylated *BCAT1/IKZF1* ctDNA. Winter *et al.*[9] compared the methylation analysis in different tumors and found the assay to be sensitive and

[a]Institute of Medicine, Tribhuvan University, Kathmandu, [b]Department of Microbiology, Tribhuvan University Teaching Hospital, Institute of Medicine, Kathmandu, [c]S.R. Sanjeevani Hospital, Siraha, Nepal, [d]Upstate Medical University, Syracuse, New York, USA, [e]Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, [f]Datta Meghe Institute of Higher Education and Research, [g]Global Consortium for Public Health and Research, Datta Meghe Institute of Higher Education and Research, Jawaharlal Nehru Medical College, Wardha, India and [h]Hamad Medical Corporation, Doha, Qatar

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*Corresponding author. Address: Institute of Medicine, Tribhuvan University, Kathmandu 44600, Nepal. Tel.: +977 9844414083.
E-mail: sangam.shah.1997@gmail.com (S. Shah).

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specific only to CRC, while that for breast and prostate cancers were statistically insignificant. These evidences further advocate prospects of the testing to CRC-specific screening. However, the appropriateness on its worldwide routine implementation is questionable until strong validation studies are performed. There lie few pitfalls on standardization on specific risk cut-offs since the methylation profiles can vary based on age, sex, and the biopsy sampling. Also, variations in the methylation levels were reported between biopsies sampled from luminal surfaces and the central bulk of cancer.

Turning to another flip, the epigenetic landscape in cancer is intricate, and methylation of multiple genes along with other epigenetic alternations, contributes to the disease[10]. Furthermore, correlation with mutations on the mismatch repair genes is highly important for its stringent association with CRC[11]. Therefore, it is essential to consider the broader genetic and epigenetic context when validating screening tests. The field of epigenetic markers in cancer is continually evolving, and the relevance and significance of *BCAT1* and *IKZF1* methylation in CRC may change as more research is conducted. There indeed lies the requirement for further research and conclusions so as to implement these methylation markers into routine CRC screening.

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