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The Association of CADM1, MAL and PAX1 Methylation with Cervical Cancer: A Meta-Analysis

Li Y*

Department of Medicine, China

***Corresponding author:**

Yan Li,

Department of Medicine, China

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1. Abstract

DNA methylation is the main epigenetic event for gene silencing and is associated with carcinogenesis. In this meta-analysis, we evaluated the association between the methylation of the promoter regions of CADM1, MAL and PAX1 genes and the risk of cervical cancer development and progression. Overall, 13 eligible studies were identified assessing the associations of promoter methylation status of aforementioned genes with low- and high-grade squamous intraepithelial lesions (LSIL and HSIL) and cervical cancer development. Promoter methylation frequencies were shown to be significantly higher in LSIL and HSIL cervical cancer cases as compared to control specimens for CADM1, MAL and PAX1 genes. A moderate association was found between the promoter methylation, Promoter methylation could be considered as a non-invasive biomarker for early cervical lesions, making them highly promising targets for a personalized therapeutic approach.

2. Introduction

Cervical cancer (CC), a common gynecological malignancy, according to the GLOBOCAN 2018 database that showed that CC was the fourth leading cause of cancer-related deaths and cased approximately 570,000 new cases and over 311,000 deaths worldwide [1]. The development of CC is characterized as a progressive process from normal epithelium to squamous intraepithelial lesion (SIL) and eventually to invasive carcinoma. SIL, a precursor lesion of CC, consists of low-grade SIL (LSIL) and high-grade SIL (HSIL)[2-3] Cervical intraepithelial neoplasia (CIN) is a premalignant lesion of CC, histologically divided as CIN1, CIN2, and CIN3 [4].The diagnosis of LSIL including productive human papillomavirus (HPV) infection, CIN1 and mild dysplasia, while the category of HSIL including CIN 2 or 3, moderate and extensive dysplasia and carcinoma in situ (CIS) on the basis of the categorization of the 2001 Bethesda System [5]. Invasive cancer development from precancerous lesions requires additional genetic and epigenetic alterations [6], and DNA methylation, considered as an important epigenetic mechanism for gene silencing, tends to accumulate with disease severity [7]. Significantly, many of the inactivated genes are tumor suppressor genes and the inhibition of these genes expression by methylation is involved in cancer initiation, development, and progression [8]. Epigenetic alterations, such as gene or DNA methylation, have been proposed as novel biomarkers for the cervical cancer detection. For discovering and developing new biomarkers for cervical cancer detection is a matter of great urgency in the clinic, we conducted a meta-analysis study to evaluate the hypermethylation status of some genes (CADM1, MAL and PAX1) involved in cervical cancer development, to assess the correlation between genes hypermethylation, and to identify genes with the major impact on the onset of cervical cancer.

3. Materials & Methods

3.1. Search Strategy

In order to identify potentially eligible trials, a systematic search was performed in the electronic databases, including PubMed, Web of Science, EMBASE for available studies. The search was performed by using following keywords and Mesh terms: “CADM1and/or MAL and/or PAX1 methylation” and “cervical cancer”

or “cervical neoplasia” or “cervical tumor” or “cervical carcinoma”. The latest search in this study was updated as of May 2023 without language restriction.

3.2. Inclusion & Exclusion Criteria

Inclusion criteria: studies assessing the association of gene methylation genes (CADM1, MAL and PAX1) with CC; detailed information about the frequency of the selected gene methylation for both the cancer group and the normal control group on human samples was reported; exclusion criteria: reviews, comments, letters, conference, case reports or animal trials; insufficient usable data reported and unpublished studies.

3.3. Data Extraction

The authors extracted data from the selected publications according to the inclusion and exclusion criteria by using the following key information: the first author, publication year, methods used in the methylation methods used in the methylation, detailed positive number of cancer cases and controls.

3.4. Statistical Methods

Review Manager Software (Version 5.3, The Cochrane Collaboration, Copenhagen, Denmark) was performed for statistical analyses. The results were calculated using pooled odds ratios (ORs) for the categorical variables with 95% CI. Forest plots of the ORs were used to present the association between gene methylation expression and CC. The heterogeneity between the studies was evaluated by Q-test and I2 tests. Fixed-effect models were performed to evaluate the ORs when evidence showed no significant heterogeneity ($p \geq 0.1$), or else, random-effect models were chosen. Funnel plots and Begg’s tests were used to assess the potential publication bias. The overall effect was represented by p-value and statistical significance was defined as the two-side $p < 0.05$.

4. Results

4.1. Study Characteristics

We searched several international databases; 349 articles were identified using our search strategy. After we reviewed all the article titles, abstracts and full text, a total of 13 studies [9-21] which met the inclusion criteria were included. (Figure 1) demonstrates the details of selection process. The studies were published from 2008 to 2016 in English, with all of 863 CC sample sizes enrolled and 865 control sample (Table 1).

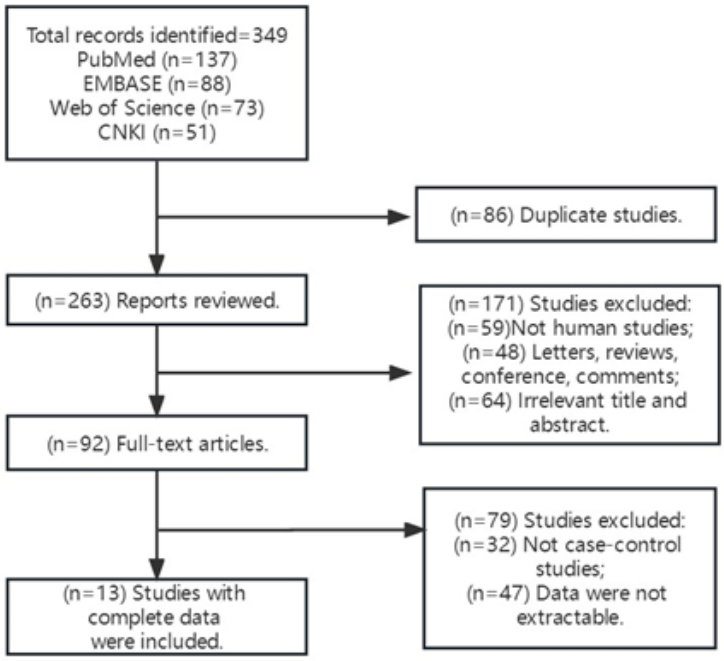


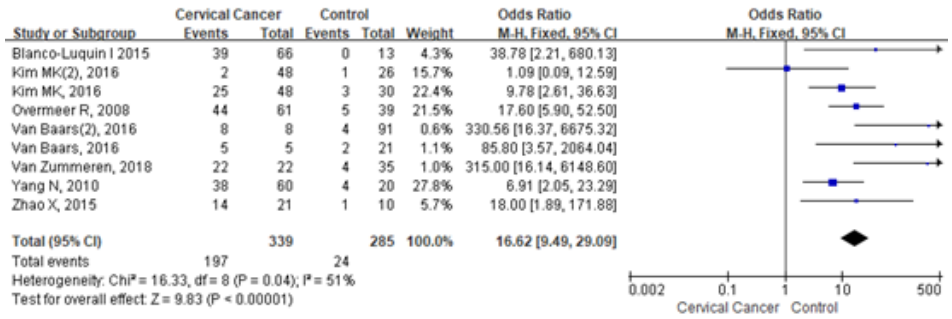
Figure 1: Flow diagram for the selection of studies in the meta-analysis.

Table 1: Characteristics of 13 included studies.

Author	Country	Gene	Cervical cancer	LSIL/CIN 1	HSIL/CIN 2-3	Control	Simple	Method
			Event/Total	Event/Total	Event/Total	Event/Total		
Blanco-Luquin I, 2015	Spain	CADM1	39/66	Oct-13	14/90	0/13	Tissue	MSP
Kim MK, 2016	Korea	CADM1	Feb-48	Jan-44	Jan-48	Jan-26	LBP	yrosequencin
Kim MK, 2016	Korea	CADM1	25/48	0/44	Jun-48	Mar-30	Tissue	yrosequencin
Overmeer R, 2008	Netherlands	CADM1	44/61	15/32	27/37	May-39	Tissue	MSP
Van Baars, 2016	Spain	CADM1	08-Aug	Feb-22	15/57	Apr-91	Tissue	qMSP
Van Baars, 2016	Spain	CADM1	05-May	0/14	May-25	Feb-21	Scrapes	qMSP
Van Zummeren, 2018	Netherlands	CADM1	22/22	Feb-19	Mar-17	Apr-35	Tissue	qMSP
Yang N, 2010	Netherlands	CADM1	38/60	Jun-20	Oct-20	Apr-20	Tissue	qMSP
Zhao X, 2015	China	CADM1	14/21	Aug-26	20/35	01-Oct	Tissue	MSP
Kim MK, 2016	Korea	MAL	34/48	Feb-44	Jan-48	Apr-30	Tissue	yrosequencin
Kim MK, 2016	Korea	MAL	Apr-48	Jan-44	Jan-48	Jan-26	LBP	yrosequencin
Overmeer R, 2008	Netherlands	MAL	111/122	Jun-66	34/64	0/22	Tissue	qMSP
Van Baars, 2016	Spain	MAL	06-Aug	Mar-22	25/57	Mar-91	Tissue	qMSP
Van Baars, 2016	Spain	MAL	04-May	Apr-14	Aug-15	Apr-21	Scrapes	qMSP
Van Zummeren, 2018	Netherlands	MAL	22/22	Feb-19	Mar-17	Apr-35	Tissue	qMSP
Huang TH, 2010	Taiwan	PAX1	14/14	01-Oct	Aug-32	0/17	Tissue	QMSP
Kan YY, 2014	Taiwan	PAX1	04-Apr	Oct-76	28/39	Jun-53	Tissue	qMSP
Kim MK, 2016	Korea	PAX1	36/48	0/44	Jul-44	Feb-29	Tissue	yrosequencin
Kim MK, 2016	Korea	PAX1	17/48	Mar-44	May-48	Feb-26	LBP	yrosequencin
Lai HC, 2008	Taiwan	PAX1	101/107	-	-	0/41	Tissue	MSP+BS
Lai HC, 2008	Taiwan	PAX1	19/22	Jan-44	24/57	0/41	Swab	MSP+BS
Lin CJ, 2010	Taiwan	PAX1	01-Jan	Apr-58	14/41	13/120	Tissue	MSP
Xu J, 2015	China	PAX1	27/27	Mar-32	15/34	0/28	Tissue	yrosequencin

4.2. Association of CADM1 Methylation Status with Cervical Cancer Risk

Seven selected studies [9-15] provided the data to evaluate the association between CADM1 promoter methylation and cervical cancer development, including 339 cervical cancer specimens and 285 controls. On the basis of the fixed effects model, the results showed that CADM1 promoter methylation was associated with an increased CC risk with a pooled OR of 16.62(95%CI 9.49---29.09), There was statistically significant heterogeneity across the included studies(I2=51%). The association between CADM1 promoter hypermethylation and HSIL risk, with an OR of 4.82 (95% CI, 2.90–7.99), I2 = 43%, P < 0.001), showed statistically significant. The association between CADM1 promoter hypermethylation and LSIL risk, with an OR of 2.30 (95% CI, 1.34–3.92), I2 = 51%, P = 0.002), also showed statistically significant (Figure 2).



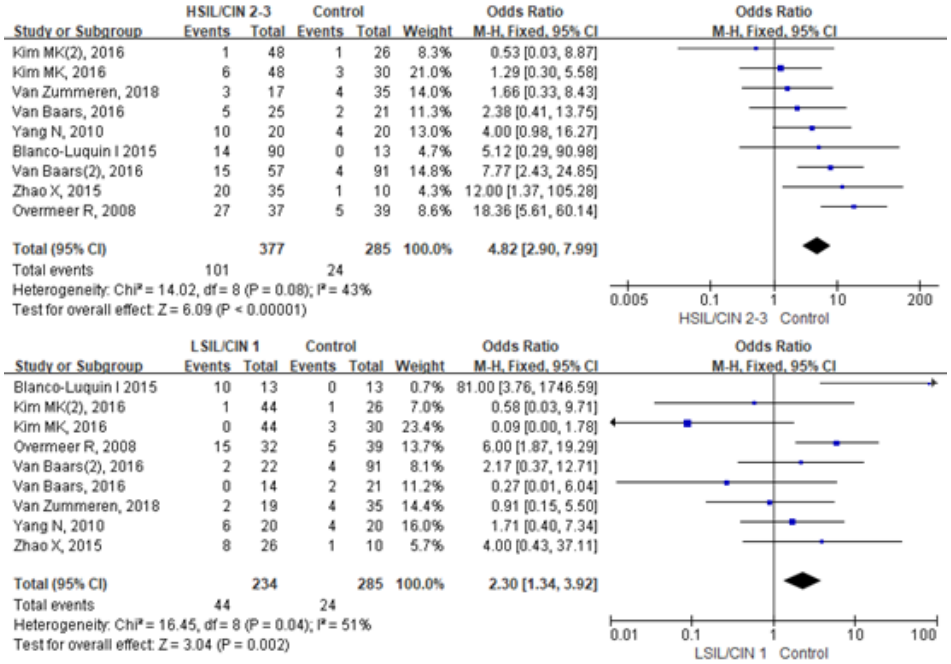


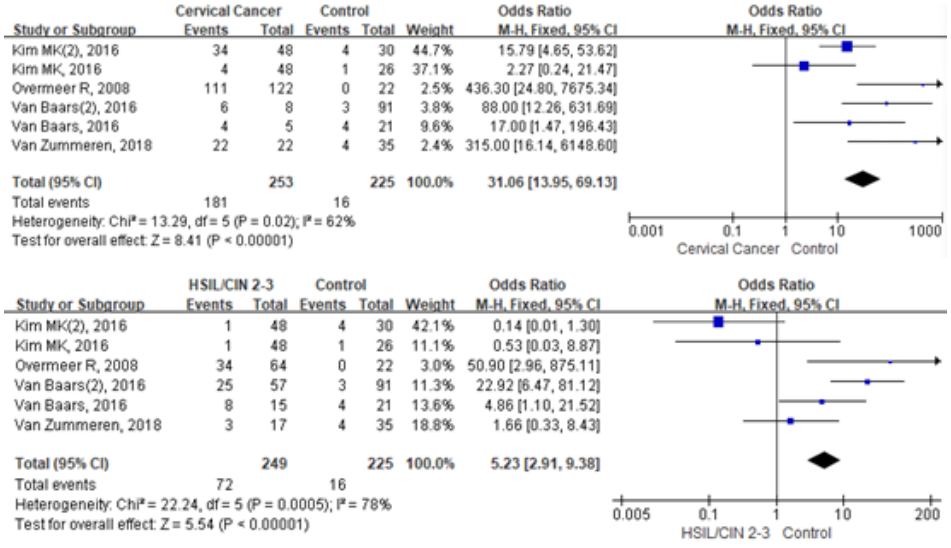
Figure 2: Forest plot for the association between CADM1 promoter methylation and cervical cancer risk

4.3. Association of MAL Methylation Status with Cervical Cancer Risk

In this meta-analysis, A total of 4 studies [9-11, 16] showed that there was a significant difference in MAL promoter methylation expression between CC tissue and normal tissue (OR: 31.06; 95% CI: 13.95–69.13) with statistically significant heterogeneity (I2 = 62%; p < 0.00001). These studies were conducted on 253 cancer cases and 225 controls. On the basis of the fixed effects model, results clearly showed that MAL promoter methylation status was not significantly associated with HSIL development (OR, 5.23; 95% CI, 2.91–9.38, I2 = 78%, P < 0.00001). There was no significant difference observed between MAL promoter hypermethylation and LSIL risk, with an OR of 1.32 (95% CI, 0.63–2.77) (Figure 3).

4.4. Association of PAX1 Methylation Status with Cervical Cancer Risk

In this meta-analysis, only six studies [9, 17-21] were selected to assess the association between PAX1 methylation status and cervical cancer risk, including 271 cervical cancer cases and 355 controls. The result indicated that the frequency of methylated PAX1 in CC samples was significantly higher than that in controls (OR, 120.07; 95% CI, 24.50–588.46). A statistically significant heterogeneity across the included studies was observed (OR, 8.49; 95% CI, 4.97–14.48, I2 = 58%, P < 0.00001) between PAX1 promoter hypermethylation and HSIL risk. There was not reach statistically significant between PAX1 promoter hypermethylation and LSIL risk, with an OR of 1.02 (95% CI, 0.55–1.87) (Figure 4).



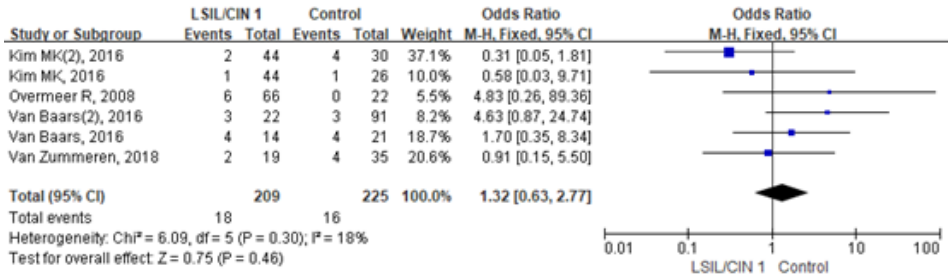


Figure 3: Forest plot for the association between MAL promoter methylation and cervical cancer risk

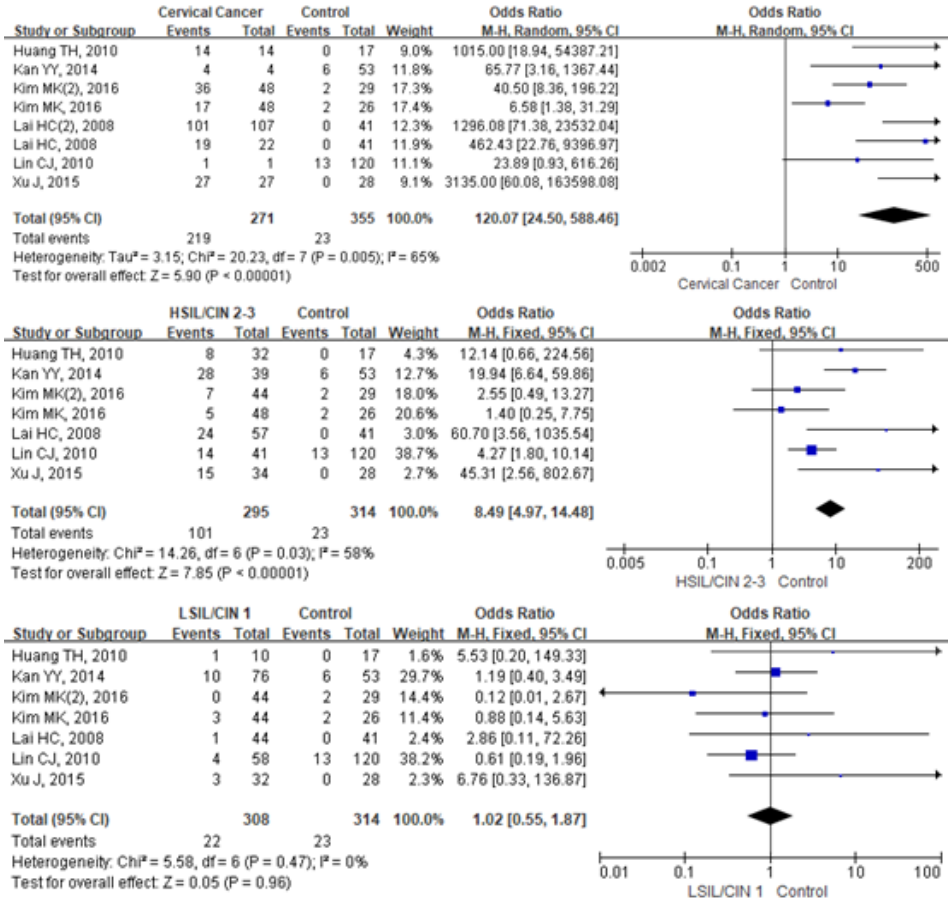


Figure 4: Forest plot for the association between PAX1 promoter methylation and cervical cancer risk

5. Discussion

DNA methylation is a crucial epigenetic process that plays a significant role in gene regulation and expression, the HPV and the host genomes showed different genetic and epigenetic alterations [22]. It involves the addition of a methyl group to the DNA molecule, which can lead to changes in chromatin structure and ultimately result in gene silencing, some genes reactivate the expression of hypermethylated and silenced tumor suppressor genes in tumor tissues, then evaluation of the methylation status [23]. This event has been linked with various diseases, including cancer. Cervical cancer is one such disease where DNA methylation patterns have been observed. In this meta-analysis study, we aimed to investigate the association between promoter region methylation of four genes CADM1, MAL and PAX1 and cervical cancer development and progression. Our findings suggest that there is an increased risk of cervical cancer associated with methylation patterns in these genes' promoter regions.

In our study, the analysis has focused on the methylation status of the promoters of CADM1, MAL and PAX1genes, provide a general methylation profile of genes which assessed on 1728 samples from patients with cancer and 863 samples from healthy controls, including 13 studies and analyzed using a model considering that promoter methylation could be a tissue-specific event. Results exhibit that methylation of CADM1, MAL and PAX1 genes promoters was significantly higher in cervical cases than in controls, and was also present in precancerous lesions, shows a significant association between the methylation of the genes promoters and the risk of CC development.

DNA methylation is a biological process that involves the addition of a methyl group to the DNA molecule, specifically to the cytosine base. This process plays a crucial role in gene regulation, Genes CADM1, MAL and PAX1 involved in cell adhesion, apical

membrane proteins transport, and cell differentiation, respectively, playing an important role during CC development [24, 25], as it can either activate or silence genes depending on the specific context [26]. DNA methylation is essential for normal development and is associated with various biological processes, such as genomic imprinting, X-chromosome inactivation, and the suppression of repetitive elements [27]. Hypermethylation of the promoters' regions of these genes is widely reported to induce loss of their functions during cervical carcinogenesis [28, 29].

CADM1 is a gene that encodes a protein involved in cell adhesion, which is the process by which cells interact and attach to neighboring cells or the extracellular matrix. CADM1 plays a role in various biological processes, including cell signaling, cell differentiation, and the formation of synapses in the nervous system. Abnormal expression of CADM1 has been associated with several diseases, such as cancer and autoimmune disorders. There is evidence to suggest that DNA methylation can regulate the expression of CADM1. In some cases, hypermethylation of the CADM1 gene promoter region has been observed, which leads to the silencing of the gene and a decrease in CADM1 protein levels. This downregulation of CADM1 has been linked to the development and progression of certain types of cancer, as it can promote cell proliferation, migration, and invasion. On the other hand, demethylation of the CADM1 promoter region can lead to the reactivation of the gene and an increase in CADM1 protein levels, which may have therapeutic potential in the treatment of certain diseases.

However, the main limitation of this investigation is the technique used for methylation status assessment. Indeed, most studies were performed using MSP, considered as the gold standard method (70.30%), in which PCR products are run on a gel and results are reported as methylated or unmethylated at the target DNA sequence. Moreover, this technique lacks to identify partial levels of methylation, which is considered as highly relevant feature both biologically and clinically. To overcome the limitation of this conventional method, qMSP was developed in recent years and was reported to be more specific and more sensitive, and allows for high-throughput analysis, making it more suitable as a screening tool.

DNA methylation and MAL are related in the context of epigenetics and gene regulation. DNA methylation is a process where a methyl group is added to the DNA molecule, usually at a cytosine base followed by a guanine base (CpG site). This modification can affect gene expression by altering the accessibility of the DNA to transcription factors and other regulatory proteins. MAL is a gene that encodes a protein involved in the formation and maintenance of myelin sheaths in the nervous system. The expression of MAL can be regulated by DNA methylation. In some cases, hypermethylation of the MAL gene promoter region has been associated with gene silencing, leading to reduced MAL expression. This epigenetic modification has been observed in various diseases, including certain types of cancer, where MAL downregulation may contribute to disease progression.

DNA methylation and PAX1 are related in the context of gene regulation and development. PAX1 is a member of the paired box (PAX) family of transcription factors, which play critical roles in embryonic development and tissue differentiation [30]. DNA methylation, as an epigenetic modification, can regulate the expression of PAX1 by adding a methyl group to the DNA molecule, usually at cytosine residues in CpG dinucleotides. This modification can lead to changes in PAX1 expression, which may have implications in various developmental processes and diseases.

However, the main limitation of this investigation is the technique used for methylation status assessment. Indeed, most studies were performed using MSP, considered as the gold standard method (70.30%), in which PCR products are run on a gel and results are reported as methylated or unmethylated at the target DNA sequence. Moreover, this technique lacks to identify partial levels of methylation, which is considered as highly relevant feature both biologically and clinically. To overcome the limitation of this conventional method, qMSP was developed in recent years and was reported to be more specific and more sensitive, and allows for high-throughput analysis, making it more suitable as a screening tool.

DNA methylation is an epigenetic modification that plays a crucial role in gene regulation and has been implicated in the development of various cancers, including cervical cancer. The genes CADM1, MAL, and PAX1 have been reported to be associated with the risk of cervical cancer, and their methylation status may contribute to this association. In conclusion, this meta-analysis gave evidence that highlights the association between the methylation status of the promoter region of some genes and promotion of cervical cancer in some cases. However, the technique used in the investigation can also generate questions related to the effectiveness of the detection of this methylation. Other analyses are, therefore, necessary to generate other data, making it possible to validate the highly promoter epigenetic markers for the diagnosis and development of therapeutic targets.

6. Financial & Competing Interests Disclosure

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflicts of interest concerning this article. No writing assistance was utilized in the production of this manuscript.

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