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Review Article

Accuracy of special histochemical staining methods in diagnosis of oral pathology: A systematic review and meta-analysis

Maryam Ghelichli[1], Abdolhalim Rajabi[2], Niyayesh Mirsaeedi[3], Masoud Mohammadi[4], Sina Sohrabi[3], Niloufar Darabi[3]

[1]Department of Oral and Maxillofacial Pathology, School of Dentistry, Golestan University of Medical Sciences, [2]Health Management and Social Development Research Center, Faculty of Health, Golestan University of Medical Sciences, [3]Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Golestan University of Medical Sciences, [4]Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

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Address for correspondence:
Dr. Niyayesh Mirsaeedi, Faculty of Dentistry, Golestan University of Medical Sciences, Gorgan, Iran.
E-mail: nmirsaeedi78@gmail.com

ABSTRACT

Clinical decision-making and biomedical research heavily rely on imaging techniques to visualize tissue morphology. To examine tissues in detail, it is necessary to use special histochemical stains to enhance contrast. This meta-analysis aimed to assess the sensitivity and specificity of these stains in diagnosing oral pathologic specimens. We conducted a search in 8 databases, including EMBASE, PubMed, Web of Science, Scopus, ProQuest, Ovid, Cinahl, and Cochrane, up to June 2022. Of 87,393 studies, 41 articles were selected for inclusion in our study. The results revealed that the specificity and sensitivity of the special histochemical stains were 86% with confidence interval (CI) 95%: 80%–90% and 83% with CI 95%: 75%–89%, respectively. Among the stains evaluated, toluidine blue, Papanicolaou, silver stain, Giemsa, Gram, feulgen, and periodic acid–Schiff (PAS) were the most frequently used for the detection of malignancy, premalignant lesions, dysplasia, and candidiasis. The specificity and sensitivity of each stain were analyzed individually, considering the type of specimen. Toluidine blue was the most commonly utilized special histochemical stain, particularly effective, for detecting malignancy, with a specificity of 97% with CI 95%: 88%–99% and sensitivity of 76% with CI 95%: 56%–89%. In conclusion, special histochemical stains are effective in diagnosing oral lesions, exhibiting reasonable specificity and sensitivity, especially in cases of premalignant and malignant lesions. Based on the reviewed articles in our study, the silver stain was identified as highly sensitive, while Giemsa and Papanicolaou stain exhibited the highest specificity.

Key Words: Accuracy, oral pathology, sensitivity, special histochemical stain, specificity

INTRODUCTION

Histopathology plays a crucial role in disease diagnosis. Accurate diagnosis is essential for effective treatment, particularly for premalignant and malignant lesions.[1] Clinical decision-making and biomedical research heavily rely on imaging tissue morphology and sample preparation. However, bright-field optical imaging often lacks sufficient contrast, necessitating staining to enhance details for observation and diagnosis. Staining is especially critical in histopathological analyses, the gold standard for diagnosing various diseases, including cancer, and most tissue-related research.[2] Conventionally, hematoxylin

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and eosin (H and E) staining has been widely used as the gold standard in the diagnostic process.[3] Nevertheless, this method is not always practical for diagnosing specific lesions. For instance, diagnosing odontogenic tumors and fibro-osseous lesions can be challenging, and routine histopathology staining procedures such as H and E may not adequately reveal the characteristics of hard tissues. Therefore, the use of special diagnostic tools and histochemical staining techniques, such as Masson's Trichrome and modified Galgo, can help detect hard tissues such as bones and other pathological calcifications.[4] Similarly, diagnosing malignant lesions with an unknown origin, such as carcinomas, has always been a common and challenging problem. An accurate and reliable diagnosis cannot be solely based on histological features and routine H and E staining in these cases.[5] Early diagnosis is vital in reducing damage and mortality caused by disease, primarily through differentiating premalignant lesions from malignant ones (such as oral squamous cell carcinoma [OSCC] and the most common oral malignancy).[6,7] The Toluidine blue test is used as an aid in the diagnosis of high-risk premalignant lesions and early asymptomatic OSCCs.[6]

Given the increasing use and diagnostic potential of special histochemical stains in the diagnosis of lesions, this meta-analysis study aimed to evaluate the accuracy of oral specimens' special histochemical staining methods in oral and maxillofacial pathology diagnosis.

MATERIALS AND METHODS

This systematic review and meta-analysis study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[8] This systematic review was approved by ethics (IR.GOUMS.REC.1401.320) in research of Golestan University of Medical Sciences.

This review aimed to assess the diagnostic accuracy of special histochemical stains for oral lesions, using the following PICO question:

- P: Patients with oral lesions of any type or etiology
- I: Special histochemical stains applied to oral samples
- C: Histopathological examination or other diagnostic gold standards
- O: Correct diagnosis of the type of oral lesions.

We aim to answer the following question: "What are the diagnostic values of staining in lesions of oral cavity patients versus controls without lesions of the oral cavity?"

Search strategy

A comprehensive approach and strategy were implemented to search PubMed, EMBASE, Scopus, Ovid, ProQuest, Web of Science, Cochrane, and Cinahl bibliographic databases. The search terms were ("Ziehl–Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou) AND (Dent* OR oral OR mouth* OR Oris*) [Appendix 1]. All review-related work was performed in June 2022. Two authors (MG and NM) independently screened studies for possible inclusion in the review by reading the titles and abstracts. We retrieved the full text of the references that seemed to satisfy our protocol inclusion criteria. We limited studies to English, but considered studies in other languages if an English abstract was provided, sufficient data were provided, and met inclusion criteria. Two authors reviewed abstracts and full text of the publication and excluded nonrelevant studies. All disagreements in the

screening and reviewing process were discussed and reviewed by a third author (AR).

Eligibility criteria

Inclusions criteria

The following were defined as criteria for inclusion: (1) Diagnostic and screening studies using staining for lesions of the oral cavity. (2) Studies with sufficient data to obtain true positive, false positive, true negative, and false-negative values.

Exclusion criteria

Based on the exclusion criteria, the following studies were excluded: (1) case reports, letters, personal opinions, reviews, book chapters, short communications, conference abstracts, and patents; (2) duplicate publications; (3) *in vitro* research that reported an association between staining and lesions of the oral cavity; and (4) studies with no existing data or incomplete information.

Following that, the authors individually reviewed the entire content of eligible studies to determine appropriateness. Disagreements among the authors were discussed until a consensus was reached.

Data extraction

Two authors extracted data individually from each eligible study. The extracted data included first author, publication year, country, number of controls and cases, study design, staining type (Periodic Acid Schiff [PAS], Toluidine Blue, Feulgen, Papanicolaou, Gram, Giemsa, and Silver Stain), type of specimen, age, and sex of participants, type of gold standard, and quantitative data.

Quality assessment

The quality of selected papers was appraised separately by authors using a checklist for diagnostic test accuracy studies of JBI.[9] If there were disagreements between evaluators, they strived for consensus through discussion. Utilizing the JBI checklist, selected studies were assessed with ten main questions. Based on the options of each question, every study that met the conditions corresponding to the question was coded 1, and the study that did not or did not specify the conditions was coded 0. Finally, the quality status was presented based on the total scores.

Statistical analyses

The diagnostic value of staining for lesions of the oral cavity was assessed by the pooled sensitivity and specificity data. These results are presented in forest plots and graphed study-specific estimates of sensitivity and specificity with 95% confidence interval (CI) in the receiver operating characteristic space. To obtain the pooled specificity and sensitivity, we used a random-effects model to combine the studies, accounting for the heterogeneity of the studies in terms of populations, outcomes, settings, and gold standard. The evaluation of heterogeneity between studies was done by using I^2 index, and Cochran's Q. Finally, we performed Deeks' funnel plot asymmetry test to investigate the potential for publication bias by visual inspection of the patterns drawn from study data, where lack of symmetry should denote high risk of reporting bias. All statistical analyses were performed using STATA 17 (College Station, TX, USA).

RESULTS

A total of 87,393 articles were found by searching the mentioned database. Out of these, 31,534 articles were removed due to duplicates leaving 55,859 articles to be examined. Among the examined articles, 54,602 were excluded as they did not meet the inclusion criteria. After reading the full text of 1257 articles, it was found that the full text of 60 articles was unavailable. In total, 1197 articles were thoroughly examined, and among these, 41 articles were selected due to their numerical value, while 1156 articles were excluded [Figure 1].

The articles included in this study comprised 27 cross-sectional studies and 14 case–control studies. Among these, seven studies were conducted in Europe, six were conducted in America and 28 studies were conducted in Asian countries, with 24 studies specially related to India. The publication year of the reviewed articles ranged until June 2022. The total number of specimens investigated in these studies was 3419 oral tissue specimens. There were 22 studies focused on malignancy, eight on premalignancy, nine on both malignancy and premalignancy, three on candidiasis, and three on dysplasia. The special histochemical stains commonly used in the reviewed articles were PAS in 2 articles, Feulgen in 2 articles, Giemsa in 3 articles, Gram in 2 articles, Papanicolaou in 10 articles, Toluidine Blue in 28 articles, and Silver stain in 3 articles.

The diagnostic gold standard employed in these articles included histopathology in 28 cases, biopsy in 10 cases, H and E in 3 cases, culture technique in

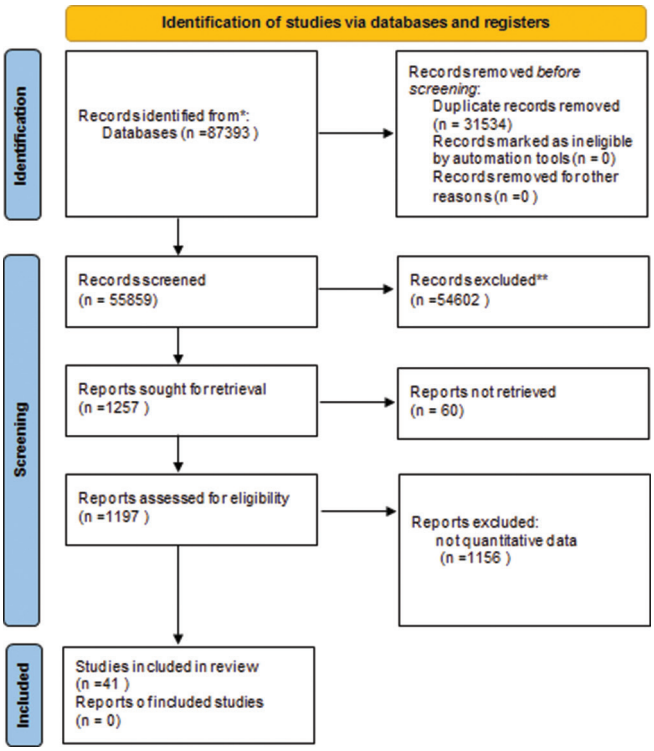

Figure 1: Flow diagram showing the study selection process. *The mentioned databases are introduced in appendix 1. **:Exclusion was based on the mantioned criterias.

2 cases, and various other methods such as clinical evaluation, cytology, fluorescent microscopy, PAS, and polymerase chain reaction in the remaining cases.

Toluidine blue was frequently used to diagnose malignant lesions in 12 studies, followed by Papanicolaou in 5 studies, Giemsa and Feulgen in 2 studies each, and silver stain in 1 study. Toluidine blue was the most commonly used stain in 7 studies, followed by Papanicolaou in 1 study for diagnosing premalignant lesions. In articles focused on malignant and premalignant lesions, Toluidine blue was used in 4 studies, while Papanicolaou and silver stain were used in 2 studies each.

Toluidine blue was the most frequently used stain in 3 studies regarding dysplasia samples.

For the diagnosis of candidiasis, Papanicolaou, Gram, and PAS stains were each used in 1 study. The evaluation results of the studies showed an average evaluation score of 4.92 ± 1.02, with a minimum score of 3 and a maximum score of 7. The characteristics of the included studies for meta-analysis are presented in Table 1.

Based on the forest plot, the overall results of the meta-analysis on special histochemical stains for diagnosing of oral lesions revealed a sensitivity of 86% with CI 95%: 80%–90%, a specificity of 83% with CI 95%: 75%–89%, and an estimated area under the curve (AUC) of 92% with CI 95%: 89%–94% [Figure 2].

Periodic acid–Schiff

Based on the meta-analysis, the sensitivity of PAS stain was 59% with CI 95%: 51%–67%, and its specificity was 56% with CI 95%: 39%–71%. All included studies, utilized this stain for diagnosing candidiasis [Figure 3].

Feulgen

According to the meta-analysis on Feulgen stain, its sensitivity was 66% with CI 95%: 56%–75%, while its specificity was 93% with CI 95%: 88%–96% [Figure 3].

The reviewed articles employed this stain for diagnosing malignant lesions.

Papanicolaou

The meta-analysis on the Papanicolaou stain revealed a sensitivity of 78% with CI 95%: 64%–88% and a specificity of 95% with CI 95%: 71%–99%. The AUC for the Papanicolaou stain was estimated to be 90% with CI 95%: 87%–92% [Figure 4].

The reviewed articles indicated the usage of this stain for detecting malignant, premalignant, and candidiasis lesions. Specifically, the diagnostic sensitivity for malignant lesions was 79% with CI 95%: 56%–92%, with a specificity of 99% with CI 95%: 53%–100%. For malignant and premalignant lesions, the sensitivity was 66% with CI 95%: 56%–74% and the specificity was 76% with CI 95%: 66%–84% [Figure 5].

Toluidine blue

Based on the meta-analysis, the sensitivity of this stain was estimated to be 89% with CI 95%: 83%–94%, and its specificity was estimated to be 78% with CI 95%: 69%–85%. In addition, its AUC was estimated to be 91% with CI 95%: 88% to 93% [Figure 6].

The studies employed this stain for diagnosing malignancy, premalignancy, and dysplasia. Its diagnostic sensitivity for malignant lesions was 97% with CI 95%: 88%–99%, with specificity of 76% with CI 95%: 56%–89%. For premalignant lesions, the sensitivity was 78% with CI 95%: 65%–87% and the specificity was 80% with CI 95%: 70%–87%.

In the case of malignant and premalignant samples, the diagnostic sensitivity was 90% with CI 95%:

Table 1: Characteristics of included studies

First author, citation	Country	Study design	Age	Sex	Gold standard	Type of specimen	Type of stain	Quality score
Yadav *et al.*, 2014[31]	India	Cross-sectional	NR	Both	Hematoxylin and eosin (H and E)	Malignant	Papanicolaou	5
Vijayakumar *et al.*, 2019[32]	India	Cross-sectional	Adolescents	Both	Histopathology	Malignant	Toluidine blue	4
Vashisht *et al.*, 2014[33]	India	Case-control	NR	Both	Histopathology	Dysplasia	Toluidine blue	4
Sivakumar *et al.*, 2021[34]	India	Cross-sectional	NR	Both	PCR	Premalignant and malignant	Papanicolaou	4
Rajmohan *et al.*, 2012[17]	India	Case–control	NR	Both	Biopsy	Premalignant and malignant	Toluidine blue	5
Patel *et al.*, 2021[14]	India	Case–control	20–80	Both	Fluorescence microscopy	Premalignant and malignant	Papanicolaou	4
Noormohammadpour *et al.*, 2020[23]	Iran	Case–control	51.14±16.12	Both	Histopathology	Other	Giemsa	5
Mehkri *et al.*, 2010[26]	India	Cross-sectional	NR	Both	Cytology	Premalignant and malignant	Silver stain	4
Padilha *et al.*, 2014[29]	Brazil	Cross-sectional	60	Both	PAS	Candida	Gram	5
Lajolo *et al.*, 2022[35]	Italy	Cross-sectional	67.4	Both	Biopsy	Malignant	Toluidine blue	4
Kore *et al.*, 2020[36]	India	Case–control	21–70	Both	Clinical evaluation	Malignant	Toluidine blue	5
Junaid *et al.*, 2012[37]	Pakistan	Cross-sectional	NR	Both	Biopsy	Malignant	Toluidine blue	5
Kumar *et al.*, 2011[38]	India	Cross-sectional	NR	Both	Histopathology	Malignant	Toluidine blue	3
Chainani-Wu *et al.*, 2015[39]	USA	Cross-sectional	61±10.6	Both	Biopsy	Premalignant and malignant/malignant	Toluidine blue	4
Chattopadhyay *et al.*, 2002[24]	India	Case–control	NR	Both	Hematoxylin and eosin (H and E)	Premalignant and malignant	Silver stain	4
Desai and Narang, 2015[40]	India	Cross-sectional	NR	Both	Histopathology	Malignant	Toluidine blue	4
Güneri *et al.*, 2011[20]	Turkey	Cross-sectional	56.2	Both	Histopathology	Malignant	Toluidine blue	4
Junaid *et al.*, 2013[41]	Pakistan	Cross-sectional	50.07±15.73	Both	Histopathology	Malignant	Toluidine blue	4
Kartheek *et al.*, 2018[28]	India	Case–control	50.81±13.44	Both	SBA culture technique followed by germ tube test	Normal	Gram/PAS	5
Kumaraswamy Naik *et al.*, 2016[27]	India	Cross-sectional	NR	Both	NR	Candida	Papanicolaou/ PAS	4
Mojsa *et al.*, 2012[42]	Poland	Cross-sectional	50.3	Both	Biopsy	Premalignant	Toluidine blue	5
Monea *et al.*, 2016[43]	Romania	Cross-sectional	NR	Both	Histopathology	Premalignant	Toluidine blue	5
Neher *et al.*, 2004[30]	Austria	Case–control	Control 37, case 59	Both	Hematoxylin and eosin (H and E)	Malignant	Feulgen	6
Onofre *et al.*, 2001[44]	Brazil	Cross-sectional	55.2±13.4	Both	Histopathology	Premalignant	Toluidine blue	7
Pallagatti *et al.*, 2013[45]	India	Cross-sectional	NR	Both	Histopathology	Dysplasia	Toluidine blue	5
Parakh *et al.*, 2017[46]	India	Case–control	18–65	Male	Histopathology	Premalignant	Toluidine blue	5
Prajeesh and Soni, 2019[47]	India	Cross-sectional	All ages	Both	Histopathology	Premalignant and malignant	Toluidine blue	5
Rahman *et al.*, 2012[48]	India	Cross-sectional	NR	Both	Histopathology	Premalignant and malignant	Toluidine blue	5
Rajput and Tupkari, 2010[25]	India	Case–control	NR	Both	Histopathology	Malignant	Papanicolaou/ silver stain	5
Santos *et al.*, 2015[15]	Brazil	Cross-sectional	>10	Both	Biopsy	Other	Papanicolaou	4
Singh and Shukla 2015[16]	India	Cross-sectional	41–60	Both	Biopsy	Malignant	Toluidine blue	6
Allegra *et al.*, 2009[49]	Italy	Cross-sectional	59	Both	Histopathology	Premalignant and malignant	Toluidine blue	5
Belgaumi and Shetty, 2013[13]	India	Case–control	18–30	Both	Histopathology	Malignant	Giemsa/ Papanicolaou	4
Adil *et al.*, 2017[50]	India	Cross-sectional	22–70	Both	Histopathology	Malignant/ premalignant	Toluidine Blue	5
Zafar *et al.*, 2020[11]	India	Cross-sectional	NR	Both	Histopathology	Malignant	Papanicolaou/ giemsa/feulgen	7
Braz-Silva *et al.*, 2012[51]	Brazil	Case–control	NR	Both	Biopsy	Premalignant	Papanicolaou	5
Aggarwal *et al.*, 2022[18]	India	Case–control	All ages	Both	Histopathology	Other	Toluidine blue	4
Epstein *et al.*, 2008[52]	USA	Cross-sectional	All ages	Both	Histopathology	Malignant	Toluidine blue	6

Contd...

Table 1: Contd...

First author, citation	Country	Study design	Age	Sex	Gold standard	Type of specimen	Type of stain	Quality score
Sharma *et al.*, 2021[53]	Nepal	Cross-sectional	45	Both	Histopathology	Premalignant	Toluidine blue	6
Prakash *et al.*, 2011[54]	India	Case–control	≥20	Both	Biopsy	Malignant	Papanicolaou	4
Awan *et al.*, 2012[19]	UK	Cross-sectional	≥16	Both	Histopathology	Dysplasia/ premalignant	Toluidine blue	7

SBA: Sabouraud dextrose agar; PAS: Periodic acid–Schiff; NR: not realized

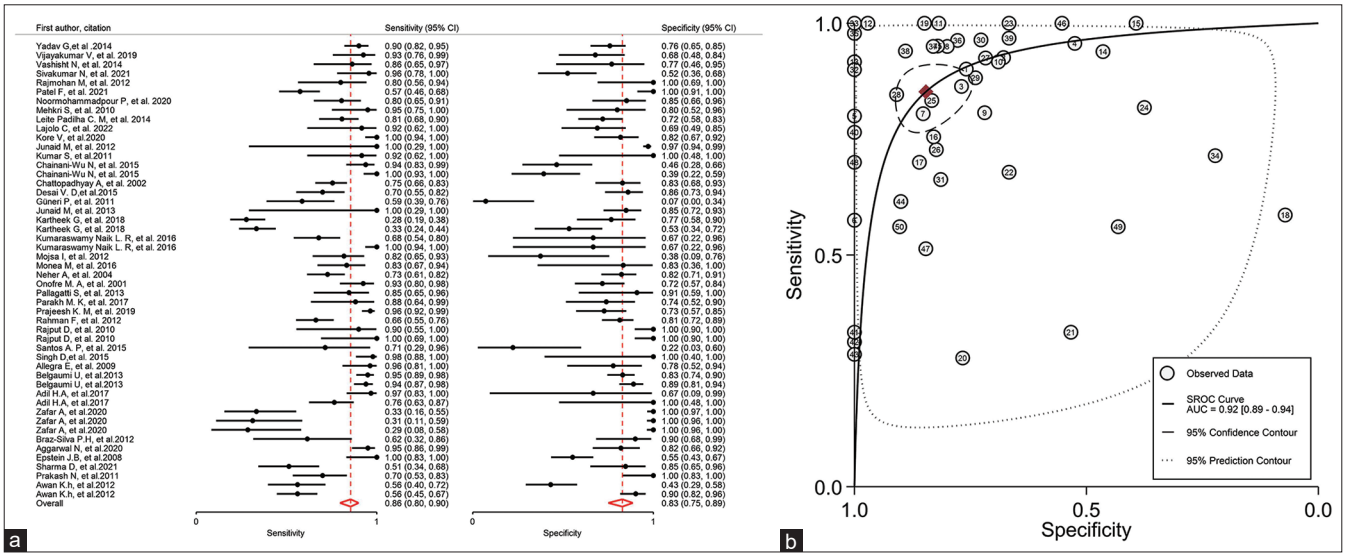

Figure 2: (a) Forest plot of sensitivities and specificities reported for special histochemical stains in all reviewed articles. (b) Summary receiver operating characteristic curve of all sensitivities and specificities reported in the reviewed articles. SROC: Summary receiver operating characteristic, AUC: Area under the curve.

77%–96%, with a specificity of 75% with CI 95%: 60%–86%. Moreover, the diagnostic sensitivity of dysplastic lesions was 77% with CI 95%: 56%–90%, with specificity of 71% with CI 95%: 41%–90% [Figure 5].

Gram

Based on the meta-analysis of Gram stain, the stain demonstrated a sensitivity of 48% with CI 95%: 40%–56% and a specificity of 74% with CI 95%: 63%–82% [Figure 3]. The studies included in the analysis employed Gram stain for diagnosing Candidiasis lesions.

Giemsa

The meta-analysis revealed that Giemsa stain exhibited a sensitivity of 77% with CI 95%: 35%–95% and a specificity of 95% with CI 95: 74%–99% [Figure 3]. In the studies included in the meta-analysis, Giemsa was used for the diagnosis of malignant lesions and oral ulcers, and the diagnostic sensitivity of this stain for malignant lesions was 86% with CI 95%: 79%–91% and its specificity was 91% with CI 95%: 87%–95% [Figure 7].

Silver stain

The meta-analysis indicated a sensitivity of 94% with CI 95%: 46%–100% and a specificity of 93% with CI 95%: 70%–99% for this stain [Figure 3]. This stain was utilized to diagnose malignant and premalignant lesions with a diagnostic sensitivity of 79% with CI 95%: 71%–85% and a specificity of 82% with CI 95%: 70%–90% [Figure 7].

Regarding publication bias, the uniform distribution of studies observed in Deek's Funnel Plot diagram and the statistical test conducted indicate the absence of significant publication bias [$P = 0.88$, Figure 8].

DISCUSSION

Since oral lesions can sometimes jeopardize a patient's health and even their life, especially in the case of premalignant and malignant lesions, accurate and early diagnosis is crucial for timely treatment. The utilization of accurate diagnostic methods, such as special histochemical stains, holds significant value and efficiency in reducing damage and mortality

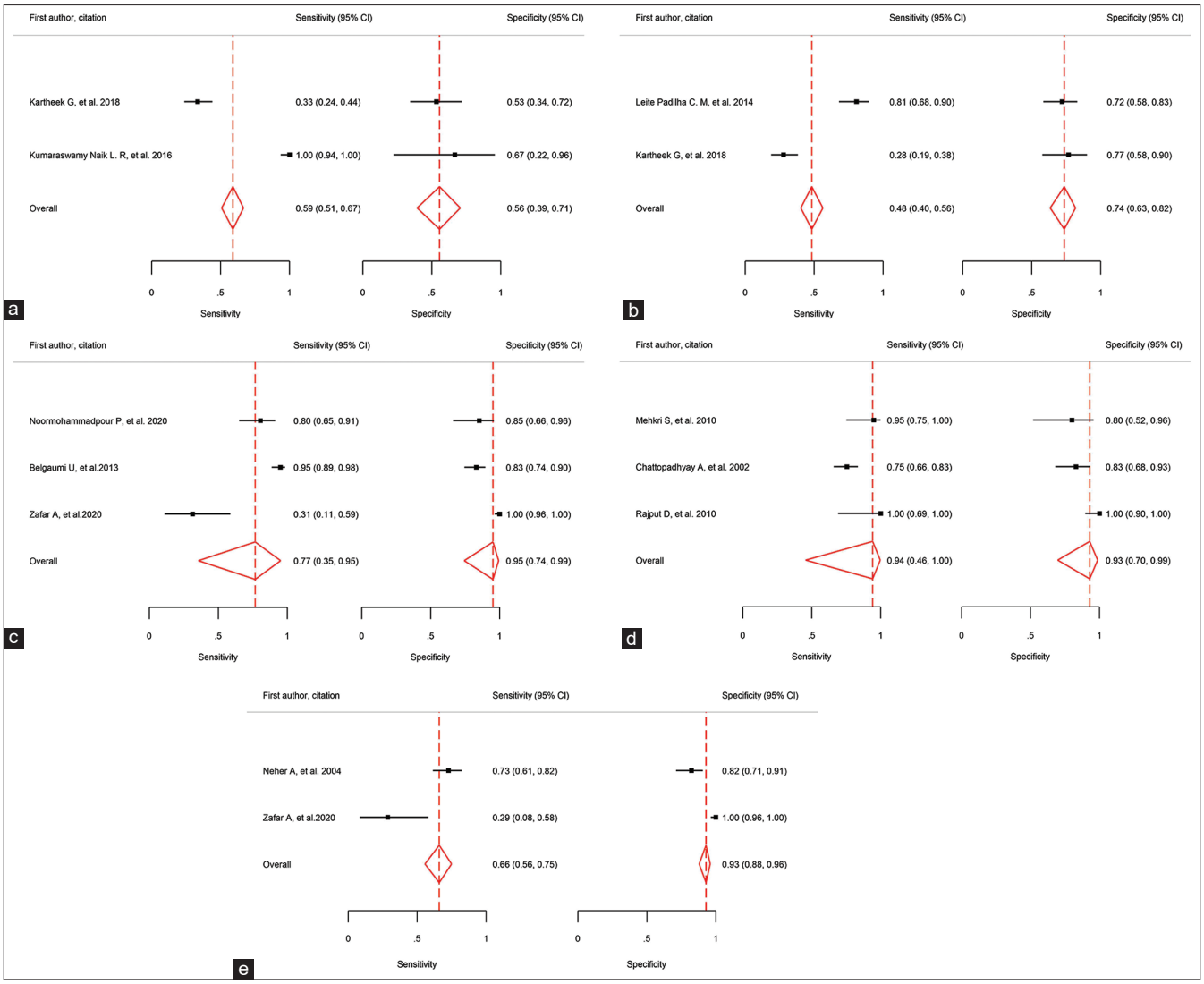

Figure 3: (a) Forest plot of sensitivities and specificities of periodic acid–Schiff stain in the reviewed articles. (b) Forest plot of reported sensitivities and specificities of Gram stain in articles. (c) Forest plot of reported sensitivities and specificities of Giemsa stain in articles. (d) Forest plot of reported sensitivities and specificities of silver stain in articles. (e) Forest plot of reported sensitivities and specificities of Feulgen stain in articles.

caused by pathologies, particularly malignant lesions. Moreover, it greatly enhances the life expectancy and prognosis of patients with oral diseases.[10-12]

There is a long, boring, and unlimited list of special histochemical stains. This study discusses common special histochemical stains used in diagnosing oral lesions, relying on available references and sources.

This study represents the first systematic meta-analysis to examine the sensitivity and specificity of various special histochemical staining methods in diagnosing oral and maxillofacial pathologies.

On reviewing articles and sources until June 2022, the stains employed in quantitative studies on human oral specimens included toluidine blue, Papanicolaou, Giemsa, silver stain, Gram, Feulgen, and PAS.

In general, the evaluation of special histochemical stains revealed high sensitivity and specificity in diagnosing oral lesions.

Nevertheless, it is worth noting that various factors can influence the reported sensitivity and specificity of the stains in different studies. These factors include the type of staining kits and their condition of use (e.g., the pH of the test), sample size, the expertise of the test performer, sampling method, gold standard, type of microscope used for sample analysis, the accuracy of the pathologist examining the stained samples under the microscope, target

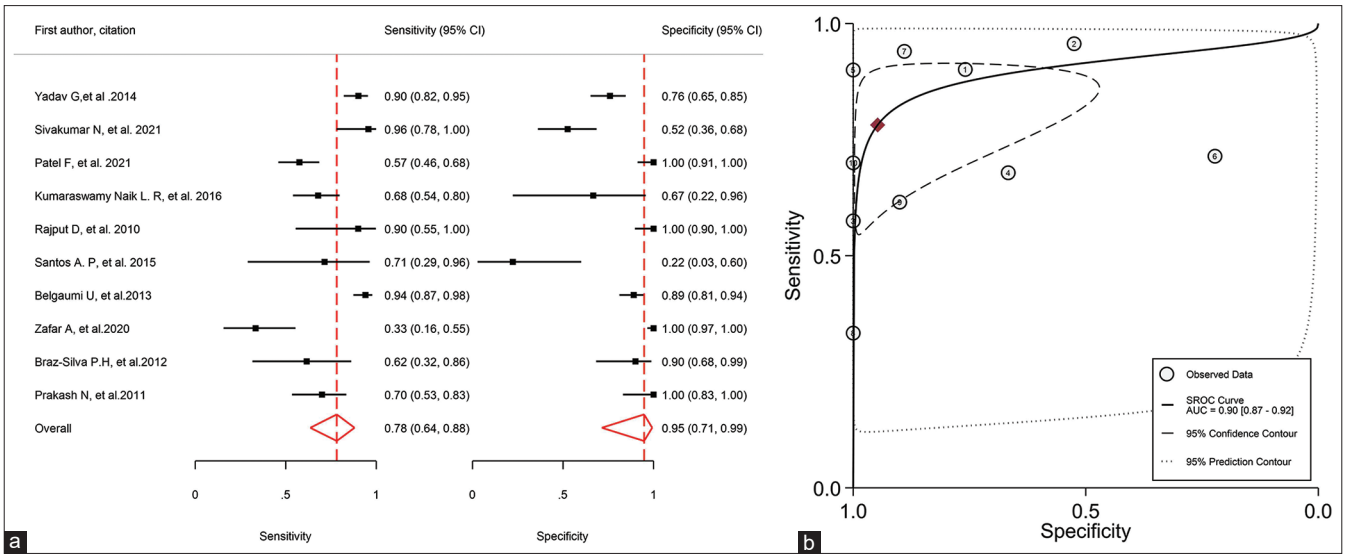

Figure 4: (a) Forest plot of sensitivities and specificities of Papanicolaou stain in the reviewed articles. (b) Summary receiver operating characteristic curve of sensitivities and specificities of Papanicolaou in the reviewed articles. SROC: Summary receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval.

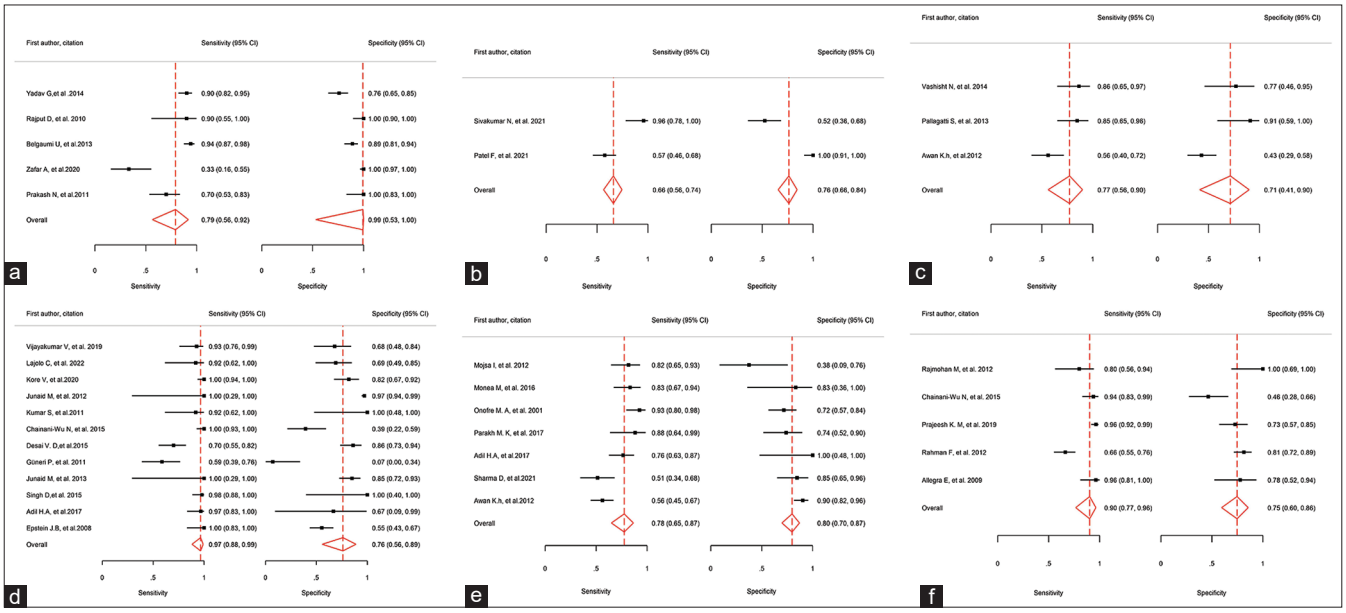

Figure 5: (a) Forest plot of sensitivities and specificities of Papanicolaou for malignant lesions. (b) Forest plot of sensitivities and specificities of Papanicolaou for malignant and premalignant lesions. (c) Forest plot of sensitivities and specificities of toluidine blue for dysplastic lesions. (d) Forest plot of sensitivities and specificities of toluidine blue for malignant lesions. (e) Forest plot of sensitivities and specificities of toluidine blue for premalignant lesions. (f) Forest plot of sensitivities and specificities of Toluidine Blue for malignant and premalignant lesions. CI: Confidence interval.

tissue of sampling, type of pathology, accuracy of statistical analysis and categorization and reporting methodologies employed.

In the present study, the sensitivity and specificity of Papanicolaou were evaluated as high and it was used to detect malignant, premalignant, candidiasis, and oral nodular lesions, with the most common tissue samples being malignant and premalignant lesions.

The results of the study by Mahajan *et al*.[10] showed that Papanicolaou is an accurate method in the diagnostic cytology of oral lesions. This finding aligns with and confirms the findings of our study.

Belgaumi and Shetty[13] also confirmed the diagnostic value of Papanicolaou for oral lesions, especially malignant types. In this study, which compared the diagnostic value of Papanicolaou, Leishman–Giemsa

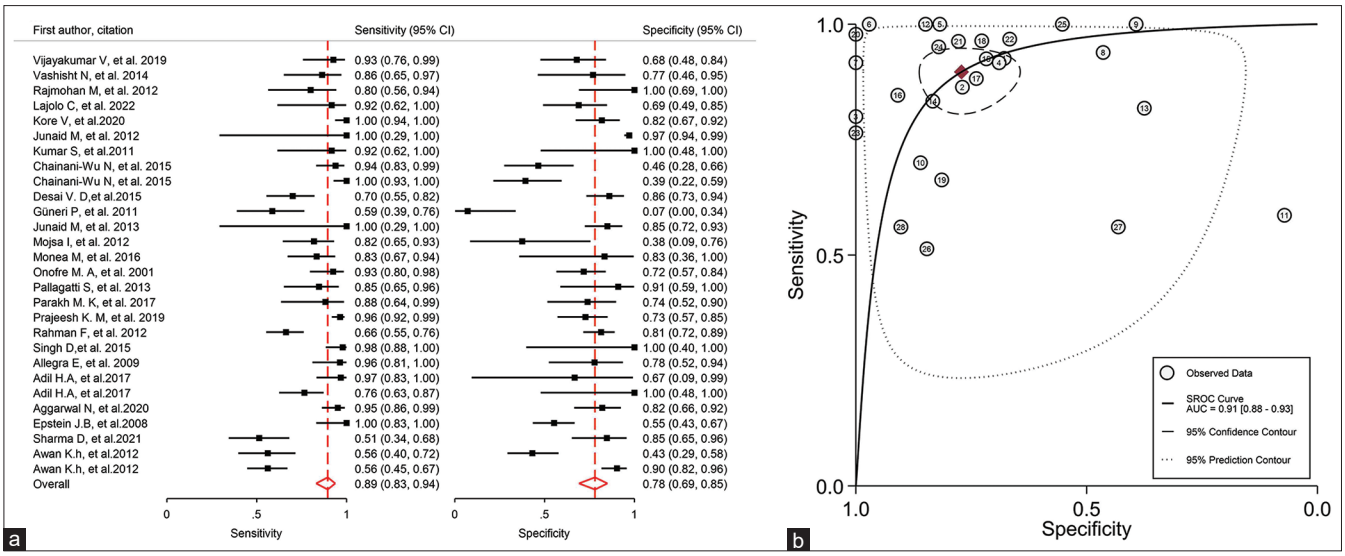

Figure 6: (a) Forest plot of sensitivities and specificities of toluidine blue in the reviewed articles. (b) Summary receiver operating characteristic curve of sensitivities and specificities of toluidine blue in the reviewed articles. SROC: Summary receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval.

cocktail, and May–Grunwald Giemsa for malignant lesions, it was found that despite acceptable results in the other two stains, Papanicolaou still exhibited high sensitivity and specificity (94% with CI 87%–98% and 89% with CI 81%–94%, respectively). In addition, Papanocolaou is easy, affordable and has a suitable and reliable research history in diagnosis.

However, a study by Patel *et al*.[14] in which samples were obtained by scraping with a flat wooden stick, showed that despite higher specificity, Papanicolaou's sensitivity was lower than our results (sensitivity was 57% with CI 46%–68% and specificity was 100% with CI 91%–100%). Despite its effectiveness in detecting malignant and premalignant lesions, Papanicolaou had a lower diagnostic value than other methods, such as acridine orange fluorescence.

Similarly, Santos *et al*.'s[15] study, which utilized the fine- needle aspiration biopsy (FNAB) method for preparing samples, showed that Papanicolaou had the same sensitivity as the usual H and E stain in diagnosing malignant lesions. However, its specificity was lower than H and E in diagnosing benign neoplastic lesions (its sensitivity was 71% with CI 29%–96% and its specificity was 22% with CI 3%–60%).

The varying results obtained from different studies can be attributed to differences in sampling methods, study design, target tissue, sample size, gold standard, and study type. In cases where there is doubt regarding the malignancy of the lesion from a clinical perspective, it is recommended to use the biopsy method, which is the gold standard for diagnosis and has exceptionally high accuracy, instead of less accurate methods such as FNAB and brush cytology.

In this study, Toluidine blue stain was a method with high sensitivity and specificity for diagnosing malignant, premalignant, and dysplastic oral lesions, with the most common tissue samples being malignant lesions.

Based on the results of studies by Singh and Shukla[16] Rajmohan *et al*.[17] and Aggarwal *et al*.,[18] the Toluidine blue stain is considered a reliable, cost-effective, and noninvasive method for detecting malignant and precancerous lesions. These findings are consistent with and confirm the findings of our study.

Furthermore, Awan *et al*.[19] demonstrated that the Toluidine blue stain is valuable for diagnosing premalignant and dysplastic lesions when used with clinical examination. It exhibits high sensitivity and specificity in detecting oral premalignant lesions. However, it lacks sufficient accuracy in diagnosing oral dysplastic lesions.

In a study conducted by Güneri *et al*.,[20] which compared the diagnostic accuracy of brush cytology and Toluidine blue, the sensitivity of Toluidine blue was lower compared to other studies, with a rate of 59% with CI 39%–76%. This discrepancy may be attributed to the utilization of brush cytology for

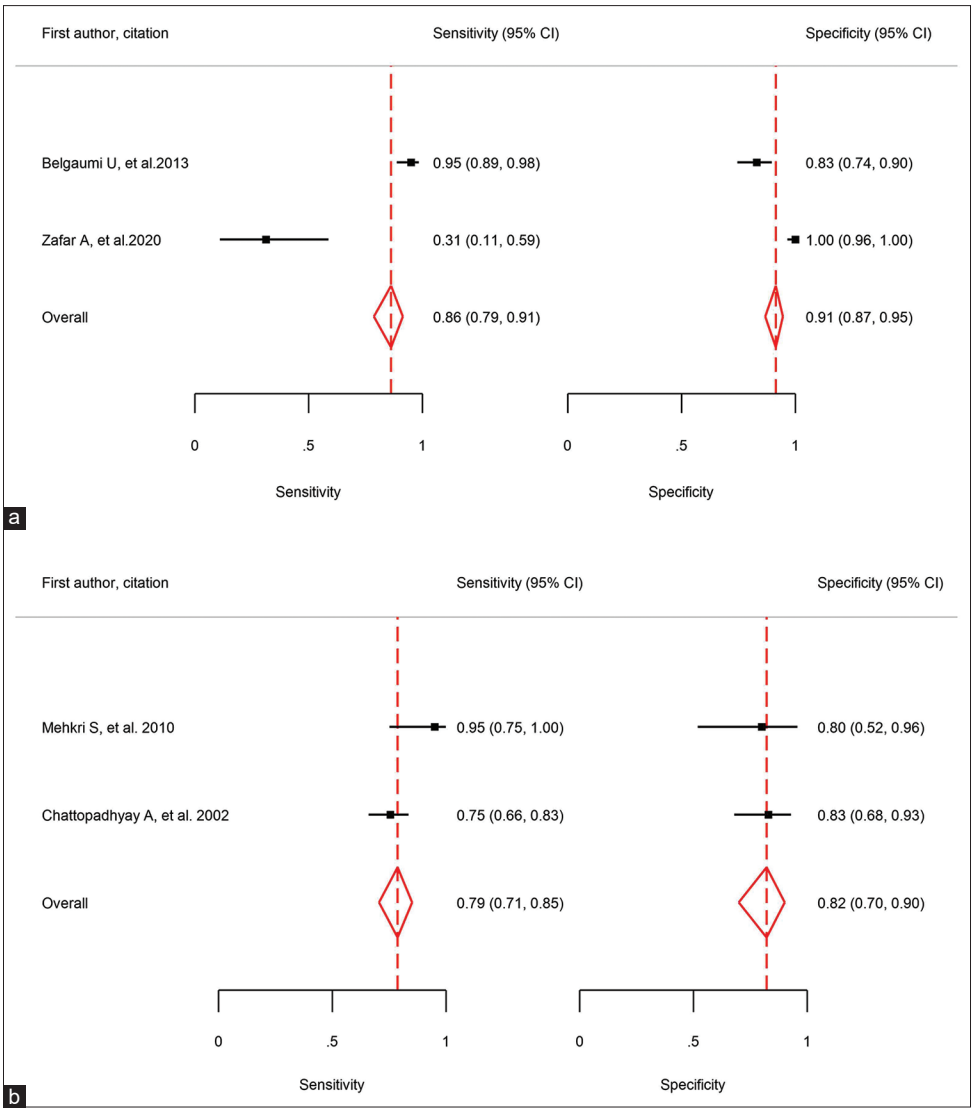

Figure 7: (a) Forest plot of sensitivities and specificities of Giemsa stain for malignant lesions in the reviewed articles. (b) Forest plot of sensitivities and specificities of silver stain for premalignant and malignant lesions in the reviewed articles. CI: Confidence interval.

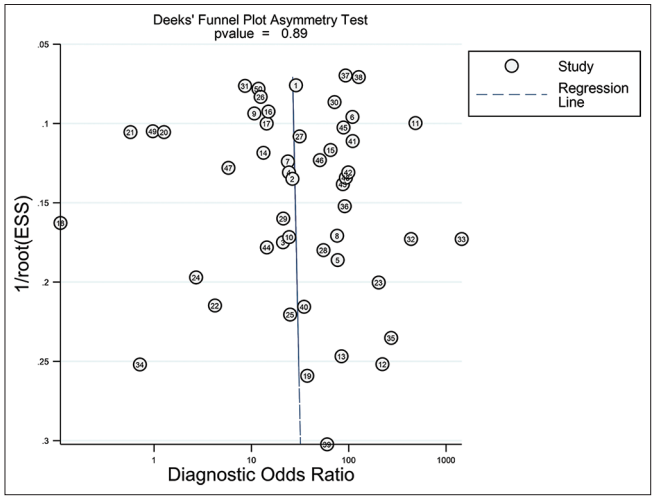

Figure 8: Deek's funnel plot asymmetry test for reviewed articled.

sample preparation instead of the more common biopsy method.

Mills[21] and Kim *et al*.[22] conducted studies exploring the effectiveness of Toluidine Blue in diagnosing malignant and premalignant oral lesions. They found that this stain exhibited higher sensitivity and specificity than clinical examination alone (Mills' study reported a sensitivity of 73% and specificity of 69%, while Kim *et al*. reported a sensitivity of 89.1% and specificity of 73.9%). Toluidine blue demonstrated greater sensitivity in diagnosing severe dysplasia and higher specificity for benign oral lesions. However, combining this stain with more accurate diagnostic methods, such as chemiluminescence, is recommended to diagnose oral malignancies.

Our study identified Giemsa stain as a diagnostic method with high sensitivity and specificity for malignant oral lesions and ulcerated lesions. Malignant oral lesions were the most frequently encountered.

These findings align with and confirm the results of the studies conducted by Belgaumi and Shetty[13] and Noormohammadpour *et al*.,[23] which compared the diagnostic sensitivity and specificity of the Leishman–Giemsa cocktail with Papanicolaou stain for malignant lesions. Giemsa stain was introduced as an easy, cost-effective, and one-step method for diagnosing malignant oral.

However, a study by Zafar *et al*.[11] indicated that Giemsa stain exhibited a relatively lower staining index, potentially attributable to its sensitivity to pH.

In this study, the silver stain was an accurate diagnostic method with high sensitivity and specificity for malignant and premalignant oral lesions.

In Chattopadhyay *et al*.'s[24] study, the silver stain was introduced as a valuable method for diagnosing dysplastic and nondysplastic leukoplakia.

The study conducted by Rajput and Tupkari[25] compared the value of silver stain and Papanicolaou stain in the brush biopsy technique, and it concluded that silver stain is an easy, noninvasive, safe, and accurate method for detecting malignancy in suspicious oral lesions.

These findings align with and support the results of our study.

However, the studies conducted by Chattopadhyay *et al*.[24] and Mehkri *et al*.[26] suggest that more extended studies with larger sample sizes are necessary to determine the value of AgNOR counting in diagnosing malignant and premalignant.

In the articles reviewed in our study, PAS stain was introduced as a special histochemical stain used for diagnosing candidiasis lesions. Its sensitivity and specificity were 59% and 56% which were lower than other stains.

Similarly, Kumaraswamy Naik *et al*.'s[27] study, which compared the value of PAS and Papanicolaou stains in the diagnosis of oral candidiasis found that PAS stain had higher sensitivity and specificity for diagnosing these lesions, consistent with our results.

Based on the results of Kartheek *et al*.'s[28] study, comparing the accuracy of Sabouraud dextrose agar (SBA) and PAS stain in the diagnosis of candidiasis, the SBA method was reported to have a higher value than the PAS stain, with low sensitivity and specificity. Although the PAS stain's sensitivity was lower in this study than ours, its specificity was close to our findings.

In the articles examined in our study, Gram stain was introduced as a special histochemical stain used for diagnosing candidiasis. It was found to have low sensitivity but high specificity compared to other stains.

Similarly, Leite Padilha *et al*.'s study[29] which compared the value of Papanocolaou and Gram stain in the diagnosis of oral candidiasis, identified Papanocolaou as the best method. However, the diagnostic accuracy of Gram stain was also considered sufficient and its sensitivity was higher than our findings. This difference in the results can be due to the use of PAS stain as the diagnostic gold standard in this study.

Based on Kartheek *et al*.'s[28] study, which compared the specificity and sensitivity of SBA and Gram stain in the diagnosis of candidiasis, the diagnostic value of the SBA method was reported to be higher than Gram.

In the articles included in the present study, the Feulgen stain was introduced as one of the special histochemical stains for the diagnosis of malignant lesions and its sensitivity and specificity were evaluated as high.

According to Neher *et al*.'s[30] study, inexpensive diagnostic tools such as cytology are methods with high diagnostic sensitivity for the diagnosis of laryngopharyngeal cancers.

In Zafar *et al*.'s[11] study, which investigated the diagnostic accuracy of Papanicolaou, Giemsa, Feulgen, and H and E in the touch imprint cytology technique for diagnosing OSCC, the diagnostic sensitivity and specificity of Papanicolaou and H and E were much higher than Giemsa and Feulgen. Moreover, this difference was statistically significant. This study suggested that the relatively low diagnostic accuracy of Feulgen stain may be due to the low number of OSCC cells in the samples examined with this stain or the presence of blood or other tissue components in the samples. Therefore, the results of this study cannot definitively indicate that Feulgen is ineffective in staining the nuclei of cells

and with suitable samples, this stain can achieve good diagnostic accuracy. In addition, the study suggests that the touch imprint cytology technique should be modified to increase the diagnostic accuracy of Feulgen when using this stain.

One strength of the present study is the comprehensive search of each literature database and the formulation of detailed inclusion and ranking criteria to ensure the quantity and quality of the included literature. Subgroup analyses were performed based on the stain type and lesion type. However, this study has some limitations, including a limited sample size in some subgroups, statistical heterogeneity in the included studies, and the use of different staining techniques across studies, which could potentially affect the accuracy and reliability of the results. Furthermore, this study does not account for potential confounding factors that may have influenced the results, such as age, sex, and smoking status).

CONCLUSION

According to this study, special histochemical stains are effective in the detection of oral lesions with reasonable specificity and sensitivity, especially in the case of premalignant and malignant lesions. Within the limitations of the present study, the results indicate that silver stain had the highest sensitivity, while Giemsa and Papanicolaou demonstrated the highest specificity. Consequently, these special histochemical stains are highly recommended, as they can significantly enhance the diagnostic accuracy of oral lesions.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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Appendix 1: Search strategies in different databases

Database	Search strategy
PubMed	(Dent*[Title/Abstract] OR oral[Title/Abstract] OR mouth*[Title/Abstract] OR Oris*[Title/Abstract]) AND ("Ziehl-Neelsen stain*"[Title/Abstract] OR "Toluidine Blue stain*"[Title/Abstract] OR "Alcian Yellow stain*"[Title/Abstract] OR "Dieterle stain*"[Title/Abstract] OR "Diff Quik stain*"[Title/Abstract] OR "Giemsa stain*"[Title/Abstract] OR "Gram stain*"[Title/Abstract] OR "Grocott's Methenamine Silver stain*"[Title/Abstract] OR "GMS stain*"[Title/Abstract] OR "Mayer's Mucicarmin stain*"[Title/Abstract] OR "Periodic Acid Schiff stain*"[Title/Abstract] OR "PAS stain*"[Title/Abstract] OR "Sayeed's stain*"[Title/Abstract] OR "Steiner stain*"[Title/Abstract] OR "Warthin Starry stain*"[Title/Abstract] OR "Gomori's one step"[Title/Abstract] OR "Trichrome stain*"[Title/Abstract] OR "Russel Movet Pentachrome stain*"[Title/Abstract] OR "Oil Red O stain*"[Title/Abstract] OR "Sudan Black B stain*"[Title/Abstract] OR "Orcein stain*"[Title/Abstract] OR "Lendrum's Method"[Title/Abstract] OR "Phosphotungstin Acid Hematoxylin"[Title/Abstract] OR "PTAH stain*"[Title/Abstract] OR "Silver stain*"[Title/Abstract] OR "Verhoeff stain*"[Title/Abstract] OR "Van Gieson stain*"[Title/Abstract] OR "Ethyl Green Pyronin stain*"[Title/Abstract] OR "Feulgen stain*"[Title/Abstract] OR "Bielschowsky Silver stain*"[Title/Abstract] OR "Congo Red"[Title/Abstract] OR "Cresyl Violet stain*"[Title/Abstract] OR "Luxol Fast Blue stain*"[Title/Abstract] OR "MBS stain*"[Title/Abstract] OR "Page's Eriochrome Cyanine R"[Title/Abstract] OR "Alizarin Red S stain*"[Title/Abstract] OR "Chloroacetate Esterase stain*"[Title/Abstract] OR "Leder stain*"[Title/Abstract] OR "Hall's stain*"[Title/Abstract] OR "Masson Fontana stain*"[Title/Abstract] OR "Perl's Prussian Blue stain*"[Title/Abstract] OR "p-dimethylaminobenzylidenerhodanine Stain*"[Title/Abstract] OR "Villanueva Osteochrome Bone Stain*"[Title/Abstract] OR "Alcain Blue Stain*"[Title/Abstract] OR "Gomori's silver stain*"[Title/Abstract] OR "Mucicarmine stain*"[Title/Abstract] OR "Periodic Acid – Silver"[Title/Abstract] OR "Methenamine stain*"[Title/Abstract] OR "PEM stain*"[Title/Abstract] OR "Masson's Trichrome"[Title/Abstract] OR "Modified Gallego's"[Title/Abstract] OR Immunohistochemistry[Title/Abstract] OR IHC[Title/Abstract] OR Trochrome[Title/Abstract] OR Mauveine[Title/Abstract] OR "Grocott Gomori"[Title/Abstract] OR "Methyl Green Pyronin stain*"[Title/Abstract] OR "Leishman Giemsa Cocktail"[Title/Abstract] OR Papanicolaou[Title/Abstract])
Web of Science	("Ziehl-Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou) AND (Dent* OR oral OR mouth* OR Oris*)
Scopus	((TITLE-ABS-KEY (("Gomori's silver stain*") OR ("Mucicarmine stain*") OR ("Periodic Acid – Silver") OR ("Methenamine stain*") OR ("PEM stain*") OR ("Masson's Trichrome") OR ("Modified Gallego's") OR (immunohistochemistry) OR (ihc) OR (trochrome) OR (mauveine) OR ("Grocott Gomori") OR ("Methyl Green Pyronin stain*") OR ("Leishman Giemsa Cocktail") OR (Papanicolaou)) AND TITLE-ABS-KEY (dent* OR oral OR mouth* OR oris*))) OR ((TITLE-ABS-KEY (("Page's Eriochrome Cyanine R") OR ("Alizarin Red S stain*") OR ("Chloroacetate Esterase stain*") OR ("Leder stain*") OR ("Hall's stain*") OR ("Masson Fontana stain*") OR ("Perl's Prussian Blue stain*") OR ("p-dimethylaminobenzylidenerhodanine Stain*") OR ("Villanueva Osteochrome Bone Stain*") OR ("Alcain Blue Stain*") OR ("Giemsa stain*")) AND TITLE-ABS-KEY (dent* OR oral OR mouth* OR oris*))) OR ((TITLE-ABS-KEY (("Sudan Black B stain*") OR ("Orcein stain*") OR ("Lendrum's Method") OR ("Phosphotungstin Acid Hematoxylin") OR ("PTAH stain*") OR ("Silver stain*") OR ("Verhoeff stain*") OR ("Van Gieson stain*") OR ("Ethyl Green Pyronin stain*") OR ("Feulgen stain*") OR ("Bielschowsky Silver stain*") OR ("Congo Red") OR ("Cresyl Violet stain*") OR ("Luxol Fast Blue stain*") OR ("MBS stain*")) AND TITLE-ABS-KEY (dent* OR oral OR mouth* OR oris*))) OR ((TITLE-ABS-KEY (("Ziehl-Neelsen stain*") OR ("Toluidine Blue stain*") OR ("Alcian Yellow stain*") OR ("Dieterle stain*") OR ("Diff Quik stain*") OR ("Giemsa stain*") OR ("Gram stain*") OR ("Grocott's Methenamine Silver stain*") OR ("GMS stain*") OR ("Mayer's Mucicarmin stain*") OR ("Periodic Acid Schiff stain*") OR ("PAS stain*") OR ("Sayeed's stain*") OR ("Steiner stain*") OR ("Warthin Starry stain*") OR ("Gomori's one step") OR ("Trichrome stain*") OR ("Russel Movet Pentachrome stain*") OR ("Oil Red O stain*")) AND TITLE-ABS-KEY (dent* OR oral OR mouth* OR oris*)))
Cochrane	"Ziehl-Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou in Title Abstract Keyword AND Dent* OR oral OR mouth* OR Oris* in Title Abstract Keyword

Contd...

Appendix 1: Contd...

Database	Search strategy
ProQuest	(ti("Ziehl-Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou) OR su("Ziehl-Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou) OR ab("Ziehl-Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou)) AND (ab (Dent* OR oral OR mouth* OR Oris*) OR su (Dent* OR oral OR mouth* OR Oris*) OR ti (Dent* OR oral OR mouth* OR Oris*))
Embase	("ziehl-neelsen stain":ti, ab, kw OR "toluidine blue stain":ti, ab, kw OR "alcian yellow stain":ti, ab, kw OR "dieterle stain":ti, ab, kw OR "diff quik stain":ti, ab, kw OR "gram stain":ti, ab, kw OR "grocott methenamine silver stain":ti, ab, kw OR "gms stain":ti, ab, kw OR "mayers mucicarmin stain":ti, ab, kw OR "periodic acid schiff stain":ti, ab, kw OR "pas stain":ti, ab, kw OR "sayeeds stain":ti, ab, kw OR "steiner stain":ti, ab, kw OR "warthin starry stain":ti, ab, kw OR "gomori one step":ti, ab, kw OR "trichrome stain":ti, ab, kw OR "russel movet pentachrome stain":ti, ab, kw OR "oil red o stain":ti, ab, kw OR "sudan black b stain":ti, ab, kw OR "orcein stain":ti, ab, kw OR "lendrums method":ti, ab, kw OR "phosphotungstin acid hematoxylin":ti, ab, kw OR "ptah stain":ti, ab, kw OR "silver stain":ti, ab, kw OR "verhoeff stain":ti, ab, kw OR "van gieson stain":ti, ab, kw OR "ethyl green pyronin stain":ti, ab, kw OR "feulgen stain":ti, ab, kw OR "bielschowsky silver stain":ti, ab, kw OR "congo red":ti, ab, kw OR "cresyl violet stain":ti, ab, kw OR "luxol fast blue stain":ti, ab, kw OR "mbs stain":ti, ab, kw OR "pages eriochrome cyanine r":ti, ab, kw OR "alizarin red s stain":ti, ab, kw OR "chloroacetate esterase stain":ti, ab, kw OR "leder stain":ti, ab, kw OR "halls stain":ti, ab, kw OR "masson fontana stain":ti, ab, kw OR "perls prussian blue stain":ti, ab, kw OR "p-dimethylaminobenzylidenerhodanine stain":ti, ab, kw OR "villanueva osteochrome bone stain":ti, ab, kw OR "alcain blue stain":ti, ab, kw OR "giemsa stain":ti, ab, kw OR "gomoris silver stain":ti, ab, kw OR "mucicarmine stain":ti, ab, kw OR "periodic acid silver":ti, ab, kw OR "methenamine stain":ti, ab, kw OR "pem stain":ti, ab, kw OR "massons trichrome":ti, ab, kw OR "modified gallegos":ti, ab, kw OR immunohistochemistry: ti, ab, kw OR ihc: ti, ab, kw OR trochrome: ti, ab, kw OR mauveine: ti, ab, kw OR "grocott gomori":ti, ab, kw OR "methyl green pyronin stain":ti, ab, kw OR "leishman giemsa cocktail":ti, ab, kw OR Papanicolaou: ti, ab, kw) AND (dent*:ti, ab, kw OR oral: ti, ab, kw OR mouth*:ti, ab, kw OR oris*:ti, ab, kw)

Contd...

Appendix 1: Contd...

Database	Search strategy
Ovid	((Ziehl-Neelsen stain or Toluidine Blue stain or Alcian Yellow stain or Dieterle stain or Diff Quik stain or Giemsa stain or Gram stain or Grocotts Methenamine Silver stain or GMS stain or Mayers Mucicarmin stain or Periodic Acid Schiff stain or PAS stain or Sayeeds stain or Steiner stain or Warthin Starry stain or Gomoris one step or Trichrome stain or Russel Movet Pentachrome stain or Oil Red O stain or Sudan Black B stain or Orcein stain or Lendrums Method or Phosphotungstin Acid Hematoxylin or PTAH stain or Silver stain or Verhoeff stain or Van Gieson stain or Ethyl Green Pyronin stain or Feulgen stain or Bielschowsky Silver stain or Congo Red or Cresyl Violet stain or Luxol Fast Blue stain or MBS stain or Pages Eriochrome Cyanine R or Alizarin Red S stain or Chloroacetate Esterase stain or Leder stain or Halls stain or Masson Fontana stain or Perls Prussian Blue stain or P dimethylaminobenzylidenerhodanine Stain or Villanueva Osteochrome Bone Stain or Alcain Blue Stain or Giemsa stain or Gomoris silver stain or Mucicarmine stain or Periodic Acid Silver or Methenamine stain or PEM stain or Massons Trichrome or Modified Gallegos or Immunohistochemistry or IHC or Trochrome or Mauveine or Grocott Gomori or Methyl Green Pyronin stain or Leishman Giemsa Cocktail or Papanicolaou).ab. or (Ziehl-Neelsen stain or Toluidine Blue stain or Alcian Yellow stain or Dieterle stain or Diff Quik stain or Giemsa stain or Gram stain or Grocotts Methenamine Silver stain or GMS stain or Mayers Mucicarmin stain or Periodic Acid Schiff stain or PAS stain or Sayeeds stain or Steiner stain or Warthin Starry stain or Gomoris one step or Trichrome stain or Russel Movet Pentachrome stain or Oil Red O stain or Sudan Black B stain or Orcein stain or Lendrums Method or Phosphotungstin Acid Hematoxylin or PTAH stain or Silver stain or Verhoeff stain or Van Gieson stain or Ethyl Green Pyronin stain or Feulgen stain or Bielschowsky Silver stain or Congo Red or Cresyl Violet stain or Luxol Fast Blue stain or MBS stain or Pages Eriochrome Cyanine R or Alizarin Red S stain or Chloroacetate Esterase stain or Leder stain or Halls stain or Masson Fontana stain or Perls Prussian Blue stain or P dimethylaminobenzylidenerhodanine Stain or Villanueva Osteochrome Bone Stain or Alcain Blue Stain or Giemsa stain or Gomoris silver stain or Mucicarmine stain or Periodic Acid Silver or Methenamine stain or PEM stain or Massons Trichrome or Modified Gallegos or Immunohistochemistry or IHC or Trochrome or Mauveine or Grocott Gomori or Methyl Green Pyronin stain or Leishman Giemsa Cocktail or Papanicolaou).kw. or (Ziehl-Neelsen stain or Toluidine Blue stain or Alcian Yellow stain or Dieterle stain or Diff Quik stain or Giemsa stain or Gram stain or Grocotts Methenamine Silver stain or GMS stain or Mayers Mucicarmin stain or Periodic Acid Schiff stain or PAS stain or Sayeeds stain or Steiner stain or Warthin Starry stain or Gomoris one step or Trichrome stain or Russel Movet Pentachrome stain or Oil Red O stain or Sudan Black B stain or Orcein stain or Lendrums Method or Phosphotungstin Acid Hematoxylin or PTAH stain or Silver stain or Verhoeff stain or Van Gieson stain or Ethyl Green Pyronin stain or Feulgen stain or Bielschowsky Silver stain or Congo Red or Cresyl Violet stain or Luxol Fast Blue stain or MBS stain or Pages Eriochrome Cyanine R or Alizarin Red S stain or Chloroacetate Esterase stain or Leder stain or Halls stain or Masson Fontana stain or Perls Prussian Blue stain or P dimethylaminobenzylidenerhodanine Stain or Villanueva Osteochrome Bone Stain or Alcain Blue Stain or Giemsa stain or Gomoris silver stain or Mucicarmine stain or Periodic Acid Silver or Methenamine stain or PEM stain or Massons Trichrome or Modified Gallegos or Immunohistochemistry or IHC or Trochrome or Mauveine or Grocott Gomori or Methyl Green Pyronin stain or Leishman Giemsa Cocktail or Papanicolaou).ti.) AND ((Dent* or oral or mouth* or Oris*).ab. or (Dent* or oral or mouth* or Oris*).kw. or (Dent* or oral or mouth* or Oris*).ti.)
Cinahl	("Ziehl–Neelsen stain*" OR "Toluidine Blue stain*" OR "Alcian Yellow stain*" OR "Dieterle stain*" OR "Diff Quik stain*" OR "Giemsa stain*" OR "Gram stain*" OR "Grocott's Methenamine Silver stain*" OR "GMS stain*" OR "Mayer's Mucicarmin stain*" OR "Periodic Acid Schiff stain*" OR "PAS stain*" OR "Sayeed's stain*" OR "Steiner stain*" OR "Warthin Starry stain*" OR "Gomori's one step" OR "Trichrome stain*" OR "Russel Movet Pentachrome stain*" OR "Oil Red O stain*" OR "Sudan Black B stain*" OR "Orcein stain*" OR "Lendrum's Method" OR "Phosphotungstin Acid Hematoxylin" OR "PTAH stain*" OR "Silver stain*" OR "Verhoeff stain*" OR "Van Gieson stain*" OR "Ethyl Green Pyronin stain*" OR "Feulgen stain*" OR "Bielschowsky Silver stain*" OR "Congo Red" OR "Cresyl Violet stain*" OR "Luxol Fast Blue stain*" OR "MBS stain*" OR "Page's Eriochrome Cyanine R" OR "Alizarin Red S stain*" OR "Chloroacetate Esterase stain*" OR "Leder stain*" OR "Hall's stain*" OR "Masson Fontana stain*" OR "Perl's Prussian Blue stain*" OR "p-dimethylaminobenzylidenerhodanine Stain*" OR "Villanueva Osteochrome Bone Stain*" OR "Alcain Blue Stain*" OR "Giemsa stain*" OR "Gomori's silver stain*" OR "Mucicarmine stain*" OR "Periodic Acid – Silver" OR "Methenamine stain*" OR "PEM stain*" OR "Masson's Trichrome" OR "Modified Gallego's" OR Immunohistochemistry OR IHC OR Trochrome OR Mauveine OR "Grocott Gomori" OR "Methyl Green Pyronin stain*" OR "Leishman Giemsa Cocktail" OR Papanicolaou) AND (Dent* OR oral OR mouth* OR Oris*)