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REVIEW ARTICLE

Methylated DNA as a Diagnostic Biomarker of Esophageal Cancer in the Indian Population and its Association with Risk Factors

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ABSTRACT
Esophageal cancer is an increasingly significant health issue, particularly in underdeveloped nations where its prevalence is rising. In India, esophageal cancer ranks among the top five cancers, highlighting the importance of understanding its molecular mechanisms for early detection. DNA methylation, an epigenetic modification, occupies a vital role in the development of many cancers, including esophageal cancer, and has emerged as a potential biomarker. This process, which involves the addition of a methyl group to DNA, can alter expression of genes without changing the DNA sequence, leading to the activation or suppression of genes involved in cancer initiation and progression. In the Indian population, where factors such as tobacco use, alcohol consumption, and specific dietary practices contribute to the increased frequency of esophageal cancer, studying the methylation patterns of DNA may provide key insights into the disease's origins and development. This review aims to investigate the potential of methylated DNA as a diagnostic biomarker for esophageal cancer in India and explore its links to disease progression and risk factors, paving the way for more personalized and effective cancer management strategies for this high-risk population.
Keywords: *Biomarker; DNA methylation; Esophageal cancer; Risk factors.*

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INTRODUCTION

Cancer, a notorious disease, has plagued humanity for decades, exacting a devastating toll on individuals and posing a significant public health challenge worldwide. In developing countries, it stands as the leading cause of disease-related deaths [1]. Esophageal cancer (EC) ranks as one of the most prevalent cancers worldwide, with 0.5 million new cases and 0.4 million deaths documented in 2022 [2]. India is particularly affected, with EC ranking as the fifth most common cancer, with approximately 70 thousand cases and 66 thousand deaths (Fig 1). DNA methylation is essential for the regulation of gene expression, influencing differential gene expression, chromosomal stability, and transcription [3, 4]. The process entails the incorporation of a methyl group to the fifth carbon of cytosine, forming 5-methylcytosine. Abnormal methylation patterns, such as hypermethylation, which silences tumor suppressor genes, and hypomethylation, which activates oncogenes, are frequently observed in cancer. These aberrations, particularly hypermethylation of genes that is involved in DNA repair, cell cycle regulation, apoptosis, and cell adhesion, are common in many cancers [5]. In recent years, methylated DNA has emerged as a valuable biomarker in cancer biology, spurring extensive research. Research has indicated that DNA methylation is a stable and heritable modification, specific to tissues or cancer types, easily detectable, and present in various body fluids [5, 6]. Additionally, epigenetic modifications such as DNA methylation, occur more frequently in the initial stages of tumorigenesis compared to genetic alterations, making it an ideal non-invasive biomarker for early cancer detection [7]. For instance, in colorectal cancer (CRC), hypermethylation of tumor suppressor genes like *NDRG4* and *BMP3* has been approved by the U.S. Food and Drug Administration for the detection of CRC and advanced precancerous lesions [8].

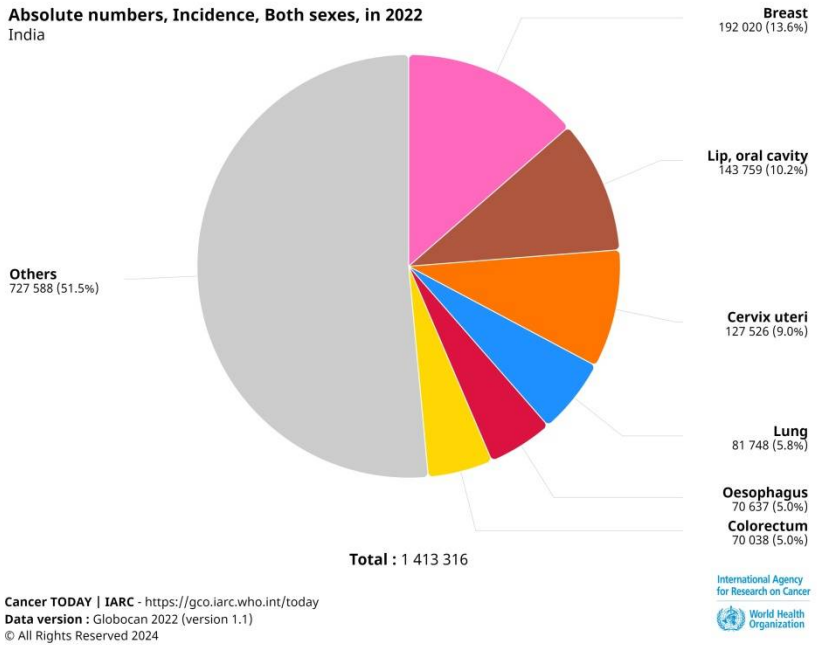

Fig 1 The top five cancers in India. Source: Global Cancer Observatory: Cancer Today, 2022 (IARC-WHO) https://gco.iarc.who.int/media/globocan/factsheets/populations/356-india-fact-sheet.pdf.

This review's primary goal is to explore the potential of DNA methylation as a diagnostic biomarker for EC in the Indian population and to analyze how it relates to risk variables, offering a pathway toward more personalized and effective approaches to cancer management in this high-risk group.

DNA METHYLATION IN CANCER

DNA methylation is one of the epigenetic modifications where methyl-group is added covalently to the fifth carbon of the cytosine nucleotide by DNA methyltransferases to form the 5-methylcytosine. It plays a crucial role in mammalian development and normal functions by facilitating the process of genomic imprinting, differential gene expression, inactivation of X-chromosome in females, and suppression of repetitive element transcription and transposons [9]. In cancer cells, aberrant methylation patterns have been reported in several studies and have become a common phenomenon in cancers. Significant promoter hypermethylation (more than normal) and hypomethylation (less than normal) has contributed to malignant transformations where hypermethylation results in transcriptional repression and reduced expression of genes while hypomethylation leads to chromosomal instability, aneuploidy, and overexpression of oncogenes [10]. In cancer, aberrant methylation patterns have been observed in genes that are crucial in cell cycle, DNA repair, tumor suppression, cell adhesion and other genes implicated in malignant transformations. *p16, DAPK*, *CDH1*, *GSTP1*, and *MGMT* are found to be hypermethylated in cancers of the mouth and stomach [4, 11-13]. These genes are implicated in cell cycle, apoptosis, cell adhesion, and DNA repair. Genes such as *TAC1, ZNF569, PAX1, ZNF582, and FRP1* were frequently methylated in EC, indicating the role of these genes in malignant transformation [14]. In colorectal cancer, methylation of *SEPT9* and the combination of *NDRG4* and *BMP3* has been approved as a methylation-based biomarker for diagnosis [15].

DESCRIPTIVE EPIDEMIOLOGY OF EC

EC has the highest incidence in Asia, with an age-standardized rate (ASR) of 6.2, accounting for 74.9% of global cases and a mortality rate of 74% [2]. Among males, Asia reports the highest number of cases (74.9%), followed by Europe (11.1%). For females, after Asia (74.9%), Africa has the next highest incidence at 9.1%. Mortality rates in males are also highest in Asia (73.9%), with Europe (11.3%) following. Among females, mortality is similarly highest in Asia (74.3%), with Africa (9.9%) ranking second. India ranks second globally, after China, contributing 13.8% of worldwide EC cases with an ASR of 5 (Table 1). The prevalence and death rate of EC are higher in males compared to females in India. According to the National Cancer Registry Programme, the highest incidence of EC is observed in the northeastern states, followed by northern and central regions of India [16].

Table 1: Epidemiological data of EC (Countries)

Countries	Number		ASR* Incidence	ASR* Mortality
	Males	***Females***		
China	167472	56540	8.3	6.7
India	45608	25029	5	4.7
Bangladesh	16578	8654	16	15.3
Japan	15658	4268	4.8	2.6
United States of America	14757	3990	2.8	2.4
Brazil	8812	2173	3.7	3.5
United Kingdom	6504	3097	5.9	4.9
Russian Federation	7107	2238	3.5	3.1
Pakistan	4776	4513	5.5	5.2
Germany	5621	1689	3.5	2.9

*Age standardized rate per 100000 individuals. Source: Global Cancer Observatory: Cancer Today, 2022 (IARC-WHO) https://rb.gy/73p4ai.

RISK FACTORS ASSOCIATED WITH EC IN INDIA

Several risk factors have been found and linked to EC, including dietary habits, consumption of betel quid, tobacco, alcohol, and gastroesophageal reflux disease [17, 18]. In the northern region, particularly Kashmir, specific dietary practices include the consumption of salted tea (Noon Chai), sun-dried (Hokh Gaard) and smoked fish (Phari), and leafy vegetables rich in amines that have been linked to an elevated risk of EC [19]. Among these, hot salted tea is the most significant dietary risk factor. The International Agency for Research on Cancer (IARC) has reported an association between hot beverage consumption and EC risk [20]. Several studies from Kashmir specifically link the consumption of hot salted tea to a heightened risk of EC development [21]. In the northeastern region, the extensive use of betel quid (with or without tobacco), smoke and smokeless tobacco, and drinking alcohol are important EC risk factors [22, 23]. A case-control study revealed that the risk of developing EC increases two to seven times when connected to the use of betel quid and tobacco [24]. Hospital-based cancer registry data also suggest an elevated risk of EC when tobacco is consumed through forms like bidis, hookahs, and cigarettes [25, 26]. Furthermore, it has been demonstrated that drinking doubles the risk of EC in a dose-dependent manner, according to another case study [27]. However, alcohol and EC have a complicated association; some research suggests a J-shaped curve rather than a linear relationship, posing challenges for healthcare providers in designing effective health policies [28, 29].

METHYLATED DNA AS DIAGNOSTIC MARKER IN INDIAN EC PATIENTS

In many cancers, methylation has been observed in genes that are crucial in DNA repair, tumor suppression, and cell cycle [30]. Therefore, DNA methylation holds a promising potential as a biomarker for the early diagnosis of various cancers. For instance, *SEPT9, GSTP1,* and *SHOX2* methylation has been shown to have high specificity and accuracy in detecting and discriminating cancers from normal in colorectal, prostate, and lung cancer, respectively [31-33]. The potential methylated-DNA biomarkers of EC with their molecular functions are shown in Fig 2. Promoter hypermethylation of *CDKN2A* was detected in 42.8% of EC samples from individuals with a history of raw betel nut chewing, compared to normal peripheral blood samples [34]. Salam et al. reported a 52% incidence of *p16* promoter methylation in EC samples, with 72% of these cases showing a correlation with protein downregulation as the pathological grade increased [35]. Another study found that 81% of EC samples exhibited *p16* promoter methylation [36]. The frequency, amount, and duration of tobacco and betel quid chewing significantly increased the risk of EC development, with hypermethylation primarily observed in individuals who chewed betel quid, used tobacco, or smoked. Similarly, methylation of the *p16* promoter was discovered in 81% of EC cases from NE, distributed across all clinical stages [37]. A significant association ($p < 0.05$) was found in the cases having betel quid/tobacco chewing habit. Therefore, *p16* methylation is a frequent event in EC and could potentially serve as a diagnostic marker in high-risk groups. However, another study detected *p16* methylation in only 37.5% of EC samples from the same region [38]. In addition to *p16*, hypermethylation of *DAPK*, *GSTP1*, and *BRCA1* was observed in 61.6%, 58.92%, and 20.53% of EC cases, respectively [38]. EC development was far more likely to occur when the methylation of these four genes was linked to with tobacco use, both smoked and smokeless. Table 2 shows the summary of the genes under review and its implication in EC. Methylation of *MGMT* as a significant diagnostic biomarker in high-risk groups has been supported by numerous studies. Das et al. reported *MGMT* hypermethylation in 70% of EC cases compared to normal controls, with a fivefold

increased risk of EC development in tobacco chewers and a threefold increase in smokers compared to non-chewers and non-smokers [39]. Rehman et al. found *MGMT* methylation in 56.25% of EC samples, noting that it increased with increasing pathological grades and correlated with poorer survival, particularly in cases involving smoking and alcohol use [40]. Additionally, non-CpG methylation of *MGMT* was observed in 84% of EC samples and was strongly linked to an increased risk of EC development [41]. The *hMLH1* gene has been demonstrated to undergo methylation early in the development of EC. A study reported *hMLH1* hypermethylation in 53.8% of premalignant tissues and 63.5% of EC tissues, with methylation levels increasing as the disease advanced [42]. Additionally, Gastroesophageal reflux disease (GERD) patients exhibited a high rate of *hMLH1* hypermethylation (88.8%), highlighting the gene's potential as an early diagnostic marker for EC. Another study found promoter hypermethylation of *hMLH1* in 56% of EC cases, compared to just 15% in normal tissues, with a significant association between hypermethylation and increased risk of EC development [43].

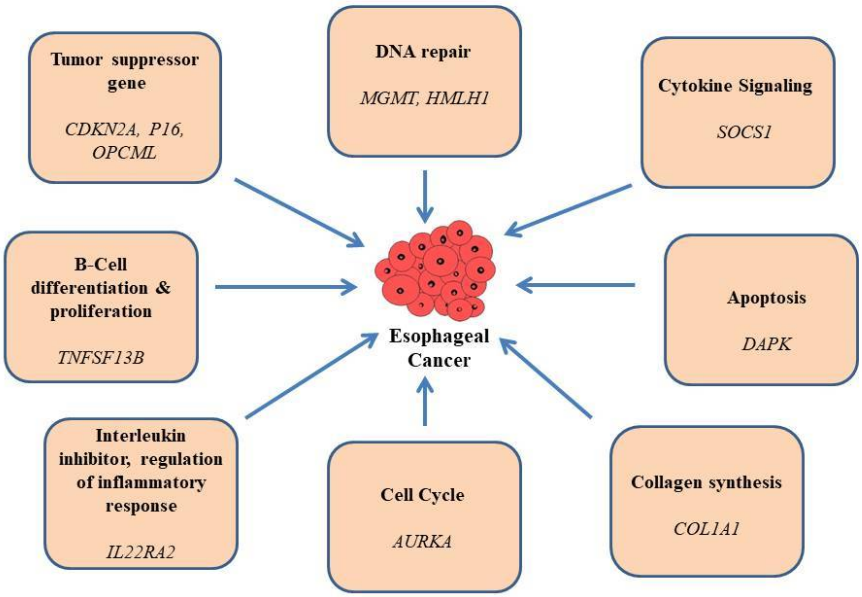

Fig 2 The molecular functions of the potential methylated gene biomarkers of EC

Hypermethylation of the *SOCS-1* gene was observed in 45% of EC tissues, while no methylation was detected in adjacent normal tissues [44]. There was a substantial correlation between this methylation and advanced cancer stages, with higher levels of methylation seen in more aggressive tumor grades, underscoring the role of *SOCS-1* methylation in EC progression. Additionally, a large proportion of cases with promoter methylation had a history of hot-salted tea consumption, suggesting a possible environmental link.

Table 2: Potential DNA methylation biomarker for the diagnosis of EC in India

Sample	Genes markers	Inference	Citations
Tissue & blood	CDKN2A	CDKN2A methylation occurred early in the pathogenesis of EC. Raw betel nut alone could induce CDKN2A methylation.	[34]
Tissue	p16	Parallel increase of promoter methylation with increasing pathological grade.	[35]
Blood	p16	Maximum hypermethylation was observed in cases with betel nut chewing, tobacco chewing and smoking. Association of p16 methylation with betel nut chewing, tobacco chewing and smoking increased	[36]

		the risk of EC by 6, 7, and 5 times respectively.	
Blood	p16	Aberrant methylation was seen in all clinical stages of the cancer. Betel quid (91%) and tobacco chewing (89%) habit was common in the EC cases with a significant p value <0.05	[37]
Tissue	p16, DAPK, GSTP1, BRCA1	Promoter methylation of the four genes when associated with both smokeless and smoked forms of tobacco consumption had the highest risk of ESCC. Cases with high methylation index had strong association with smoke and smokeless tobacco with increased risk of 5 and 6 times respectively.	[38]
Tissue	MGMT	Methylation increased parallelly with increasing pathological grade of EC Cases with smoking and alcoholism were associated with poorer survival.	[40]
Blood	MGMT	Methylation increased the risk of EC development by five times and three times when associated with tobacco chewing and smoking, respectively.	[39]
Tissue	MGMT	The chance of developing EC was substantially connected with methylation.	[41]
Tissue	hMLHl	The methylation is an early event occurring in premalignant tissue and EC. GERD cases showed high methylation. The methylation increased in parallel with disease progression.	[42]
Tissue	hMLH1	Methylation had a strong correlation with the risk of EC development.	[43]
Tissue	SOCS-1	Methylation had a strong correlation with advanced stage of cancer. Increase in methylation level was seen with disease progression. Most cases had hot salted tea consumption habit	[44]
Tissue	AURKA	Gradual increase in methylation from premalignant to EC tissues. Tobacco consumption and methylation increased the risk of EC development.	[45]
Tissue	TAC3, TNFSF13B, SERPINA4, COL1A1, and IL22RA2	The panel methylation was associated with cancer progression and were identified as potential early diagnostic marker	[46]
Tissue	OPCML	Methylation associated with high grade EC	[47]

A study observed a gradual increase in *AURKA* gene methylation from normal tissues to precancerous tissues, with the highest levels of methylation found in EC and the lowest in normal tissues [45]. The risk of developing EC was significantly heightened when tobacco use and *AURKA* promoter methylation were analyzed together as contributing factors. Study of whole genome methylation utilizing the Infinium 450K Array in EC identified 16,135 hypermethylated and 16,105 hypomethylated CpG sites. Through integrome analysis, 23 genes that are strongly linked to cancer progression were highlighted. Further evaluation using the Methylation Efficiency Index identified five top candidate genes—*TAC3, TNFSF13B, SERPINA4, COL1A1*, and *IL22RA2*—with potential as diagnostic biomarkers in EC [46]. Furthermore, the polymerase chain reaction test showed eight TSGs with significant promoter methylation differences [47]. Of these, *NEUROG1*, *OPCML, TERT*, and *WT1* were hypermethylated, while *CDH1, SCGB3A1, THBS1*, and *VEGFA* were hypomethylated. Notably, *OPCML* exhibited the highest promoter methylation, correlating with high-grade EC.

CHALLENGES AND FUTURE IMPLICATIONS

EC is an increasingly concerning public health issue, particularly in India, where lifestyle and dietary habits contribute significantly to its prevalence. Given the aggressive nature of EC and the typically late-stage diagnosis, early detection strategies are crucial. One promising avenue is the use of DNA methylation as a biomarker, offering a non-invasive and potentially highly sensitive method for early detection [48]. DNA methylation is a stable modification and detectable in bodily fluids such as saliva and blood as well [49]. This makes it a useful tool for non-invasive screening. This could prove highly beneficial for countries such as India as access to healthcare is frequently restricted and intrusive diagnostic techniques might not be practical for mass screening. Screening of high-risk groups through this method could revolutionize EC identification and greatly increase survival rates through early care. DNA methylation-based biomarkers also come with a few setbacks. One challenge is the standardization of the method. Different studies report varying percentages of methylation frequencies for the same genes. This could most likely arise due to variations in sampling sources, detection techniques, and also the population demographics [50]. This setback calls for the necessity of extensive multi-center research, to confirm these results and create consistent diagnostic standards. Another challenge is the mechanisms underlying methylation changes with risk factors. Though aberrant methylations are associated with carcinogenesis, it is still unclear as to how these risk factors contribute to methylation changes [51]. To map the exact mechanisms connecting environmental exposures to certain methylation patterns, more mechanistic research is required. Future directions should involve cooperation amongst researchers, physicians, and legislators to incorporate DNA methylation biomarkers into clinical practice. This is crucial in high-risk areas with high incidence such as NE India and Kashmir which need affordable, dependable, and widely available methylation-based screening methods for EC. This also presents an opportunity to integrate biomarker screening with public health initiatives to address and combat the use of tobacco and betel quid and encourage dietary improvements.

CONCLUSION

In conclusion, methylated DNA as a biomarker in EC diagnosis will prove to be a significant advancement in India's efforts to identify and prevent cancer. Though developing the biomarker is challenging, the challenge will prove to be worthwhile due to the advantages of enabling early detection, non-invasiveness, and better patient outcomes. Improving the knowledge of the methylation patterns associated with EC and risk factors unique to India will help to identify EC at the early stage, improve treatment outcomes, and eventually reduce the burden of EC.

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