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ORIGINAL ARTICLE

Expression of Minichromosome Maintenance Protein 2 (MCM2) in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: A Systematic Review

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Abstarct The histopathological investigations of oral lesions are a basic approach for diagnosing ongoing cancer or pre-cancer associated pathological attributes in the dissected biopsy. The early detection and management of potentially malignant disorders of the lip and oral cavity that require intervention may reduce malignant transformations, or in case any malignancy is detected during surveillance, the appropriate treatment may improve survival rates. This would guide the clinicians to decide the appropriate treatment modality or lesion to achieve a more favorable prognosis. MCM2 protein is involved in DNA replication providing additional information about the prognosis of neoplasms. Some authors have pointed out that MCM proteins have been inversely correlated with salivary tumour differentiation and therefore could be an indicator of proliferation potential. Therefore, it is essential to find the expression of the MCM2 gene in oral leukoplakia and oral squamous cell carcinoma. Electronic databases like Ebscohost, Livivo, Google Scholar and PubMed were searched. Based on the inclusion and exclusion criteria, 2 reviewers (MS and SN) independently selected the relevant articles. Any disagreement was discussed until a consensus was reached. We used the QUADAS-2 tool to assess the quality of the included studies over four key domains: patient selection, index test, reference standard and flow and timing of participants through the study. 10 out of 57 titles were found to meet the eligibility criteria. Biopsied tissue with immunohistochemical staining or advanced diagnostic studies were included. A total of 901 samples were included in the study and different groups were normal oral mucosa (NOM), oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). MCM2 proteins are useful diagnostic markers for distinguishing malignant from benign epithelial dysplasia and for early detection and diagnosis of OSCC as an adjunct to clinicopathological parameters.

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Introduction

Oral cancer is a group of malignancies which arises in the oral cavity and is one of the most significant causes of cancer deaths worldwide [1, 2]. It is a heterogeneous group that occurs in oral cavities and generally occurs in the lip cheek, salivary gland, soft and hard palate, uvula, gums, tonsils, tongue, and inner tongue [3]. Oral carcinoma has been ranked 16 globally, with an incidence rate of 354,864 (2%) and a mortality rate of 177,384 (1.9%). In 2019 it was reported in the Indian subcontinent, the extent of new cases of oral carcinoma was 1,19,992 and reported more than 72,616 deaths annually [4]. One of the major causes of the high incidence of oral cancer in the Indian subcontinent is the extensive tissue-abusive habit of chewing betel quid (or paan) and related areca nut use. There are various reports that state that the risk profile of head and neck cancer has been changing [5] and therefore pattern (incidence and subsite predilection) of head and neck cancer is also expected to change.

Most studies have reported that more than ninety percent of all the oral cancer cases studied, oral squamous cell carcinoma is the most prevalent cancer of all [6, 7]. Oral squamous cell carcinoma (OSCC) develops in the oral cavity and oropharynx and can occur due to many etiological factors but smoking and alcohol remain the most common risk factors especially in the western world [8]. In South Asian countries, consumption of smokeless tobacco and areca nut products are the main etiological factors associated with OSCC [9]. Recent epidemiological literature has also demonstrated that the risk of oral cancer increases with the intake of alcohol. The use of alcohol has been shown to have additive and synergistic effects with tobacco.

Oral cancer is caused by concurrent changes in biochemical, cellular, and molecular alterations in conjunction with clinical developments affecting epithelial tissues. Gene mutations may also cause cancer development in the pharynx and oral cavity; however, it has been difficult to target a specific gene in OSCCs [10]. Activation of proto-oncogenes (ras, myc, EGFR) or inhibition of tumour suppressor genes (TB53, pRb, p16) by environmental factors such as smoking, irradiation, and viral infection may increase the risk of oral and oropharynx OSCC [11]. Most of the oral and oropharynx OSCC cases occur in elderly male patients, with the tonsils and tongue being the most affected sites [12].

The early detection and management of potentially malignant disorders of the lip and oral cavity that require intervention may reduce malignant transformations, or in case any malignancy is detected during surveillance, the appropriate treatment may improve survival rates. But overlapping features could make diagnosis difficult. The minichromosome maintenance (MCM) proteins are essential for the initiation and elongation of DNA replication and comprise six proteins [13]. They were first discovered in yeast mutants that were defective in the maintenance of circular minichromosomes related to abnormal function of the replication origin [14]. MCM proteins have been demonstrated to move quickly from the cytoplasm into the nucleus as mitosis is completed and persist there until the next round of division is initiated. This observation suggested that MCM proteins may be a licensing factor for DNA replication to ensure that the genome is replicated only once in each cell cycle and no DNA is re-replicated until passage through mitosis into the next S-phase [15]. MCM proteins may also provide additional information about the prognosis of neoplasms.

There are significant challenges faced by oral pathologists and oncologists which include delayed diagnosis of OSCC, high metastatic rate, and low five-year survival rate due to cancer recurrences [16]. The histopathological investigations of oral lesions are a basic approach for diagnosing ongoing cancer or pre-cancer-associated pathological attributes in dissected biopsy [17]. The histopathologically apparent, cellular modulations responsible for the initiation and progression of oral cancer are cumulative aftermaths of prior molecular aberrations induced by various OSCC etiologies [18]. Some authors have expressed that MCM proteins can be known to inversely correlated with salivary tumor differentiation and therefore could be an indicator of proliferation potential [19]. Therefore, we planned a review to find the expression of gene MCM2 in squamous cell carcinoma.

Materials and Methods

Review Question

Is there a change in gene expression of MCM2 in Oral leukoplakia/ Oral epithelial dysplasia and oral squamous cell carcinoma?

The reviewers tried setting the review question in PICO format (Population, Intervention, Comparison, and Outcome) keeping mind in that all the included studies had a prognostic approach.

The following PICO framework was developed for a systematic review of the existing literature.

PIC Model

Patient	Patients with squamous cell carcinoma
Intervention	MCM2 gene
Comparison	N. A
Outcome	Effectiveness of expression of MCM2 gene

Protocol and Registration

The present systematic review was registered at the National Institute for Health Research PROSPERO International Prospective Register of Systematic Reviews.

The search protocol is designed based on the PRISMA (Preferred Reporting Items for Systematic Reviews and meta-analysis) guidelines 2009.

Inclusion Criteria

1. Studies including Squamous cell carcinoma or Oral epithelial dysplasia/ Oral leukoplakia
2. Studies which included only the MCM2 gene, or which had other comparators genes
3. Any method which identified the MCM2 gene was included
4. Lesion pertaining only to the oral cavity
5. Patient's age group if any 18–60 years
6. Gender male as well as female.

Exclusion Criteria

1. Animal studies and Randomized controlled trials or any studies not including human sample
2. Patients below 18 years of age
3. Cases with recurrence of squamous cell carcinoma or Oral epithelial dysplasia/ Oral leukoplakia
4. Preoperative and postoperative chemotherapy and radio therapy
5. Articles other than the English language

Criteria for Considering Studies for this Review

Type of Studies

All study designs with the required intervention were included. The resulting initial hits were screened, and the first preselection by title was undertaken. Titles were sequentially excluded if they indicated irrelevant content or no mention of the required MCM2 gene.

In case of any uncertainty or confusion, an additional step of abstract reading was performed. Abstracts of the selected titles were inspected for the relevance of the wanted content. The screened articles were again inserted in the pool, in case of any further doubt both the reviewers MS and SN performed a full-view analysis of the entire articles to determine their eligibility.

If studies were published by an author or institution several times, then the manuscripts were thoroughly read and compared to avoid the inclusion of duplicate data. After a full-text selection and data extraction, it was decided whether the publication was adequate for the intended systematic review. As mentioned earlier study selection and data extraction were performed independently by two reviewers (MS and SN), and any disagreement was solved by discussion between the two.

Type of Participants

All the studies which mentioned participants above 18 years to 60 years of age were included in the review. Those studies which did not mention participants' ages were also included to avoid excluding relevant articles. Only care was taken to exclude those studies which included participants below the age of 18 years. The reviewers agreed on including male and female participants in the study.

Types of Interventions

Due to the lack of interventions on the MCM2 gene and few authentic publications, the reviewers decided to include all the possible study designs to avoid excluding articles. Search engine riders on the date of publication were also kept open to include maximum articles.

Types of Outcome Measures

Primary Outcomes

Was to check the efficacy of expression of the MCM2 gene in squamous cell carcinoma.

Search Methods for Identification of Studies

Electronic databases like Ebscohost, Livivo, Google Scholar and PubMed were searched. Based on the inclusion and exclusion criteria, 2 reviewers (MS and SN) independently selected the relevant articles. Any disagreement was discussed until a consensus was reached.

Using the PICO-formatted question, methodological Medical Subject Heading (MeSH) terms were generated to

make the search strategy more sensitive in the identification of studies. These strategies were revised appropriately for each database. The search strategy used a combination of controlled vocabulary and free-text terms and was linked with the Cochrane.

Data Collection and Analysis

Selection of Studies

Two review authors (MS and SN) assessed titles and abstracts for inclusion in the review. Selection criteria were used for selecting papers suitable for inclusion. A downloaded set of records from each database was imported to the bibliographic software package Zotero and merged into one core database to remove duplicate records and facilitate the retrieval of relevant articles.

Data Extraction and Management

Data extraction was carried out on a specially designed form independently by two review authors who were blinded to each other's data. Results were compared to check for inconsistencies and disagreements resolved by discussion. The following details for each trial were recorded on the data extraction form:

Authors, Year of publication, Study design, Sample size, Study group, Type of sample, Outcome and Inference.

Assessment of Risk of Bias in Included Studies

We used the QUADAS-2 tool to assess the quality of the included studies over four key domains: patient selection, index test, reference standard and flow and timing of participants through the study. The QUADAS-2 tool was tailored specifically for this review. Review-specific guidance was used to facilitate documentation of the pertinent descriptive information contained in the studies. Customised instructions to aid the judgement of the signalling questions was used. Core signalling questions were removed:

Results

Reviewers (MS and SN) searched 3 data bases Pubmed, Ebscohost and Livivo libraries independently and found a total of 57 articles. The relevant database searchers were Pubmed 20 articles followed by Ebscohost 15 and Livivo 22 articles. Reviewer MS also screened through other grey literature for relevant articles present in the library, but no relevant articles were retrieved.

Later, in the literature search, the reviewers identified 22 articles that were potentially relevant to the topic and not duplicated. These 22 articles were subjected for title review. Records included after title screening were 16; 6 articles were excluded after title screening. These 16 articles were considered for further abstract screening off which 6 articles were excluded after abstract screening. Finally, 10 articles were retrieved and considered for further assessment. (Fig. 1 flow chart). Next, both the primary and secondary reviewers assessed the full text of the 10 studies. All the articles that were included were Diagnostic studies in nature that evaluated or included MCM2 protein keeping in mind the inclusion and exclusion criteria set by the reviewers.

All the included studies collected data from countries like Japan, UK, China, Egypt, USA and Iran. These studies were published from 2001 to 2017. A total of 901 samples were included in the study. 6 of the 10 included studies mentioned study designs, most of them were analytical studies which were case control studies either with a prospective design or retrospective design.

Only 1 study by Li J et al. [21] specifically mentioned about the specific site (tongue) from which the sample was collected, most of the studies just mentioned the collected sample was from the oral cavity. Off all the included 8 studies used IHC to identify MCM2 protein Kodani I et al. in (2001 and 2003) [22, 23], Scott I et al. [24], Rendon AT et al. [25], Shalash HN et al. [26], Razavi S et al. [27], Zhakaria S et al. [28], Jenson E et al. [29]. In a study by Li J et al. [21] used RTPCR for evaluating MCM2 gene, where as a study by Zargoun IM et al. [30] used 2 techniques to evaluate MCM2 genes which were Tissue microarray and IHC.

Methodological Quality of Included Studies

Protein Expression in Normal Oral Mucosa

5 studies evaluated protein expression of MCM2 in normal mucosa. (Kodani I et al. [22], Rendon A et al. [25], Razavi SM et al. [27], Zakaria SH [28], Zargoun IM et al. [30]). According to Rendon A et al. [25] in most of the NOM samples there was a characteristic absence of protein expression in the basal layer. Similarly, Kodani I et al. [22], Razavi SM et al. [27] and Zakaria SH [28] the MCM-2 protein was generally restricted to the basal and parabasal compartments. MCM-2 protein was expressed at a higher frequency in the basal and parabasal compartments and extended to the mid-prickle cell region. In another study, Zargoun IM et al. [30] showed NOM samples positive nuclear staining for MCM2 mainly in the basal cell layers. Zakaria SH et al. [28] also found MCM-2 immuno-expression in the prickle cells.

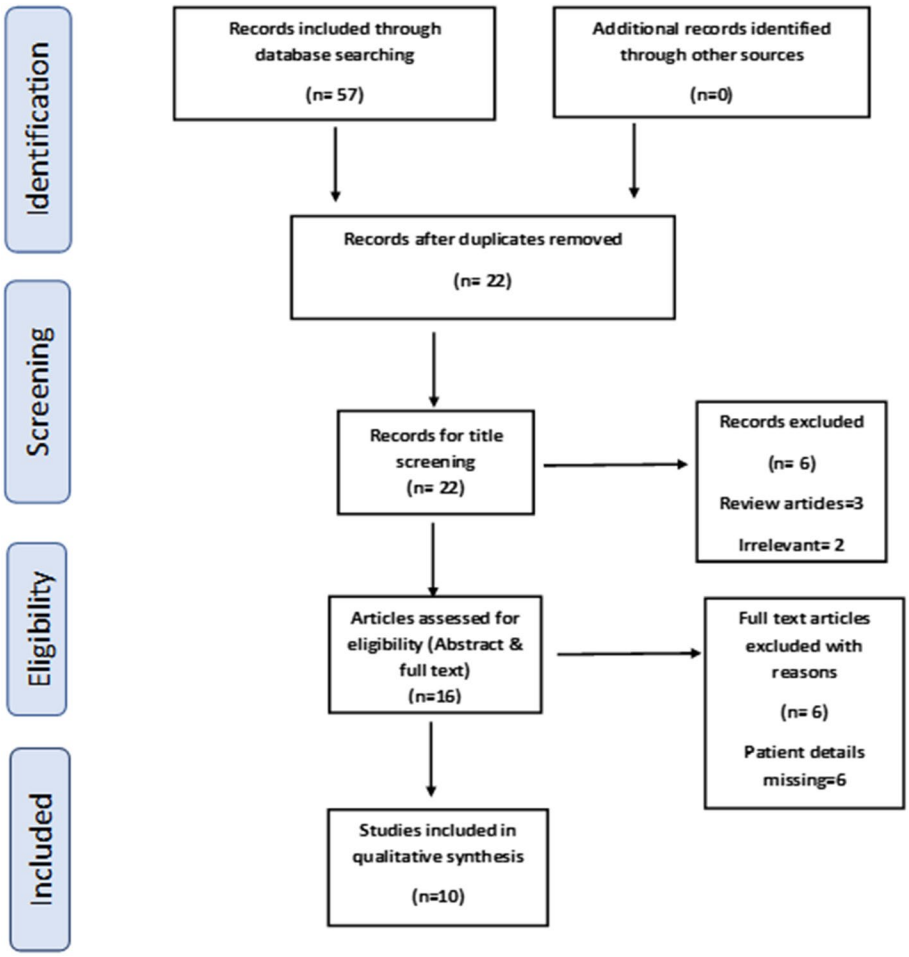

Fig. 1 PRISMA flow diagram

Protein Expression in Squamous Cell Carcinoma

Scott IS et al. [24] found a very widespread expression of MCM-2, with an overall MCM-2 LI of 92% (range 80–98%). The author also noted high MCM-2 LI values in the surface layers in all cases. Moreover, small clumps of sloughed immune positive epithelial cells were frequently identified at the surface of OSCCs. According to Shalash HN [26] all cases of OSCC demonstrated positive MCM-2 immunoreactivity with variable degree & site specificity of positivity. Most of the well differentiated cases showed a nuclear MCM-2 expression, which was expressed along the periphery of the epithelial cell nests, and at the invasive fronts. Zargoun IM et al. [30] found expression of MCM2 high and significant in all samples. Similarly, Zakaria HS et al. [28] found early invasion of squamous cell carcinoma (SCC), MCM-2 was distributed in all dysplastic epithelial cells and in cells invading the connective tissue.

Rendon A et al. [25] expression of MCM2 in the OSCC samples was seen in a high number of epithelial cells with stronger staining intensities at the invasive front. The authors also noted expression of MCM2 around the periphery of the islands in presence of keratin pearls; these findings were also reported by Razavi SM et al. [27]. He also reported that the MCM-2 expression in the OSCC samples was seen in a high number of epithelial cells with stronger staining intensities at the invasive front.

According to Li Ji et al. [21] the overall difference in MCM2 mRNA expression among the different grades of the precancerous epithelial dysplasia of the tongue was highly statistically significant. MCM2 mRNA levels were significantly higher in OSCC than in mild epithelia dysplasia. Kodani I et al. [22, 23] noted MCM2 immunoreactivity in the OSCCs, mainly in the peripheral portions of the cancer nests in the well and moderately differentiated OSCCs. MCM2 LI increased with progression to poorly differentiated squamous cell carcinoma, and this increase was significant.

Gene Expression in Oral Epithelial Dysplasia

5 studies evaluated Gene expression of MCM2 in oral epithelial dysplasia (OED) Li Ji [21], Kodani I [22], Scott IS [24], Rendon AT [25] and Zakaria HS [28]. According

to Scott IS et al. [21] MCM-2 was expressed at a higher frequency in all layers of the epithelium. When labelling index scores of MCM-2 and Ki-67 were compared, a similar pattern of variation was observed, although the overall MCM-2 LI values were consistently higher than those for Ki-67.

Rendon AT et al. [25] found that in all OED samples there was a higher expression of MCM2 when compared to Ki-67 and geminin. MCM2 expression extended from the basal and supra basal compartments to the mid-prickle cell region and in some cases to the surface layers. Expression of MCM2 was higher in the OED that progressed to OSCC than in those that did not progress. However, according to Li Ji et al. [21] the overall difference in MCM2 mRNA gene expression among the different grades of the precancerous epithelia dysplasia of the tongue was highly statistically significant. MCM2 mRNA levels were significantly higher in SCC than in mild epithelia dysplasia. In contrast, in another study by Zakaria HS [28] in mild dysplasia, the cases showed intense nuclear MCM-2 expression in both the basal and the supra-basal cells. In moderate dysplasia, the cases showed positive immunostaining of MCM-2 till the middle third of the epithelium. In severe dysplasia, all cases revealed intense MCM-2 immunoreactivity in all layers of the epithelium. However, in 2001 Kodani I et al. [22] noted high values of MCM2 in dysplasia.

Risk of Bias

Figure 2 summarises the results of the quality assessment of the included studies. 10 studies were classed as being at low risk of bias across all domains, only 1 study by Shalash HN [26] 2012 was unclear in mentioning the method of selection of patients. All 10 studies were at low concern for applicability across the three domains in patient selection, the index test, and the reference standard used. Individual assessment for each study is provided in Fig. 3.

Discussion

Oral carcinomas are known to have an extremely varied heterogenous nature rendering them difficult to manage. This has created a desire for much needed prompt early diagnosis in identifying the tumour. Early diagnosis could help establish tumour control and reduce the need for cosmetic restoration after invasive surgeries. Advances in understanding the molecular biology of oral carcinomas has led; to the search for molecular markers that are useful in diagnosis and predicting oral treatment outcomes. The survival rates of patients presenting with advanced stage tumours fluctuates between 30 and 40% and hence novel

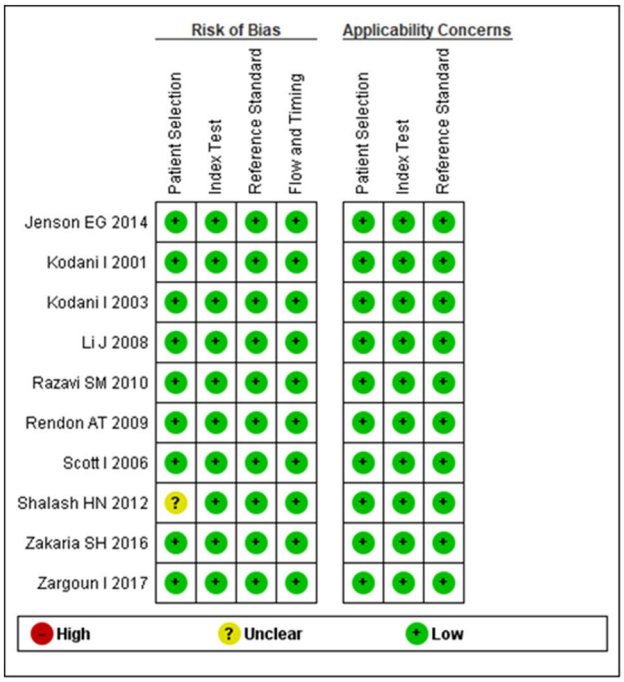

Fig. 2 Risk of bias and applicability concerns summary

strategies need to be developed for management of these patients.

Ten studies were identified for inclusion, evaluating the expression of diagnostic accuracy of MCM2 protein in squamous cell carcinoma. These studies were diverse in nature with substantial variations in population setting from countries like Japan, UK, China, Egypt, USA, and Iran and published over a wide span of 16 years from 2001 to 2017. The type of include sample also varied in nature, 6 of the included studies only mentioned the tissue and not the specific site, for instance Kodani I et al. [22] in 2001 and 2003, Scott I [24], Rendon A [25], Shalash H [26] mentioned only inclusion of oral tissues. Whereas only 1 author Li J et al. [21] specifically mentioned the included tissue site i.e., Tongue. When we compared the method of identification used for identifying MCM2 gene expression 7 studies mentioned that they had used IHC technique they were Kodani I [22], Scott I [24], Rendon A [25], Shalash H [26], Razavi S [27], Zakaria S [28] and Jenson E [29]. The remaining 2 studies used different techniques Li J et al. [21] used RTPCR and Zorgoun I [30] used microarray technique to identify MCM2 gene. However, Zorgoun I et al. [30] used both techniques IHC technique and Microarray to identify MCM2 gene.

Studies have suggested that molecular markers may be able to reveal the presence of cellular abnormalities within epithelium, or residual tumour following surgical resection, and that such identification may have utility in the clinical decision-making process following resection. Kodani I et al.

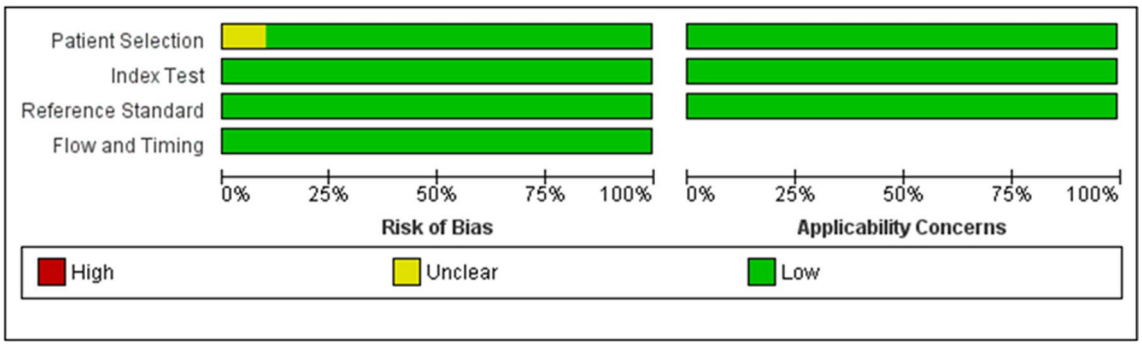

Fig. 3 Risk of bias and applicability concerns graph

[23] in 2001 indicated that the rate of cell proliferation and cell death altered with morphological changes. According to the authors, the frequency of TUNEL-positive cells increased from normal to dysplasia and then decreased in SCC. The oral dysplasia was characterized by a significantly lower rate of cell proliferation and a higher rate of cell death than oral SCCs. 13 of the dysplasia included in the study subsequently developed invasive SCCs during an interval of 48 months. The 13 dysplasia which developed SCCs showed significantly higher LI of MCM2.

Kodani I et al. [23] also compared their findings with a few other reports and found that MCM immunoreactivity had been reported as a novel marker for proliferating cells [31, 32]. In a separate study, Todorov et al. [33] examined a variety of human tumours and found that MCM2 was detectable by immunoblotting in 97% of the examined tumours but was present only in 27% of the corresponding normal tissues. In the same study, Todorov T et al. [33] also reported that the levels of MCM2 mRNA and protein remained constant during the cell cycle in tested human cell lines but decreased markedly in cells with a lower proliferation rate. The level of MCM2 mRNA was found to decrease dramatically during in vitro differentiation of human myeloblastic HL-60 cells. This could explain the higher LI of MCM2 in the dysplasias and SCCs.

Another study in 2002 by Kodani I et al. [23] demonstrated that the T-size, histologic differentiation, and the mode of carcinoma invasion in oral SCCs correlated with the frequency of tumor metastasis. According, to the authors morphologic and histopathologic findings, provide useful information on patient prognosis with oral SCCs. Kodani I et al. [23] showed that the formation of poorly histologically differentiated carcinomas or diffused invasions was likely with SCC expression or high levels of MCM2. The LI of MCM2 was higher than that of the other markers included in the study, regardless of the histologic differentiation type and the mode of carcinoma invasion grade. In another study Davis RJ et al. [34] showed the usefulness of MCM2, the authors developed a non-invasive, stool-based assay that could identify colorectal cancer by the detection of MCM2 expression in colonocytes retrieved from the faecal surface.

MCM2 is expressed in normal or neoplastic cells in the early G1 phase. Thus, MCM2 provides a more reliable and useful means of rapidly evaluating the growth fraction of normal and neoplastic cell populations during other phases than G0 and the mitotic phase. Thus, Kodani I et al. [23] in 2001 and 2002 in both the study concluded that MCM2 might be useful markers to predict the malignant transformation of oral dysplasias to SCCs.

Razavi SM et al. [27] in 2015 investigated three different oral pathologic lesions and compared them with normal oral mucosa. Their findings indicated that the MCM-2 expression was significantly higher in OSCC than in other oral categories. According to the authors, MCM-2 expression in NOM and OBK epithelium was mainly in the basal and parabasal layers, while it was absent from other layers. In a similar study, Rendon TR et al. [25] also investigated MCM-2 expression in NOM, OED, and OSCC and reported that MCM-2 was a useful marker. According to Rendon TR, the location of MCM-2 in NOM cases was mainly at the supra-basal compartment [5], and these results were similar to the findings of Razavi SM et al. [27]. Similarly, Shalash HN et al. [26] found that the immunohistochemical reactivity of MCM-2 in the normal control specimens was expressed mainly in the basal and supra-basal cells of the normal stratified squamous epithelium, with very few reactive cells in the middle third and a negative reaction in the superficial third. These findings indicate that cell division was confined to the basal and supra-basal cells, whereas the superficial cells had lost their proliferative ability.

However, Scott IS et al. [24] indicated a higher MCM-2 expression in the superficial layers of moderate/severe dysplasia and OSCC compared to benign keratosis/mild dysplasia. And a mild dysplasia with a high frequency of MCM-2 expression at all layers of the epithelium in this study. Scott IS et al. observed and suggested that MCMs markers were more likely to be more sensitive biomarkers for cytological diagnosis of oral malignancy. Their data also

suggested that the value of MCM immunocytochemistry in the analysis of oral smears is likely to be similar to that for other cytological samples, such as smears of the cervix and larynx, where detection of MCMs enables dysplastic and malignant cells to be detected with a high degree of sensitivity and specificity [35]. Whereas Zakaria S et al. [28] found that immunohistochemical reactivity in the normal control specimens was expressed mainly in the basal cells and a few cells of the prickle cell layer. According to the authors, all cases of mild and moderate dysplasia showed MCM-2 immunoreactivity in the basal, the supra-basal, and most of the prickle cell layer.

Five studies evaluated expression of MCM2 in Oral epithelial dysplasia. MCM2 was increased in cases of OED transforming into OSCC as compared to those without progression. In mild OED expression was seen in basal and suprabasal layers. In moderate OED, middle third, and in severe OED, all layers showed expression of MCM2. Hence it can be concluded that MCM2 protein can be used as a diagnostic marker for the detection of OSCC and chances of OED transforming into OSCC.

A higher Mcm-2 LI in OSCC compared to NOM was also reported by 5 other authors included in the review they were Kodani I [23], Rendon AT [25], Razavi SM [27], and Zargoun IM [30]. Most of these studies indicated that an increasing number of cells enter the proliferation cycle during tumorigenesis. The invasive front is composed of tumour subpopulations with higher proliferative activity. Furthermore, Li J et al. [21], Rendon AT [25] and Razavi SM [27] also reported that the MCM-2 was significantly higher in OSCC than in OED, OBK and NOM confirming that the application of this marker in differentiating malignant lesions from benign lesions is advantageous.

Li J et al. [21] pointed out that MCM2 expression was dependent on the grade of dysplasia, lymph node status and clinical stage. Quantitative real-time PCR analysis showed that MCM2 mRNA expression was significantly higher in tongue SCC than in epithelia dysplasia. These observations were also agreed by Kodani I et al. [22] who reported that overexpression of MCM2 was detected in dysplastic squamous epithelia cells, and the level increased with the advancement of dysplasia. An in a detailed analysis by Li J et al. [21] revealed a highly significant relationship between the level of MCM2 mRNA expression in mild epithelia dysplasia, severe epithelia dysplasia, and malignant tongue SCC. These findings revealed that MCM2 overexpression correlated with the severity of dysplasia and associated with progression of lymph node and clinical stage in patients with tongue SCC. Thus, making MCM2 a useful marker for evaluating tumor aggressiveness in tongue SCC.

Shalash H et al. [26] detected MCM-2 in all 30 tissue sections of OSCC. Almost similar percentages of MCM-2 expression were previously detected in laryngeal squamous epithelial lesions by Gouvea AF et al. [37] in 2010, in epithelial ovarian tumours by Gakiopoulou HP et al. [38] in 2007, breast cancers Gonzalez MA et al. [39] in 2003. This positive MCM-2 immunoreaction was detected in the nuclei of the tumour cells in all the grades of OSCC, and this was in accordance with the findings obtained by Kodani I et al. [23], Rendon AT et al. [25], Zakaria S et al. [28], Chatrath P et al. [35]. This could be explained by the notion that when cells exit mitosis, these newly synthesized MCM proteins accumulate in the nucleus (early G1 phase) and assemble into pre-replicative complexes [40, 41]. These findings suggest that MCM-2 played a significant role in oral carcinogenesis.

When epithelial thickness was taken into consideration Zakaria S et al. [28] in 2016 found all cases of severe dysplasia showed intense MCM-2 expression in the whole epithelial thickness (top to bottom). In cases of early SCC, the expression was much more intense. MCM-2 was expressed in the whole thickness of the epithelium in severe cases, early SCC showed higher and more intense expression; this could be of importance in the early detection of SCC. According to Zakaria S et al. [28] MCM-2 expression increased from normal mucosa to hyperplasia and from hyperplasia to dysplasia. Similar results were also obtained when different body specimens were taken into consideration. Tan DF et al. [42] concluded that MCM-2 is a promising marker for premalignant lesions of the lung. Similarly, Sirieix PS et al. [43] who studied MCM-2 expression in dysplastic and nondysplastic Barrett epithelium, suggested that the expression of MCM-2 increased gradually as the tissue progressed through the tumorigenic stages.

From the findings, it can be established that MCM2 can be used as a therapeutic target for treatment of cancer by slowing down the tumorigenesis and preventing the conversion of OED to OSCC.

Summary of Findings

MCM2 protein is involved in DNA replication and prognosis of neoplasm. This systematic review was performed with an aim to evaluate the expression of MCM2 in Oral epithelial dysplasia and Oral squamous cell carcinoma. After applying a stringent search strategy, 10 studies were included in the review. Study data was collected for studies conducted in Japan, UK, China. Egypt, USA, Iran from 2001–2017. Total 901 samples were included. 6 out of 10 studies mentioned the study design mostly were analytical study. Only one study design mentioned the site. 8 study design used IHC. 1 study design used RT-PCR. One design used both tissue microarray and IHC. Three groups were assessed for expression of MCM2 protein: normal oral mucosa, Oral epithelial dysplasia and Oral squamous cell carcinoma.

In normal oral mucosa, there was absent to mild staining mostly restricted to basal and parabasal layers. In OSCC, widespread expression of MCM2 with increased expression in surface layer was seen. Five studies evaluated expression of MCM2 in Oral epithelial dysplasia. MCM2 progressively increased in cases of mild to moderate to severe dysplasia. Hence it can be concluded that MCM2 protien can be used as a diagnostic marker for detection of OSCC and chances of OED transforming into OSCC.

Conclusion

We found that, overall, the LI of all proteins increased progressively from NOM to OED to OSCC. Most studies indicated that Mcm-2 has the potential to be applied as a marker in differentiating oral pathologies. Considering its overexpression in OSCC, there exists the possibility of applicability of Mcm-2 in molecular target therapy in patients with OSCC. These proteins are useful diagnostic markers for distinguishing malignant from benign epithelial dysplasia and for early detection and diagnosis of SCC as an adjunct to clinicopathological parameters. More studies with greater sample size and different grades of pathologies are recommended to achieve more precise results in this field.

Author Contributions MSA, ZID, SRN: Study concepts; SRN, MSA: Study Design; JVT, SMD: Data acquisition.

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Availability of Data and Material (Data Transparency) On request.

Declarations

Conflict of interest No conflict of interest to disclose.

Ethics Approval No procedures were involved on the participants as the part of the study as it is systematic review on the data available.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

References

1. Gupta B, Johnson NW (2017) Oral cancer: Indian pandemic. Br Dent J 222:497
2. Wen B, Zhang F-L, Yang W, Zhou W-W, Wei Y-C, Deng S-C, Li N, Huang Y-X, Cheng X-J (2017) High-resolution array based heterogeneity and clonality profiling of oral squamous cell carcinoma: genetic relationship between metastatic primary tumor and the paired lymph node metastases. Int J Clin Exp Pathol 10(3):3677–3690
3. Ganly I, Ibrahimpasic T, Patel SG, Shah JP (2009) Tumors of the oral cavity. Princ Pract Head Neck Surg Oncol 2:180–191
4. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F (2019) Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 144(8):1941–1953
5. Schantz SP, Yu GP (2002) Head and neck cancer incidence trends in young Americans, 1973–1997, with special analysis for tongue cancer. Arch Otolaryngol Head Neck Surg 128:268–274
6. Choi S, Myers JN (2008) Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res 87(1):14–32
7. Patthi B, Kumar JK, Singla A, Gupta R, Prasad M, Ali I, Dhama K, Niraj LK (2017) Global search trends of oral problems using google trends from 2004 to 2016: an exploratory analysis. J Clin Diagn Res: JCDR 11(9):ZC12
8. Graham S, Dayal H, Rohrer T et al (1977) Dentition, diet, tobacco, and alcohol in the epidemiology of oral cancer. J Natl Cancer Inst 59(6):1611–1618
9. Muttagi SS, Chaturvedi P, Gaikwad R et al (2012) Head and neck squamous cell carcinoma in chronic areca nut chewing Indian women: case series and review of literature. Indian J Med Paediatr Oncol 33(1):32–35
10. Krishna A, Singh S, Kumar V et al (2015) Molecular concept in human oral cancer. Natl J Maxillofac Surg 6(1):9–15
11. Neville BW, Damm DD, Allen CM et al (2009) Oral and maxillofacial pathology Saunders. Elsevier, St. Louis, Mo
12. Weatherspoon DJ, Chattopadhyay A, Boroumand S et al (2015) Oral cavity and oropharyngeal cancer incidence trends and disparities in the United States: 2000–2010. Cancer Epidemiol 39(4):497–504
13. Forsburg SL (2004) Eukaryotic MCM proteins: beyond replication initiation. Microbiol Mol Biol Rev 68:109–131
14. Maine GT, Sinha P, Tye BK (1984) Mutants of S. cerevisiae defective in the maintenance of minichromosomes. Genetics 106:365–85
15. Blow JJ, Dutta A (2005) Preventing re-replication of chromosomal DNA. Nat Rev Mol Cell Biol 6:476–486
16. Kerdpon D, Jantharapattana K, Sriplung H (2018) Factors related to diagnostic delay of oral squamous cell carcinoma in southern Thailand: revisited. Oral Dis 24(3):347–354
17. Boguspour N, Goldstein LB (2018) Oral cancer diagnosis: from biopsy to metabolomics. EC Dent Sci 17:800–803
18. El-Bayoumy K, Chen K-M, Zhang S-M, Sun Y-W, Amin S, Stoner G, Guttenplan JB (2017) Carcinogenesis of the oral cavity: environmental causes and potential prevention by black raspberry. Chem Res Toxicol 30(1):126–144
19. Alison MR, Hunt T, Forbes SJ (2002) Minichromosome maintenance (MCM) proteins may be pre-cancer markers. Gut 50:290–291
20. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB et al (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Int Med 155(8):529–36
21. Li JN, Feng CJ, Lu YJ, Li HJ, Tu Z, Liao GQ, Liang C (2008) mRNA expression of the DNA replication-initiation proteins in epithelial dysplasia and squamous cell carcinoma of the tongue. BMC Cancer 30(8):395
22. Kodani I, Shomori K, Osaki M, Kuratate I, Ryoke K, Ito H (2001) Expression of minichromosome maintenance 2 (MCM2), Ki-67, and cell-cycle-related molecules, and apoptosis in the normal-dysplasia-carcinoma sequence of the oral mucosa. Pathobiology 69(3):150–158

23. Kodani I, Osaki M, Shomori K, Araki K, Goto E, Ryoke K, Ito H (2003) Minichromosome maintenance 2 expression is correlated with mode of invasion and prognosis in oral squamous cell carcinomas. J Oral Pathol Med 32(8):468–474
24. Scott IS, Odell E, Chatrath P, Morris LS, Davies RJ, Vowler SL, Laskey RA, Coleman N (2006) A minimally invasive immunocytochemical approach to early detection of oral squamous cell carcinoma and dysplasia. Br J Cancer 94(8):1170–1175
25. Torres-Rendon A, Roy S, Craig GT, Speight PM (2009) Expression of Mcm2, geminin and Ki67 in normal oral mucosa, oral epithelial dysplasias and their corresponding squamous-cell carcinomas. Br J Cancer 100(7):1128–1134
26. Shalash HN, Draz A, El-Rouby D (2012) Immunohistochemical evaluation of the proliferation marker Mcm-2 in oral squamous cell carcinoma. Aust J Basic Appl Sci 6(12):445–452
27. Razavi SM, Jafari M, Heidarpoor M, Khalesi S (2015) Minichromosome maintenance-2 (MCM2) expression differentiates oral squamous cell carcinoma from pre-cancerous lesions. Malays J Pathol 37(3):253–258
28. Zakaria SH, Farag HA, Khater DS (2018) Immunohistochemical expression of MCM-2 in oral epithelial dysplasias. Appl Immunohistochem Mol Morphol 26(7):509–513
29. Jenson EG, Baker M, Paydarfar JA, Gosselin BJ, Li Z, Black CC (2014) MCM2/TOP2A (ProExC) immunohistochemistry as a predictive marker in head and neck mucosal biopsies. Pathol Res Pract 210(6):346–350
30. Zargoun IM, Bingle L, Speight PM (2017) DNA ploidy and cell cycle protein expression in oral squamous cell carcinomas with and without lymph node metastases. J Oral Pathol Med 46(9):738–743
31. Williams GH, Romanowski P, Morris L, Madine M, Mills AD, Stoeber K, Marr J, Laskey RA, Coleman N (1998) Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc Natl Acad Sci USA 95:14932–14937
32. Freeman A, Morris LS, Mills AD, Stoeber K, Laskey RA, Williams GH, Coleman N (1999) Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin Cancer Res 5:2121–2132
33. Todorov IT, Werness BA, Wang HQ, Buddharaju LN, Todorova PD, Slocum HK, Brooks JS, Huberman JA (1998) HsMCM2/BM28: a novel proliferation marker for human tumors and normal tissues. Lab Invest 78:73–78
34. Davies RJ, Freeman A, Morris LS, Bingham S, Dilworth S, Scott I, Laskey RA, Miller R, Coleman N (2002) Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. Lancet 359(9321):1917–1919
35. Chatrath P, Scott IS, Morris LS, Davies RJ, Rushbrook SM, Bird K, Vowler SL, Grant JW, Saeed IT, Howard D, Laskey RA, Coleman N (2003) Aberrant expression of minichromosome maintenance protein-2 and Ki67 in laryngeal squamous epithelial lesions. Br J Cancer 89(6):1048–1054
36. Tamura T, Shomori K, Haruki T, Nosaka K, Hamamoto Y, Shiomi T, Ryoke K, Ito H (2010) Minichromosome maintenance-7 and geminin are reliable prognostic markers in patients with oral squamous cell carcinoma: immunohistochemical study. J Oral Pathol Med 39(4):328–334
37. Gouvea AF, Vargas PA, Coletta RD, Jorge J, Lopez MA (2010) Clinicopathological features and immunohistochemical expression of p53, Ki-67, MCM-2 and MCM-5 in proliferative leukoplakia. J Oral Pathol Med 39:447–452
38. Gakiopoulou H, Korkolopoulou P, Levidou G, Thymara I, Saetta A, Piperi C, Givalos N, Vassilopoulos I et al (2007) Minichromosome maintenance proteins 2 and 5 in non-benign epithelial ovarian tumours: relationship with cell cycle regulators and prognostic implications. Br J Cancer 97:1124–1134
39. Gonzalez MA, Pinder SE, Callagy G, Vowler SL, Morris LS, Bird K, Bell JA, Laskey RA, Coleman N (2003) Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer. J Clin Oncol 21:4306–4313
40. Labib K, Kearsey SE, Diffley JF (2001) MCM2-7 proteins are essential components of prereplicative complexes that accumulate cooperatively in the nucleus during G1-phase and are required to establish, but not maintain, the S-phase checkpoint. Mol Biol Cell 12:3658–3667
41. Going JJ, Keith WN, Neilson L et al (2002) Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. Gut 50:373–377
42. Tan DF, Huberman JA, Hyland A et al (2001) MCM2—a promising marker for premalignant lesions of the lung: a cohort study. BMC Cancer 1:6
43. Sirieix PS, O'Donovan M, Brown J et al (2003) Surface expression of minichromosome maintenance proteins provides a novel method for detecting patients at risk for developing adenocarcinoma in Barrett's esophagus. Clin Cancer Res 9:2560–2566