SYSTEMATIC REVIEW

Promoter hypermethylation patterns of P16, DAPK and MGMT in Oral Squamous Cell Carcinoma: A systematic review and meta-analysis

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a common cancer world-wide that is highly lethal due to its recurrence and metastasis. Methylation is a common epigenetic mechanism that leads to gene silencing in tumors and could be a useful biomarker in OSCC. The prevalence of P16, death-associated protein kinase (DAPK) and O^6-methylguanine-DNA-methyltransferase (MGMT) promoter hypermethylation in OSCC has been evaluated for several years while the results remain controversial.

Objective: The aim of this systematic review is to critically analyze and perform a meta-analysis on the various studies in the literature that have reported the promoter hypermethylation of P16, DAPK and MGMT genes in OSCC.

Search Strategy: Articles were searched and selected through PubMed. Hand search from the relevant journals was also performed. Articles were reviewed and analyzed.

Results: The estimated prevalence of P16 methylation was 43%, DAPK methylation was 39.7% and MGMT methylation was 39.8%. Heterogeneity in methylation prevalences and correlations with the clinical outcomes of the disease prevailed in various studies.

Conclusion: We can conclude from our systematic review that a higher prevalence of methylation of P16, DAPK and MGMT occur in OSCC. Further studies are required to substantiate the role of methylation of P16, DAPK and MGMT as a marker in OSCC.

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The term "oral cancer" refers to malignancy arising from oral tissues. Carcinoma is the term for a malignant tumor of epithelial origin. Oral cancer is the most frequent cancer of the head and neck region, with squamous cell carcinoma being by far the most common single entity, accounting alone for about 90–95% of all malignancies of the oral cavity.

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy in the world. Approximately, 405,000 cases of OSCC are diagnosed each year, with a rising incidence in many countries.[1] Due to its relative high mortality and low cure rate, OSCC represents a major public health problem. The WHO acknowledged that the 5 years survival rate of these patients has not improved over the past few decades despite treatment advances.[2,3] Early detection of OSCC is important to reduce mortality rates and to help provide successful cancer treatment.

The etiology of OSCC is multifactorial and involves intrinsic and extrinsic factors. The major risk factors include tobacco and alcohol intake, as well as human papillomavirus infection.[4,5] These predisposing factors may lead to a wide range of genetic and epigenetic events that promote genomic instability and tumor development and progression.

The genetic alterations involved in the development and progression of OSCC are caused by irreversible changes in DNA sequence including gene deletions, amplifications and mutations leading both to oncogenes activation or tumor-suppressor genes inactivation.[6,7]

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Epigenetics is another major event in the development and progression of OSCC. The epigenetic changes refer to any reversible heritable modifications in gene expression without alterations of the DNA sequence. They occur more frequently than gene mutations.[8]

Methylation is a common epigenetic mechanism that leads to gene silencing in tumors and could be a useful biomarker in OSCC. DNA methylation refers to the covalent addition of a methyl group which usually takes place at the 5' position of the cytosine residues of CpG nucleotides. The CpG dinucleotides are found in 1/80 dinucleotides in 98% of the human genome. They are usually present in regions called CpG islands, which are usually located at promoter regions of the gene.[9] CpG islands have >55% GC content and span > 500 bp. Methylation serves to decrease expression of a gene. CpG islands are often found hypermethylated in tumors, causing the transcriptional "silencing" of tumor-suppressor genes, contributing to cancer progression. On the contrary, it also serves as a mechanism of oncogene activation by hypo/demethylation.

The enzymes directly responsible for CpG island hypermethylation of tumor-suppressor genes are known as DNA-methyltransferases. The methylated-cytosine-guanine sequences are recognized by methyl-cytosine binding proteins, which in turn help in binding of histone deacetylase enzyme. This results in the removal of acetyl groups from histone. This leads to aggregation of nucleosomes to form the heterochromatin, which results in transcriptional silencing of gene.

The genes found hypermethylated in OSCC cover a wide range of cellular processes, including cell cycle control (P16, P15, P14), apoptosis (death-associated protein kinase [DAPK], RASSF1A), Wnt signaling (APC, WIF1), cell-cell adhesion (E-cadherin), and DNA-repair (O^6-methylguanine-DNA-methyltransferase [MGMT], hMLH1).[10]

In this review, we assessed the promoter region hypermethylation of P16, MGMT, and DAPK in OSCC.

The cyclin-dependent kinase inhibitor (CDKI) p16 belongs to INK4 family and is involved in cell cycle control. INK4 family of CDKIs include p16 (CDKN2A), p15 (CDKN2B), p18 (CDKN2C), and p19 (CDKN2D). INK4 family has selective effects on cyclin D/CDK4 and cyclin D/CDK6. P16 (INK4a) binds to cyclin D-CDK4 and thereby inhibits CDK4 and promotes the inhibitory effects of retinoblastoma protein (RB) by preventing phosphorylation of RB. CDKN2A gene maps on chromosome 9p21.3 and induces cell cycle arrest in the G1 phase.

O^6-methylguanine-DNA-methyltransferase gene is located on chromosome 10q26, which encodes MGMT, a DNA-repair enzyme that removes O^6-guanine-DNA adducts caused by alkylating agents. CpG island hypermethylation of the MGMT promoter region results in gene silencing with loss of MGMT repair capacity which is thought to drive cancer progression. Epigenetic silencing of MGMT has been associated with OSCCs where tobacco exposure and betel quid chewing are suspected to be etiological factors.[11] Elevated MGMT expression has been associated with resistance to alkylating chemotherapeutic agents.[12]

Death-associated protein kinase 1 gene maps on chromosome 9q34.1. DAPK encodes a pro-apoptotic calcium/calmodulin regulated serine/threonine kinase that is required for apoptosis induced by interferon-gamma (IFN-γ).[13] Loss of its expression via promoter hypermethylation has been associated with the formation of metastases and advanced disease stages in multiple cancer types, including head and neck cancers.[14] Regarding OSCCs, DAPK hypermethylation has been reported as associated with an increased likelihood of lymph node involvement.

METHODS

Search strategy for identification of studies

The search strategy was in accordance with the Cochrane guidelines for systematic reviews. Articles relevant to the search strategy were identified from search databases of PubMed, Medline till the year 2013. Due to the scarcity of methylation pattern studies in OSCC, we wished to exhaust all the possible articles; therefore, a timeline was not included in the search. The article search included only those published in the English literature. The internet search was also done to obtain the relevant articles of our interest. The title of the articles and abstracts was reviewed. The full text of selected articles were retrieved and further analyzed.

Search methodology

The search methodology applied in PubMed was using the following keywords:

Search (((((((hypermethylation) OR DNA hypermethylation) OR CpG island hypermethylation) OR promoter hypermethylation)) AND ((((((((((P16) OR P16 gene) OR P16 INK4a) OR P16 expression) OR inhibitory kinase4A) OR CDKN2A) OR cyclin CDKI2A) OR INK4A) OR MTS1) OR multiple tumor-suppressor 1)) OR ((((((DAPK) OR DAPK1) OR DAPK gene) OR DAPK expression) OR DAPK) OR DAPK gene)) OR (((((MGMT) OR MGMT gene) OR MGMT expression) OR MGMT) OR MGMT gene)) AND ((((((((((oral cancer) OR oral carcinoma) OR oral squamous cancer) OR oral squamous carcinoma) OR oral squamous cell cancer) OR OSCC) OR squamous cell carcinoma of oral cavity) OR SCC of oral cavity) OR OSCC).

In addition, the internet search was also done using the key words "promoter hypermethylation" and "P16 DAPK and MGMT" and "OSCC." Articles in which patients had

confirmed with a diagnosis of OSCC with or without the control group regardless of the stage of the tumor were considered for this review.

Selection of studies

Inclusion criteria

- Studies that evaluated the promoter hypermethylation patterns of P16, DAPK and MGMT genes in OSCC
- Studies in which P16, DAPK and MGMT methylation status was examined using methylation-specific PCR (MSP) or quantitative MSP or restriction-multiplex PCR or nested PCR
- Studies in which the specimens used for methylation analysis include fresh cancer tissues samples or formalin fixed paraffin-embedded tissues
- Studies in which the same patient population reported in several publications, only the most recent report or the most complete one with more number of sample size was included in this analysis in order to avoid overlapping between cohorts
- Studies that have undertaken a minimal of 20 samples of OSCC patients
- Studies in English language were included.

Exclusion criteria

- Studies in which methylations examined in the cell lines were excluded
- Studies conducted on animal models were excluded
- Studies conducted on patients who were under radiotherapy and chemotherapy was excluded.

Methods of review

The selection and exclusion of the reviewed studies are summarized in Figure 1. The search strategy identified 16 studies that evaluated the promoter hypermethylation of P16, DAPK and MGMT genes in OSCC. The description of the individual studies is shown in Table 1 and that of the excluded studies in Table 2.

Data extraction

Once the articles to be reviewed were finalized, data were extracted from each article, tabulated and was verified and interpreted, and a meta-analysis was performed.

Outcomes

The outcomes assessed in this review examined and analyzed the promoter hypermethylation patterns of P16, DAPK and MGMT genes in OSCC.

RESULTS

Included studies

Out of the 16 included studies, the frequencies of promoter hypermethylation of P16 gene in OSCC were evaluated in 15 studies, the frequencies of promoter hypermethylation of DAPK gene in OSCC were evaluated in five studies and the

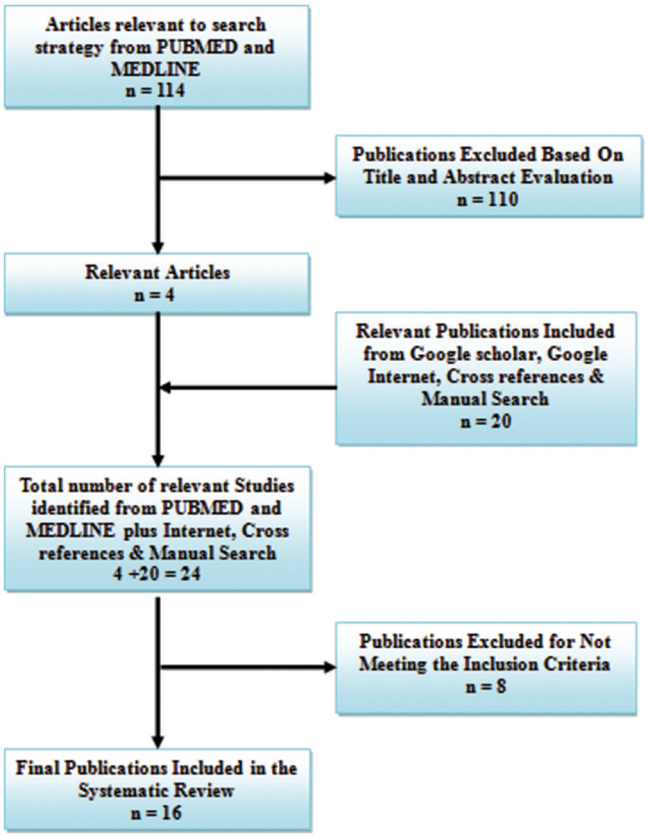

Figure 1: Flow chart showing selection and exclusion of the reviewed studies

frequencies of promoter hypermethylation of MGMT gene in OSCC were evaluated in eight studies. So, three separate meta-analysis was performed to assess the methylation status of P16, DAPK and MGMT genes in OSCC. The description of the individual studies included for each meta-analysis is shown in Tables 3-5 respectively. The data of the studies were analyzed to check for heterogeneity and publication bias.

Outcomes

Our meta-analysis data of P16 methylation status showed that the overall estimated pooled prevalence of P16 methylation among 932 OSCC cases in 15 studies was 43% (confidence interval [CI] = 40–46%). Heterogeneity of results among studies prevailed [Figures 2 and 5].

The funnel plot analysis for prevalence of P16 methylation in OSCC cases showed heterogeneity. Only 6 studies out of 15 fall within the funnel clearly indicating publication bias [Figure 6].

Our meta-analysis data of DAPK methylation status showed that the overall estimated pooled prevalence of DAPK methylation among 330 OSCC cases in five studies was 39.7% (CI = 15.0–64.3%). Heterogeneity of results among studies prevailed [Figures 3 and 7].

The funnel plot analysis for prevalence of DAPK methylation in OSCC cases showed heterogeneity. None

Table 1: Description of included studies

Author	Year	Location of study group	Patient age range	Patient gender male/female	Sample size		P16 methylated/total number of cases (%)			DAPK methylated/total number of cases (%)			MGMT methylated/total number of cases (%)		
					Patient	Control	Case	Adjacent normal mucosa	Control	Case	Adjacent normal mucosa	Control	Case	Adjacent normal mucosa	Control
Nakahara *et al.*[15]	2001	Japan			32		16/32 (50)								
Yakushiji *et al.*[16]	2001	Japan	46-84	20/5	25		12/25 (48)								
Huang *et al.*[17]	2002	Taiwan			48		20/48 (41.7)	8/48 (17)							
Ogi *et al.*[18]	2002	Japan			96	2	28/96 (29)		0/2 (0)	7/96 (7)		0/2 (0)			
Viswanathan *et al.*[19]	2003	India			99		23/99 (23)						41/99 (41)		
Kulkarni and Saranath[20]	2004	India	25-71	44/16	60	20	40/60 (66.7)	30/60 (50)	0/20 (0)	41/60 (68.3)	36/60 (60)	0/20 (0)	31/60 (51.7)	16/60 (26.7)	0/20 (0)
Ishida *et al.*[21]	2005	Japan	32-88	27/22	49		17/49 (34.7)						6/49 (12.2)		
Tran *et al.*[22]	2005	Vietnam	65-87		27		17/27 (63)								
Kato *et al.*[23]	2006	Japan	43-86		51	18	25/51 (49.0)	6/22 (27.27)	0/18 (0)				27/51 (52.9)	9/22 (40.91)	0/18 (0)
Sailasree *et al.*[24]	2008	India	36-85	82/34	116		34/112 (29.3)								
Supic *et al.*[25]	2009	Serbia	39-80	64/13	77		45/77 (58.4)			28/77 (36.8)			26/77 (33.8)		
Ohta *et al.*[26]	2009	Japan			44		28/44 (63.6)								
Kordi-Tamandani *et al.*[27]	2010	Iran	25-53	36/40	76	57							56/76 (73.7)		31/57 (54.4)
Su *et al.*[28]	2010	Taiwan	37-82	47/5	52		15/52 (28.8)			14/33 (42.4)			7/33 (21.2)		
Kaur *et al.*[29]	2010	India	23-72	73/19	92	30	44/92 (47.8)	5/48 (10.4)	0/30 (0)						
Wong *et al.*[30]	2011	Taiwan	26-77	58/6	64	20	43/64 (67.2)	20/64 (31.3)	0/20 (0)	29/64 (45.3)	26/64 (40.6)	0/20 (0)	20/64 (31.3)	16/64 (25)	0/20 (0)

MGMT=O⁶-methylguanine-DNA-methyltransferase, DAPK=Death-associated protein kinase

of the studies fall inside the funnel clearly indicating publication bias [Figure 8].

Our meta-analysis data of MGMT methylation status showed that the overall estimated pooled prevalence of MGMT methylation among 509 OSCC cases in eight studies was 39.8% (CI = 25.2–54.3%). Heterogeneity of results among studies prevailed [Figures 4 and 9].

The funnel plot analysis for prevalence of MGMT methylation in OSCC cases showed heterogeneity. Five studies out of eight fall inside the funnel indicating little publication bias [Figure 10].

Correlation of studies with clinicopathological data

Yakushiji *et al.* reported that there was no statistical significance on comparing methylation of P16 with clinicopathological stages.[16]

Ogi *et al.* reported that methylation of P16 correlated with younger age ($P = 0.043$) and T-category ($P = 0.038$).[18]

Viswanathan *et al.* reported that abnormal methylation of P16 and MGMT was detected in tumors irrespective of stage and location in the oral cavity.[19]

Kulkarni and Saranath reported that there was no significant association between the clinicopathological profile of the patients, including the size of the tumor, presence of lymph node metastasis, differentiation of the tumors, tumor-node-metastasis staging of the cancer, and sex or age of the patients, and hypermethylation of P16, DAPK and MGMT.[20]

Ishida *et al.* reported that tumor size, degree of differentiation, clinical stage, and frequency of metastasis to nodes were significantly associated with hypermethylation of P16. He reported that there was no significant correlation between MGMT and clinicopathological factors. He also reported that an apparent correlation between overall gene hypermethylation status of P16 and MGMT, tobacco use, alcohol consumption, and concurrent exposure to both substances was observed.[21]

Kato *et al.* reported that there was no relationship between methylation status of P16 and MGMT with clinicopathological features.[23]

Sailasree *et al.* reported that promoter methylation of P16 was associated with tumor size, nodal involvement and increased disease recurrence.[24]

Supic *et al.* reported that there was no correlation of methylation status of P16, DAPK and MGMT genes with clinicopathological features but reported that hypermethylation of P16 gene promoter showed tendency

Table 2: Description of excluded studies

Citation	Reasons for exclusion
Watts *et al.* 1997[31]	Does not satisfy our inclusion criteria. Methylation status was examined in the cell culture
Lo *et al.* 1999[32]	Does not satisfy our inclusion criteria. Methylation status was examined in the cell lines
Esteller *et al.* 2000[33]	Does not satisfy our inclusion criteria. Methylation status was examined in colorectal cancer
Shaw *et al.* 2006[34]	Does not satisfy our inclusion criteria. Methylation status was examined both in oral and oro-pharyngeal cancer. No statistical data given separately for oral and oro-pharyngeal cancer
Ruesga *et al.* 2007[35]	Does not satisfy our inclusion criteria. Samples were taken from the patients who had a previous OSCC but did not have any lesion in the oral cavity when the sample was taken
Sawhney *et al.* 2007[36]	Does not satisfy our inclusion criteria. Methylation status was examined using immunohistochemical analysis
Supic *et al.* 2011[37]	Does not satisfy our inclusion criteria. Same author conducted the same study on the same patient population in 2009. In this study, statistical data is incomplete with lesser number of sample size when compared to the other study conducted in 2009
Deep *et al.* 2012[38]	Does not satisfy our inclusion criteria. Methylation status was examined among smokers and not in oral cancer patients

OSCC=Oral squamous cell carcinoma

Table 3: Studies included in meta-analysis of P16 methylation status

Author	Year	Number of cases	P16 methylated/ total number of cases	P16 methylated %
Nakahara *et al.*	2001	32	16/32	50
Yakushiji *et al.*	2001	25	12/25	48
Huang *et al.*	2002	48	20/48	41.7
Ogi *et al.*	2002	96	28/96	29
Viswanathan *et al.*	2003	99	23/99	23
Kulkarni and Saranath	2004	60	40/60	66.7
Ishida *et al.*	2005	49	17/49	34.7
Tran *et al.*	2005	27	17/27	63
Kato *et al.*	2006	51	25/51	49.0
Sailasree *et al.*	2008	116	34/112	29.3
Supic *et al.*	2009	77	45/77	58.4
Ohta *et al.*	2009	44	28/44	63.6
Su *et al.*	2010	52	15/52	28.8
Kaur *et al.*	2010	92	44/92	47.8
Wong *et al.*	2011	64	43/64	67.2

Table 4: Studies included in meta-analysis of DAPK methylation status

Author	Year	Number of cases	DAPK methylated/total no. of cases	DAPK methylated %
Ogi *et al.*	2002	96	7/96	7
Kulkarni and Saranath	2004	60	41/60	68.3
Supic *et al.*	2009	77	28/77	36.8
Su *et al.*	2010	33	14/33	42.4
Wong *et al.*	2011	64	29/64	45.3

DAPK=Death-associated protein kinase

Table 5: Studies included in meta-analysis of MGMT methylation status

Author	Year	Number of cases	MGMT methylated/total number of cases	MGMT methylated %
Viswanathan *et al.*	2003	99	41/99	41
Kulkarni and Saranath	2004	60	31/60	51.7
Ishida *et al.*	2005	49	6/49	12.2
Kato *et al.*	2006	51	27/51	52.9
Supic *et al.*	2009	77	26/77	33.8
Kordi-Tamandani *et al.*	2010	76	56/76	73.7
Su *et al.*	2010	33	7/33	21.2
Wong *et al.*	2011	64	20/64	31.3

MGMT=O^6-methylguanine-DNA-methyltransferase

of increase with age, with cutoff point selected according to the median value of 58.[25]

Kordi-Tamandani *et al.* reported that there was no correlation of methylation status of MGMT with clinical features (age and sex) and stages of cancer. He also reported that MGMT methylation may be considered as a potential molecular marker for the poor survival in advanced OSCC.[27]

Su *et al.* reported that the hypermethylation status of P16, DAPK and MGMT in tumors did not depend on clinicopathological features such as gender, lifestyle, tumor stage, recurrence, or histologic differentiation. Nevertheless, he reported that the mean age of patients with hypermethylated P16 was lesser than those without ($P = 0.027$). Multiple logistic regression predicted patients with hypermethylated P16 have higher risks of lymph node invasion (adjusted odds ratio [OR] = 6.21, $P = 0.030$) in young patients and distant metastasis (adjusted OR = 19.23, $P = 0.007$) in older patients. Moreover, P16 promoter hypermethylation was significantly associated with shortened disease-free survival ($P = 0.034$) in older patients.[28]

Kaur *et al.* reported that P16 promoter methylation was significantly associated with nodal involvement ($P = 0.04$, OR = 3.3, 95% CI = 1.1–10.2).[29]

Wong *et al.* reported that frequencies of P16, DAPK, and MGMT gene promoter hypermethylation did not differ based on the tumor site ($P > 0.05$). Promoter hypermethylation rates of the P16, DAPK, and MGMT genes were not correlated with tumor size, differentiation, betel nut chewing, tobacco smoking, or alcohol consumption ($P > 0.05$). Methylation rates of MGMT (50%) and DAPK (55.6%) in metastasized OSCC were higher than those of MGMT (23.9%) and DAPK (41.3%) in nonmetastasized OSCC. He also reported that hypermethylated P16 promoters were found in 63% of nonmetastasized tumors and in 77.8% of metastatic tumors (but was not statistically significant).[30]

DISCUSSION

Oral squamous cell carcinoma is the sixth most common malignancy in the world. Due to its relative high mortality and low cure rate, OSCC represents a major health problem. Early detection of OSCC is important to reduce mortality rates and

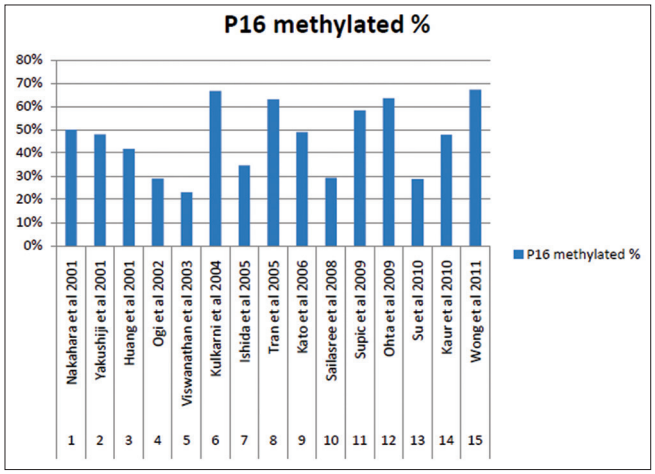

Figure 2: Chart depicting the frequency of P16 methylation found by different authors

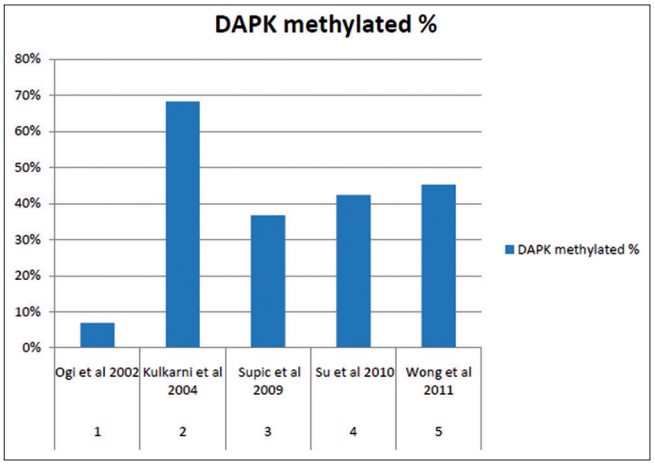

Figure 3: Chart depicting the frequency of death-associated protein kinase methylation found by different authors

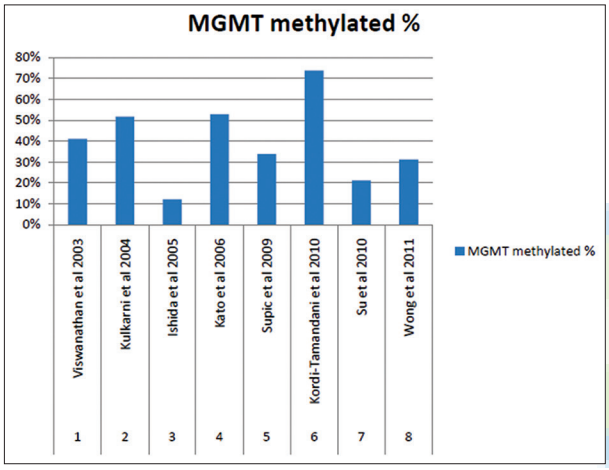

Figure 4: Chart depicting the frequency of O^6-methylguanine-DNA-methyltransferase methylation found by different authors

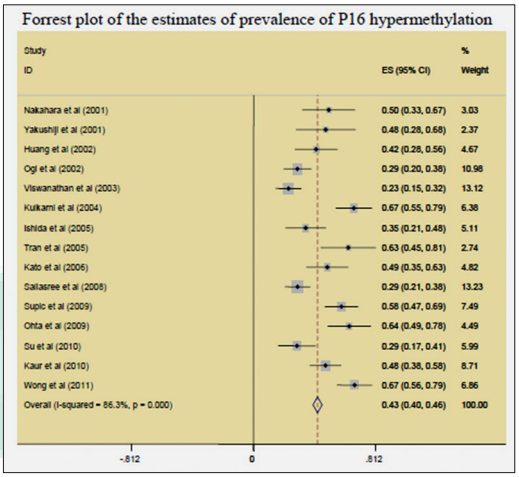

Figure 5: Forrest plot of P16 promoter methylation in oral squamous cell carcinoma

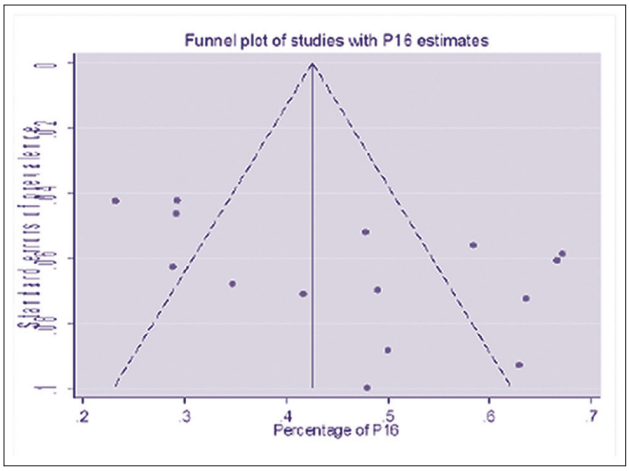

Figure 6: Funnel plot of studies with P16 promoter methylation

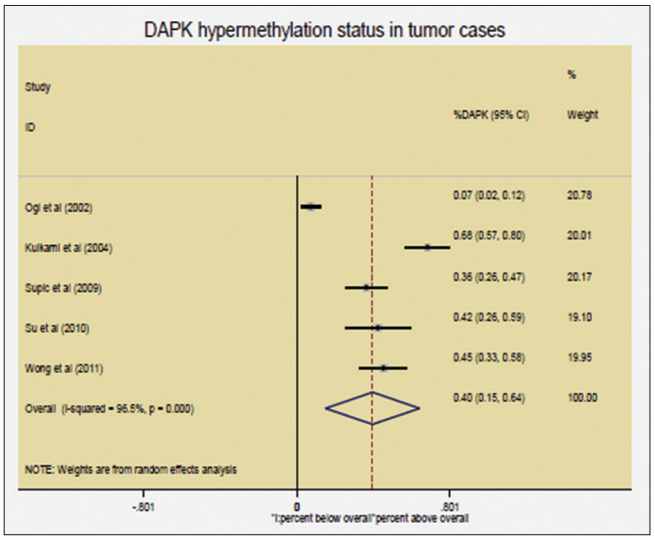

Figure 7: Forrest plot of DAPK promoter methylation in oral squamous cell carcinoma

to help provide successful cancer treatment. Carcinogenesis is a multistep process. The genetic and epigenetic alterations are involved in the development and progression of OSCC.

Gene-specific promoter alterations are common epigenetic aberrations found in human tumors. Hypermethylation of CpG

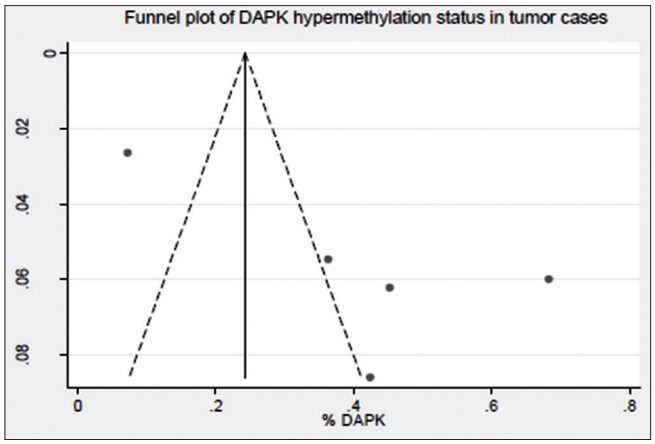

Figure 8: Funnel plot of studies with death-associated protein kinase promoter methylation

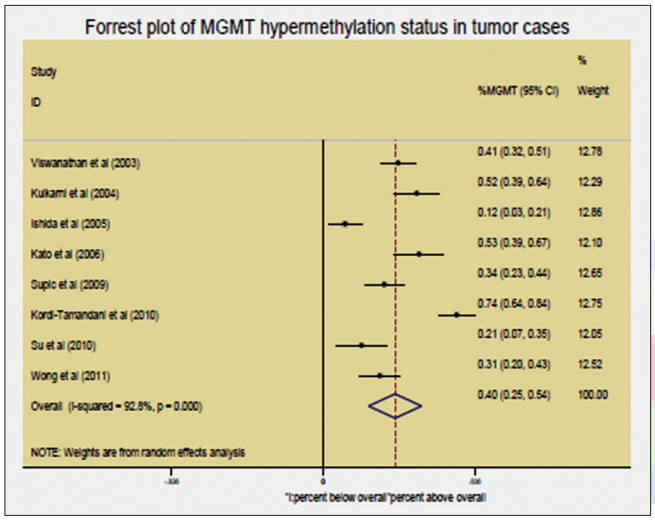

Figure 9: Forrest plot of O^6-methylguanine-DNA-methyltransferase promoter methylation in oral squamous cell carcinoma

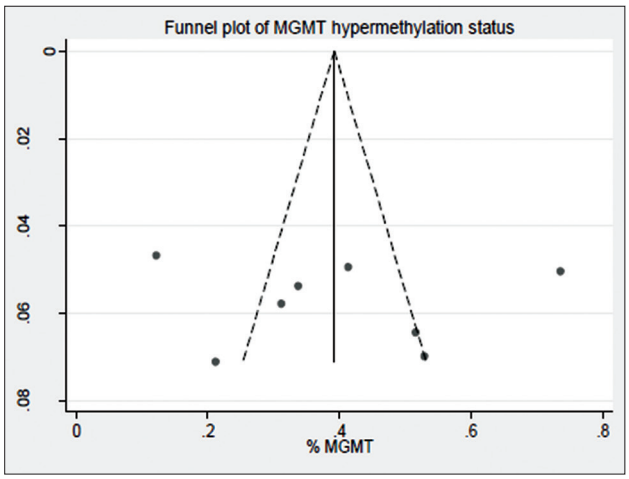

Figure 10: Funnel plot of studies with O^6-methylguanine-DNA-methyltransferase promoter methylation

islands in promoter regions is one of the important mechanisms for inactivation of tumor-suppressor genes involving apoptosis, cell cycle control, DNA-repair, cell-cell adhesion and Wnt signaling. The prevalence of P16, DAPK and MGMT promoter hypermethylation in OSCC has been evaluated for several years while the results remain controversial.

The effects of methylation have been studied in various genes over recent years, extracting genetic material from cells in tumors and potentially malignant disorders, saliva, serum, or healthy tissue adjacent to the tumor. This is because methylation can be detected in tumors and potentially malignant disorders and even in clinically and histologically healthy tissue adjacent to the tumor, suggesting that methylation may occur early in oral carcinogenesis and might serve as an early marker of the disease.[39]

P16/INK4A is known as one the most important tumor-suppressor genes which plays an important role in regulating the cell cycle. Hypermethylation of the CDKN2A promoter region has been extensively evaluated in oral cancers with the frequency of hypermethylation being reported from 28% to 86%.[15,20] Aberrant methylation of P16 gene has not been detected in noncancer controls.[19,34]

Tran *et al.* reported that in betel chewing individuals with oral cancer, P16 methylation was detected in 63% of OSCCs and 67% of verrucous carcinomas.[22]

A correlation was also found between P16 methylation and higher-grade dysplasia.[35] In studies on precancerous lesions, it was reported that P16 methylation was not related to the malignant transformation of lichen planus but was significantly associated with the malignant transformation of leukoplakia, especially in relation to tobacco use.[6]

Death-associated protein kinase encodes a serine/threonine kinase that is required for apoptosis induced by IFN-γ. Sanchez-Cespedes *et al.* reported that the promoter hypermethylation of DAPK has been associated with the formation of metastasis and advanced stages of cancer.[14]

Supic *et al.* reported that the detection of DAPK promoter hypermethylation at resection margins of oral tumors has been significantly associated with decreased overall survival, suggesting that it may have utility as a biomarker for guiding patient follow-up strategies.

O^6-methylguanine-DNA-methyltransferase is a DNA-repair gene that protects from toxicity and mutations that occur by alkylating agents through the removal of O^6-guanine-DNA adducts. MGMT hypermethylation has been reported for many cancer types.

Zuo *et al.* reported that MGMT promoter hypermethylation has also been associated with poorer outcomes for oral cancer, including a greater likelihood of nodal metastases, tumor recurrence, and decreased survival.[40]

Although many studies have reported the prevalence of P16, DAPK and MGMT gene hypermethylation in OSCC, the results remain inconclusive with the reasons of small sample size. Thus, a meta-analysis was performed by pooling data from published studies, which can increase the statistical power.

In the present study, a total of 16 articles were selected based on inclusion and exclusion criteria, from which the pooled prevalence of methylation in OSCC cases was calculated. Three separate meta-analysis was performed to assess the methylation status of P16, DAPK and MGMT genes in OSCC.

Our meta-analysis data of P16 methylation status showed that the overall estimated pooled prevalence of P16 methylation among 932 OSCC cases in 15 studies was 43% (CI = 40–46%). Heterogeneity of results among studies prevailed. From the present analysis, we found that Kulkarni and Saranath, Tran *et al.*, Supic *et al.*, Ohta *et al.* and Wong *et al.* studies showed a higher P16 methylation prevalence which range from 58% to 67% and Ogi *et al.*, Viswanathan *et al.*, Sailasree *et al.* and Su *et al.* studies showed a lower P16 methylation prevalence which range from 23% to 29%.

Our meta-analysis data of DAPK methylation status showed that the overall estimated pooled prevalence of DAPK methylation among 330 OSCC cases in five studies was 39.7% (CI = 15.0–64.3%). Heterogeneity of results among studies prevailed. From the present analysis, we found that Kulkarni and Saranath study showed a higher DAPK methylation prevalence which was 68.3% and Ogi *et al.* study showed a lower DAPK methylation prevalence which was 7% only.

Our meta-analysis data of MGMT methylation status showed that the overall estimated pooled prevalence of MGMT methylation among 509 OSCC cases in eight studies was 39.8% (CI = 25.2–54.3%). Heterogeneity of results among studies prevailed. From the present analysis, we found that Kulkarni and Saranath, Kato *et al.* and Kordi-Tamandani *et al.* studies showed a higher MGMT methylation prevalence which range from 52% to 74% and Ishida *et al.* and Su *et al.* studies showed a lower MGMT methylation prevalence which range from 12% to 21%.

The heterogeneity in methylation patterns in different studies may arise from difference in age, gender, ethnicity, and sample size, the location of the study group, smoking status, other adverse habits status, tumor stages, histopathology types and methods of methylation detection.

Despite significant epigenetic alterations found in OSCC, hypermethylation prevalences and correlations with the clinical outcomes of the disease in various studies are inconsistent. These differences probably reflect the heterogeneity of OSCC in their histology and clinical behavior, with different etiologies and associated risk factors, and known tissue and tumor-type specificity of methylation pattern. OSCCs originated from different locations of the oral region showed different methylation pattern.

The higher percentage of methylation in India may reflect the inherent differences in the prevalent molecular pathway in a majority of the chewing tobacco-associated cancers, as compared to oral cancers in USA, UK, Japan, and other developed countries, where the cancer is primarily associated with tobacco smoking and with/without alcohol consumption.[20]

To summarize, multiple studies show that a higher prevalence of methylation of P16, DAPK and MGMT occur in OSCC and the promoter hypermethylation of P16, DAPK and MGMT can be used for early detection of oral cancer and play a role in oral cancer progression.

Limitations of the review

We acknowledge the potential presence of publication bias within this review. The number of articles reviewed is minimal. This is due to the scarcity of studies available in promoter methylation pattern in OSCC. Our search also included publications in the English literature only. No unpublished data were included. The data used for pooled analysis were taken from published articles instead of original data. Further studies must be performed with similar outcome measures that could be compared in order to generate a more homogenous group of data. This could aid in giving better systematic reviews in the future in this field of study.

CONCLUSION

We can conclude from our systematic review that a higher prevalence of methylation of P16, DAPK and MGMT genes occur in OSCC cases. The promoter hypermethylation of P16, DAPK and MGMT genes play a role in oral cancer progression and can be used for early detection of oral cancer.

Heterogeneity in methylation patterns in different studies prevail which may arise from difference in age, gender, ethnicity, and sample size, the location of the study group, smoking status, other adverse habits status, tumor stages, histopathology types and methods of methylation detection.

Further studies must be performed with large sample sizes and with similar outcome measures that could be compared in order to generate a more homogenous group of data. This could aid in giving better systematic reviews in the future in this field of study.

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