Innovations

Chemiluminescence as a diagnostic aid for dysplasia in oral potentially malignant disorders- A systematic review

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Abstract: Aim-*The aim of this systematic review is to evaluate the existing literature if there are studies done using chemiluminescence as a diagnostic tool for detecting dysplastic changes in oral potentially malignant disorders.* ***Background-****Chemiluminescence is the production of light as a result of chemical reaction. Oral Potentially Malignant Disorders (OPMDs) includes both precancerous lesions and conditions which carry an increased risk of cancer. Chemiluminescence is a light based detection system and is very useful for detecting OPMDs for earlier intervention. This technique detects metabolic and structural changes in the mucosal tissues which by nature have different absorbable and reflective properties when exposed to various forms of light sources. Since it is a non-invasive procedure, it can be used to evaluate dysplastic changes in various OPMDs at their initial stage for timely intervention.* ***Methods-****The search was done using the MesSH terms and keyword search in the electronic databases namely PubMed, PubMed Central and Science Direct.A total of 23 articles were chosen after initial screening of the title.Then based on the inclusion and exclusion criteria and the availability of the full texts,a total of 6 articles were included in this systematic review.* ***Conclusion-****Literature based evidence states that chemiluminescence based screening devices serve as non-invasive, simple chair side diagnostic tools for detecting dysplasia in suspicious lesions which may carry an increased risk for malignancy.It can also be used in remote rural setups where health facilities are limited due to resources and mass screening camps as a screening tool for identifying dysplasias in patients with oral potentially malignant disorders to aid in timely intervention.*

Key words*: 1.Chemiluminescence, 2.dysplasia, 3.OPMDs*

Research question

Can chemiluminescence be used as a diagnostic aid for detecting dysplastic changes in Oral Potentially Malignant Disorders?

Structured question

P (Population): Patients with Oral Potentially Malignant Disorders (OPMDs)

I (Intervention): Chemiluminescence

C (Comparison): Histopathological examination

O (Outcome): Dysplasia

1. Introduction

1.1 Background

The World Health Organisation (WHO) workshop in 2005 redefined all oral lesions with a potential for malignant transformation to be grouped under the title "Potentially Malignant Disorders. The traditional terminologies of premalignant lesions and premalignant conditions have been abandoned. Oral Potentially Malignant Disorders (PMD) includes a variety of lesions and conditions characterised by an increased risk of malignant transformation to oral squamous cell carcinoma.It is generally accepted that the histopathological features of a given lesion,especially the presence of epithelial dysplasia are currently the most useful indicators of malignant transformation risk.In this regard,the clinical features of PMD can show considerable variation within the same histopathologically defined entity that may be critical to the likelihood of progression towards malignancy.[1]It has been reported in literatures that The International Agency for Research on Cancer(IARC) and the World Health Organisation (WHO) have stated that out of 15 million oral cancer cases, one third of it can be prevented by timely intervention with the help of appropriate screening strategies. Oral cancer is the sixth most common malignancy reported worldwide and makes around 40 % of the cancers globally. [2]

The main risk factors for oral cancer include tobacco and alcohol consumption. [3]The prognosis of these patients are also further complicated by the rate of second primary tumours with the emerging concept of 'field cancerisation'. [4] A thorough, detailed case history and appropriate clinical examination always serves as the first gold standard of examination. It has been a widely accepted fact that histopathological examination always remains as the confirmatory and standard methodology for diagnosis of potentially malignant disorders, to predict the risk of their malignant transformation rate, for diagnosis of carcinoma in-situ and carcinomas. [5]Various non-invasive screening tools are now being developed and they serve as adjunct diagnostic aids for detecting potentially malignant disorders and oral cancers. [6] Recent trends suggest that salivary biomarkers like micro RNA also play an important role in the prediction of the malignant risk transformation of these OPMDs. [7] Light based optical systems work on principles of chemiluminescence and others that utilise the tissue auto fluorescence property.[8]

Chemiluminescence is the process of emission of light as a result of chemical reaction wherein there may some amount of heat production also.[9] The term 'chemiluminescence' was coined by Eilhardt Weidemann in 1888 and the first incidence of chemiluminescence was reported in literatures by Henning Brand, a German physician with his discovery of phosphorous.[10] Chemiluminescence is characterized by a chemical reaction which causes the transition of an electron from its ground state to an excited electronic state.The two commonly used chemiluminescent systems include the luminol based and peroxy-oxalate based systems.[11]

Chemiluminescence has various applications such as it has been used in gas analysis for detecting impurities in air wherein chemiluminescence monitors have been used as universal nitrogen and sulfur

detectors for gas chromatography and capillary electrophoresis. [12] It has also been used for analysing inorganic and organic species in liquid phasesuch as in enzymatic and non-enzymatic reactions. [13] It is also useful in the detection and assay of biomolecules in ELISA and Western Blot.Chemiluminescence Western blots are usually probed with a primary antibody against the target protein. A secondary antibody labelled with HRP (horseradish peroxidase) enzyme is done next in the sequence. It is a highly sensitive protein detection method. The reporter enzymes combined with the chemiluminescent substrate make this method very ideal for quantitative assays.[14] Chemiluminescence also finds its applications in DNA sequencing and pyro sequencing and there are studies where they have used chemiluminescence detection method that used alkaline phosphatase label to the genomic DNA sequencing.[15] It is also used in combustion analysis where the rate of heat that is released is calculated by measuring the amount of light radiated from a flame at those particular wavelengths. Image processing algorithms have also been developed to characterize thiscombustion chemiluminescence. [16]Apart from this it is also available as toys, chemiluminescent kites, glow sticks for party decorations and is also used in emergency lighting. [17]

The technique of chemiluminescence applied in oral oncology for the detection of pre cancer and oral cancer is termed as lumenoscopy. The oral mucosal tissues by nature have different absorbance and reflective properties and when exposed to different light sources the metabolic and structural changes of these oral tissues can be detected. [18]

The procedure of chemiluminescent examination begins by rinsing the oral cavity with usually 1% acetic acid solution. The acetic acid solution acts as a cytoplasmic dehydrating agent .It removes the debris and also disrupts the glycoprotein barrier on the epithelium for the penetration of the light during chemiluminescent examination. Chemiluminescent illumination produces a blue-white light of wavelength around 430 nm and this light is absorbed by the cells of the normal mucosa whereas it is reflected back abnormal cells with altered nuclear cytoplasmic ratio as acetowhite hue. After the examination is done, the patient is asked to rinse the oral cavity with water for 20-30 seconds. [19]

Certain dyes like toluidine blue, tolonium chloride, and lugol's iodine can also be used in combination with this chemiluminescent examination for delineating the pathologic region. Literature reports various cross sectional studies where they had use this combination of chemiluminescence and vital staining procedures in diagnosis of dysplastic changes in oral potentially malignant disorders. The sensitivity, specificity and the overall accuracy was more when this combination was used in comparison to just chemiluminescent examination. [20]

This systematic review focuses on the studies done on chemiluminescence for the identification of dysplastic changes in oral potentially malignant disorders. In all the included studies, the results of the chemiluminescent examination have been compared with histopathological examination which was taken as the reference standard for the identification of these dysplastic changes.

2. Methodology

2.1 Search strategy for the identification of the studies

The search strategy was in accordance with the Cochrane guidelines for systematic reviews. Articles relevant to the search strategy were identified from search data bases of PubMed, PubMed Central, ScienceDirect, Cochrane Library and Google Scholar. Due to the limitation of studies on chemiluminescence as a diagnostic aid for dysplasia in oral potentially malignant disorders; therefore a timeline was not included in the search. The article search included only those published in English literature. Articles were screened on the basis of their titles following which duplicates were removed from other databases. The title of the articles and abstracts were reviewed. The full text of the selected articles were retrieved and further analysed.

2.2 Search methodology

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

Search (Eruption, Lichenoid) OR Eruptions, Lichenoid) OR Lichenoid Eruption) OR Licheniform Eruptions) OR Eruption, Licheniform) OR Eruptions, Licheniform) OR Licheniform Eruption) OR Oral Lichen Planus) OR Fibroses, Oral Submucous) OR Oral Submucous Fibroses) OR Submucous Fibrosis, Oral) OR Submucous Fibrosis, Oral) OR Oral Leukoplakia) OR Oral Leukoplakias) OR Leukokeratosis, Oral) OR Leukokeratosis, oral) OR Oral Leukokeratosis) OR Oral Leukokeratosis) OR Keratosis, Oral) OR Keratoses, Oral) OR Oral Keratoses) OR Oral Keratosis) OR Condition, Precancerous) OR Conditions, Precancerous) OR Precancerous Condition) OR Condition, Preneoplastic) OR Preneoplastic Condition) OR Preneoplastic Conditions) OR Conditions, Preneoplastic) OR Oral potentially malignant condition) OR Oral potentially malignant conditions) OR potentially malignant conditions) OR potentially malignant condition) OR potentially malignant lesions) OR Oral potentially malignant lesions) OR Oral potentially malignant disorder) OR Oral potentially malignant disorders) OR potentially malignant disorders) OR potentially malignant disorder) OR potentially malignant lesion) OR Oral potentially malignant lesion) OR Erythroplakia) OR Erythroplasia) OR Hairy Leukoplakia) OR Hairy Leukoplakia) OR Leukoplakias, Hairy) OR Leukoplakia, Hairy, Oral) OR Leukoplakia, Oral Hairy) OR Leukoplakias, Oral Hairy) OR Oral Hairy Leukoplakia) OR actinic cheilitis) OR actinic cheilosis) OR xeroderma pigmentosum) OR Kaposi Disease) OR Kaposis Disease) OR Kaposi's Disease) OR dyskeratosis congenital) OR candidal leukoplakia) OR candidal leukoplakia) OR sublingual keratosis) OR syphilitic leukoplakia) AND (Chemiluminescence) OR Chemiluminescence, Physical) OR Physical Chemiluminescence) OR Chemiluminescent Measurements) OR Chemiluminescent Measurement) OR Measurements, Chemiluminescent) OR Chemiluminescent Assays) OR Assay, Chemiluminescent) OR Assays, Chemiluminescent) OR Chemiluminescent Assay) OR Chemoluminescence Measurements) OR Chemoluminescence Measurement) OR Measurement, Chemoluminescence) OR Measurements, Chemoluminescence) OR Chemiluminescence Measurements) OR Chemiluminescence Measurement) OR Measurement, Chemiluminescence) OR Measurements, Chemiluminescence) AND (Biopsy) OR biopsy) OR pathologies) OR histopathology) OR oral pathology) OR oral biopsy) OR oral biopsies) OR oral histopathology) AND (Dysplasia) OR Dysplasias) OR Dysplastic changes) OR Dysplastic change) OR Dysplastic lesions) OR Dysplastic lesion) OR Dysplastic condition) OR Dysplastic conditions) OR Conditions, Dysplastic) OR Condition, Dysplastic) OR Lesion, Dysplastic) OR Lesions, Dysplastic) OR Dysplastic Potential)

2.3 Selection of studies

a) Inclusion criteria

1) Original studies onchemiluminescence as a diagnostic aid for dysplasia in oral potentially malignant disorders.
2) Studies which included histopathologically confirmed cases of oral potentially malignant disorders.
3) Studies which had included adjunct staining techniques along with chemiluminescent examination.
3) Studies in English literature were included.
4) Availability of full text articles

b) Exclusion criteria

1)Studies done in Oral Squamous Cell Carcinoma.
2) Studies where they have not considered histopathological examination as the reference standard.
3) Animal studies

3.Results

3.1 Literature evaluation

A total of seven studies were included in this systematic review (Fig 1) and were analysed in detail (Table 1). The distribution of the samples in the included studies is illustrated in Fig 2.

3.2Quality assessment of the studies

The search strategy yielded 7 articles based on the selection criteria following which their quality were assessed using QUADAS tool2. Quality assessment of diagnostic accuracy studies has four domains namely patient sampling, index test, reference standard and flow and timing. Each of these domains consists of two to four questions which were answered as "yes", "no" or "unclear". This data was fed into Review manager software namely in Revman5.3 to obtain a colour coded chart of risk of bias and applicability concern. (Fig 3)

3.2 Risk of bias and applicability concern

Studies done by Epstein et al(2007), Awan et al (2011), Kaemmerer et al (2014) and Bagga et al. (2018) had a low risk of bias whereas studies done by Chaudhry et al. (2014) and Nidhi Jain et al. (2017) had moderate risk of bias. A study by Suyambukesan et al. (2014) had high risk of bias. (Fig 4)

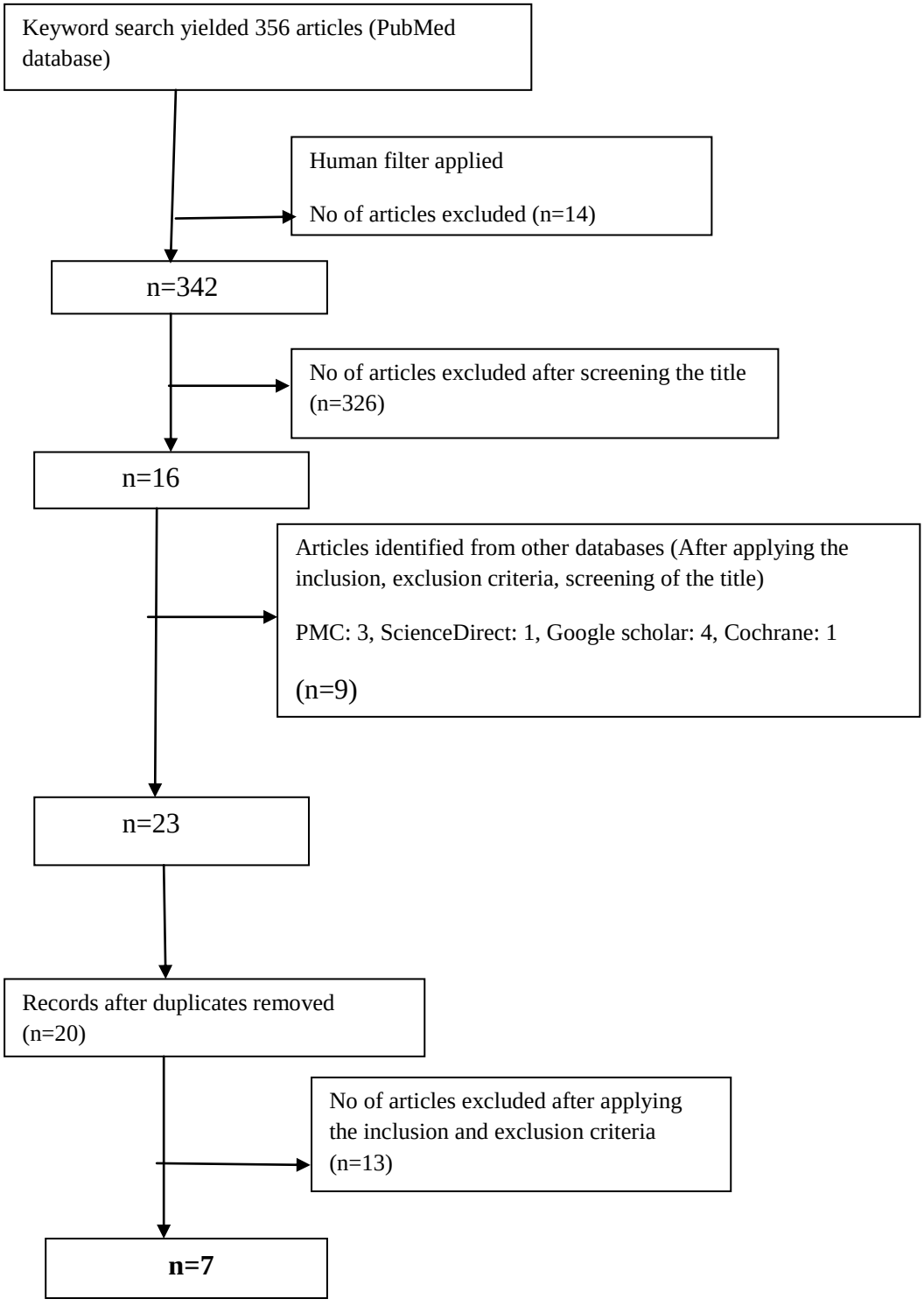

Fig 1: PRISMA Flow Diagram

Table 1: Characteristics of included studies

S.No	Author,Year	Study participants	Clinical Features	Habit history	Chemiluminescence examination	H/P examination	Inferences
1	Epstein et al. 2007	97 clinically suspicious lesions from 84 patients ,mean age:59.64(Standard deviation=12.53)	Wide variety of lesions such as lichen planus, lichenoid dysplasia, hyperplastic candidiasis,ulceration, hyperkeratosis, verrucous hyperplasia were included	i)15.48 %:current cigarette smokers ,41.67%:prior cigarette smokers ii)2/3rd of pts:consumed alcohol on regular basis iii) <10% of pts used cigar,pipes,chew or snuff	True positive : 20,False positive : 76	No dysplasia: 43,Mild dysplasia:19,Moderate dysplasia:15,Severe dysplasia:7,Carcinoma-in-situ:4,Squamous cell carcinoma:9	Chemiluminescent illumination increased the brightness and sharpness of the oral lesion in 61.8% of identified lesions.
2	Awan et al. 2011	126 patients: male-70,female-56(mean age:58.5+/-11.9yrs)	70 : Oral leukoplakia/erythroplakia, 32:Oral lichen planus,9:chronic hyperplastic candidiasis,13:Oral submucous fibrosis/frictional keratosis	61:current smokers,28:ex-smokers,92:alcohol users	Sensitivity: 77.3 %,Specificity:27.8%,PPV:39.5%, NPV:66.7%,AUC:0.53	No dysplasia:82,mild dysplasia:29,moderate dysplasia:8,severe dysplasia:7	Chemiluminescent illumination(Vizilite)had the ability to detect OPMD, it does not accurately delineate dysplastic lesions.The device can be used as a general oral mucosal examination system and may in particular improve the visualisation of leukoplakias.
3	Chaudhry et al. 2014	50 study subjects, males:37,females:13, age gp: 20-100 yrs.	37:Homogenous leukoplakia,13:Non-homogenous(speckled)leukoplakia	Smokeless tobacco(including betel nut):30,Smoking tobacco:16, Both:4	Sensitivity:93.75%,Specificity:55.56%,PPV:78.95%,NPV:83.3%,Overall accuracy:80%	36%:no dysplasia,64%:dysplastic(mild dysplasia-42%,moderate dysplasia-14%,severe dysplasia-4%,carcinoma in situ-4%	Chemiluminescent examination serves as an easy ,safe,minimally time consuming and non invasive technique ,it has only adjunctive utility and does not replace biopsy for the diagnosis of leukoplakia
4	Kaemmerer et al. 2014	44 pts(male:25,female:19),mean age: 60.4 yrs(SD:9.9)	50 oral lesions(as seen in 44 pts): oral lichen planus-31,leukoplakia-10,OSCC-9	Not mentioned	VL : Sensitivity: 100%,specificity:30%,PPV:26%, NPV:100% TB+VLP:Sensitivity:80%,specificity:97.5%, PPV: 89%, NPV: 95%	40: Reactive/inflammatory nature, 3:moderate dysplasia,7:OSCC	The adjunct of TB to VL reduces the number of false positives without increasing the rate of false negatives
5	Suyambukesan et al. 2014	70 pts:50-OPMD,20-no apparent lesions,males:59,females:11	20-normal oral mucosa,21-leukoplakias,16-oral lichen planus,13-oral submucous fibrosis	All 70 participants had H/O tobacco usage	Leukoplakia: Sensitivity-100%,specificity-100%,PPV:1,NPV:1 Oral lichen planus:Sensitivity-67%,specificity-77%,PPV:0.4,NPV:0.9,Oral submucous fibrosis: Sensitivity-100%,Specificity:57%,PPV:0.67, NPV:0.77	Dysplasia positive:24(23-True positive,1-False negative),Dysplasia negative:26(6-False positive, 20-True negative),Sensitivity: 96%, specificity: 77%, PPV: 0.79,NPV:0.95	Chemiluminescent(Vizilite)examination helps in mere visualisation of the oral cavity without identification of new lesions. It does not differentiate between keratotic,inflammatory, malignant or OPMDs.
6	Nidhi Jain et al. 2017	40 subjects,males-38,females-2,mean age:47.25 +/-8.89 yrs.	Clinically diagnosed having leukoplakia(sites:buccal mucosa(n=22),commissures(n=14),labial mucosa(n=2),tongue(n=1), palate(n=1)	27-tobacco chewers, 13-smokers	Sensitivity:100%,specificity:97.3%,PPV:100%,NPV:75%,Accuracy: 97.5%	Biopsy(positive)-37,Biopsy(negative)-3	Chemiluminescent examination can be used as a general oral mucosal examination system.The limitation of the study is that only leukoplakic lesions only were considered and did not include other lesions such as erythroplakia,oral lichen planus,oral submucous fibrosis,keratotic lesions and oral squamous cell carcinoma.
7	Bagga et al. 2018	100 subjects(93 % males,7% females),Age:15-72 yrs,(mean age of 34.92 yrs,S.D. +/-13.11)	Group A (Oral leukoplakia):50,Group B (oral submucous fibrosis):50	Group A (Betel nut quid mixed with lime chewers):28, Group B(Areca nut chewers):30,presence of beedi smoking,gutka chewers were also there in the study subjects	Chemiluminescence:-sensitivity:75 %,specificity:54.7% ,accuracy:68%, Toluidine blue:- sensitivity:57.4 %,specificity:44.1%,accuracy: 44 %	On comparison of chemiluminescence and toluidine blue with histopathology,no significant correlation was observed.	Chemiluminescence and toluidine blue cannot be compared with histopathology as these are adjunctive aids in early diagnosis of oral precancer and cancer. Hence their adjunctive value is of great importance to be used as a chair-side investigation and for mass screening of oral cancer.

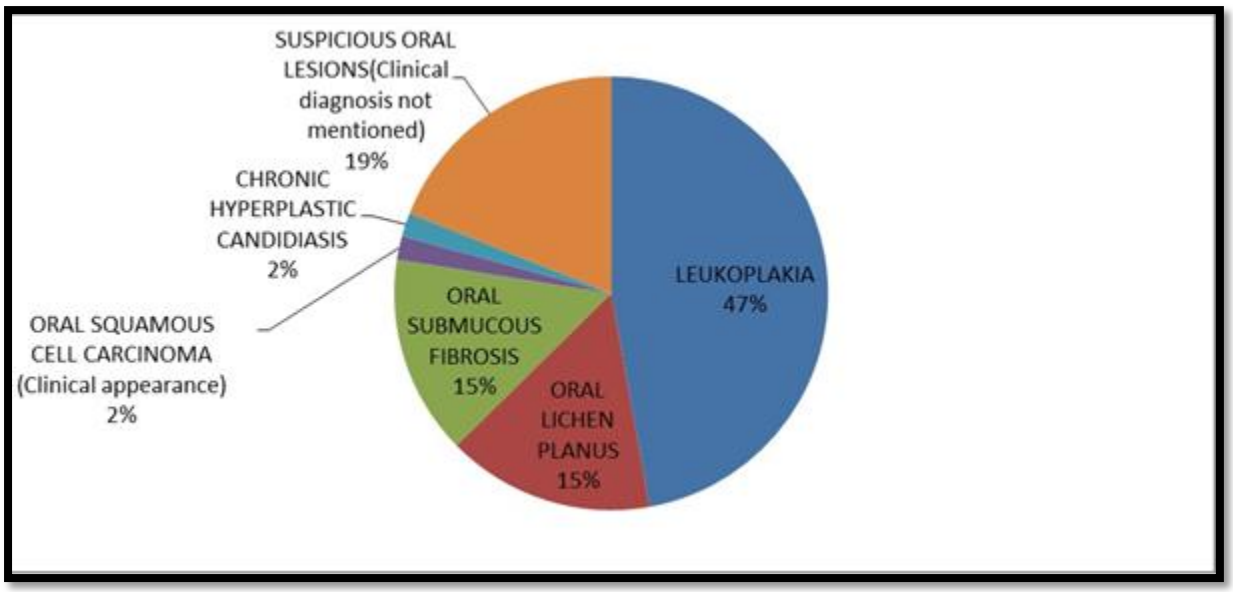

Fig 2: Sample distribution of included studies

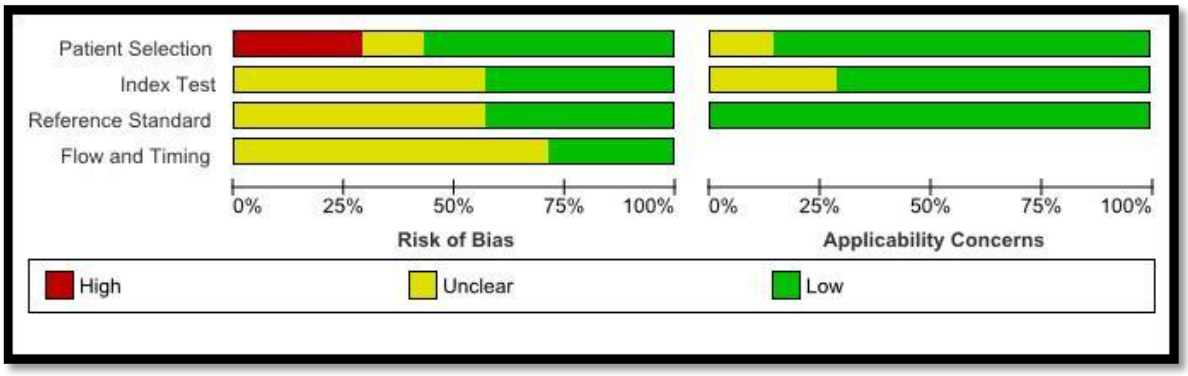

Fig 3: Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies

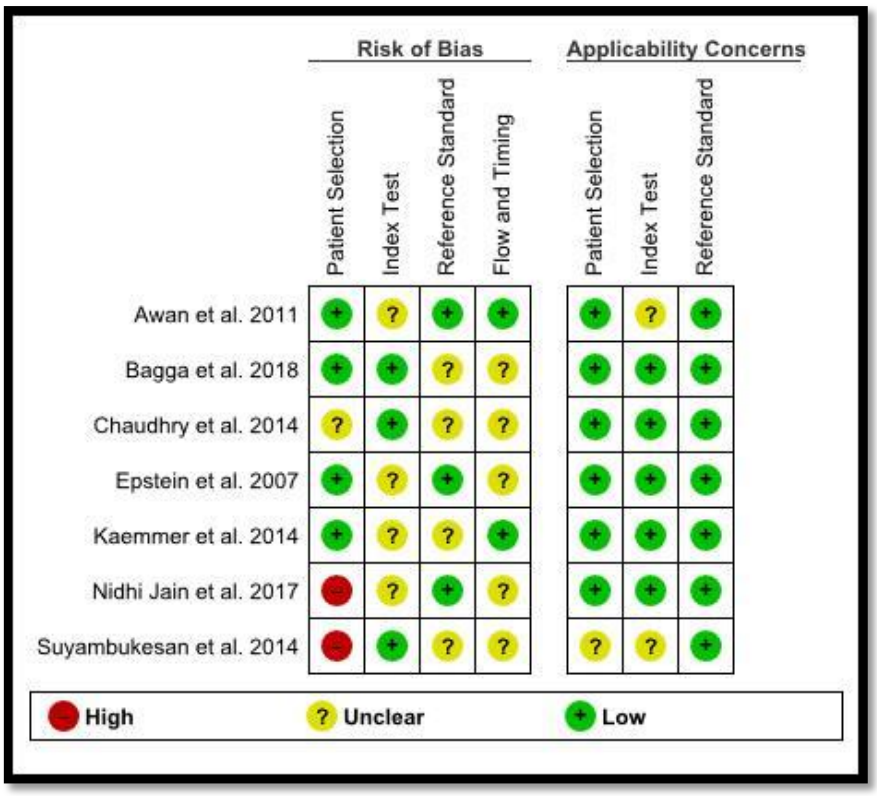

Fig 4: Risk of bias and applicability concerns summary: review authors' judgements about each domain for the included studies

4. Discussion

Studies on the application of chemiluminescence in oral potentially malignant disorders emphasise its usefulness as a non-invasive effective chair side diagnostic tool. Chemiluminescencent examination is a simple, non-invasive chair side diagnostic procedure for detecting dysplastic changes in oral potentially malignant disorders. [21] Awan et al. in 2011 evaluated the utility of chemiluminescence in the detection of OPMDs and benign keratosis. They found that chemiluminescent examination had a sensitivity of 77.3 %, however the specificity was only 27.8%.It was because they observed that non-dysplastic lesions (confirmed on histopathological examination) also showed acetowhitening leading to increase in the number of false positive results. In their study, they had used ViziLite as the chemiluminescent source and found that ViziLite did not have the ability to discriminate high risk and low risk lesions among OPMDs. [22] Chaudhry et al. in 2014 evaluated the efficacy of chemiluminescence for the diagnosis of leukoplakia. They found that chemiluminescence had 93.75% sensitivity and 55.56 % specificity in detecting dysplastic changes in leukoplakia in comparison to histopathological examination. The sensitivity and specificity was increased when non homogenous leukoplakias were screened with chemiluminescent examination. [23]A study on the analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue by Epstein et al. in 2007 evaluated four characteristics of lesions under chemiluminescent examination namely brightness, sharpness, texture and size. They found that in 97 clinically suspicious lesions, the chemiluminescent examination improved the brightness and/or sharpness of the margin in 61.8% of the identified lesions. [24] Kaemmerer et al. in 2014 used a chemiluminescent light system in combination with toluidine blue to assess suspicious oral lesions. They found that sensitivity was 100 % and specificity was 30 % when chemiluminescence was

used to dysplastic lesions. They also found that when a combination of both chemiluminescent examination and toluidine blue stain were used the sensitivity was 80% while specificity was 97.5 %.They also did a systematic review to validate their results and it was reported in literatures that chemiluminescent illumination (ViziLite) had a mean sensitivity of 82 % and specificity of 25.8 %. and when a combination of ViziLite illumination and toluidine blue stain were used as Vizilite Plus system, the mean sensitivity and specificity were 74 % and 69 % respectively which states the specificity (the number of false positives) can be increased when toluidine blue stain was used with chemiluminescent illumination. [25] A study on the critical scrutiny of the visualization and detection of Oral Potentially Malignant Disorders by chemiluminescent illumination by Suyambukesan et al. in 2014 was conducted on 50 patients with OPMDs and 20 subjects with no apparent lesions. They found that chemiluminescent (ViziLite) examination had 100 % sensitivity and specificity for leukoplakia, however it wasn't quite for lichen planus and oral submucous fibrosis. [26] Nidhi Jain et al. in 2017 evaluated the role of chemiluminescence examination as non-invasive diagnostic tool in early detection of leukoplakia. They found that the chemiluminesecent examination (ViziLite) and toluidine blue combination demonstrated that the sensitivity was 100 % and specificity was 97.3 % in detecting dysplastic changes in leukoplakia lesions.[27] A study on the comparative morphological analysis of precancerous lesions and conditions by clinical examination, chemiluminescence and toluidine blue by Bagga et al. in 2018 found that the sensitivity , specificity and accuracy of chemiluminescence was 75 %, 54.7 % and 68 % respectively in comparison to the 57.4 %, 44.1 % and 44% of toluidine blue. However they had concluded that chemiluminescence and toluidine blue can only serve as adjunctive aids for early diagnosis of oral precancer and cancer and cannot be compared to histopathological examination. Their adjunctive role is of great importance to be used as a chair side investigation and for mass screening of oral cancer. [28] When oral cancer is diagnosed is diagnosed at a late stage, the prognosis is poor and the risks of morbidity and mortality rates are also significantly higher. It has been stated that the mortality rates in oral cancer can be reduced by primary prevention, secondary prevention by early screening and detection methods and by improving the treatment methodologies.[29] Diagnostic adjunct aids in the detection of oral cancer include brush biopsy, vital tissue staining, narrow emission tissue fluorescence , confocal in-vivo microscopy, tissue fluorescence spectroscopy, colposcopy, salivary biomarkers, cell and tissue markers, elastography, surface enhanced Raman spectroscopy, optical coherence tomography, positron emission tomography , rose Bengal staining, bio-nanochip, DNA ploidy analysis and chemiluminescence.[30] Chemiluminescence was developed with the purpose of improving the visualization, identification and monitoring of oral potentially malignant disorders. This chemical reaction mostly involves the interaction of hydrogen peroxide and acetyl salicylic acid in alight capsule stick. [31] Although chemiluminescent examination serves as a non-invasive, chair side diagnostic aid for early detection of dysplastic changes in OPMDs particularly in the detection of edge of a suspicious lesion it has certain disadvantages as well. It can be quite expensive and also can be non-specific as it does not identify the etiology of the lesion- whether the lesion is inflammatory, benign or malignant.

5. Conclusion

Diagnostic procedures of OPMDs begin with a detailed case history followed by clinical oral examination. Histopathological examinations have always remained as the gold standard for the confirmatory diagnosis of dysplastic changes in these lesions. Chemiluminescent examination techniques only serve as adjuncts with the clinical oral examination and cannot replace the histopathological examination. They do offer several advantages as they are chair side, non-invasive diagnostic aids and can help in the early diagnosis of dysplastic changes for earlier intervention.

6. Future recommendations

More studies with larger sample size and statistically significant results are required. Chemiluminescent examination procedures can be useful for mass screening of suspicious oral lesions. It can be used as diagnostic screening tools in rural and remote settings where primary health facilities are not available for creating awareness and educating the people regarding the harmful effects of these tobacco products and their principal role in the etiology in OPMDs and oral cancer.

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