REVIEW

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Diagnostic Accuracy of DNA Ploidy for Oral Potentially Malignant Disorders: A Systematic Review and Meta Analysis

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Abstract

Objective: to compare the diagnostic accuracy of DNA ploidy compared to biopsy followed by histopathological investigation in patients with oral potentially malignant disorders (OPMDs). **Method:** The review protocol is registered under PROSPERO(CRD42024524656) and performed in accordance to Preferred Reporting Items for Systematic Reviews and Meta-Analysis – Diagnostic Test Accuracy (PRISMA- DTA) checklist. Databases like PubMed, Google Scholar, EBSCOhost were searched from 2000 to 2024 to identify the diagnostic potential of DNA ploidy for various OPMDs. True-positive, false-positive, true-negative, false-negative, sensitivity, specificity values were calculated manually if not present for each study. Quality assessment of included studies was evaluated based on Quality assessment of diagnostic accuracy studies (QUADAS)- 2 tool using review manager (RevMan ver. 5.3). Meta-analysis was performed in Meta-Disc 1.4 software for pooled sensitivity and specificity. Additional analysis was performed in terms of positive likelihood ratio (+LR), negative likelihood ratio (-LR), diagnostic odds ratio (DOR) and summary receiver operating characteristics (SROC) with Area Under Curve (AUC) and $p<0.05$ as statistically significant. **Results:** Nine studies were included for qualitative synthesis and seven studies for meta-analysis. Included studies reported low risk of bias (ROB). Various OPMDs (oral leukoplakia (OL), oral lichen planus (OLP), erythroplakia (OE), oral erythro-leukoplakia and other oral dysplastic lesions were evaluated. The meta-analysis revealed an overall pooled sensitivity of 0.71 (CI 0.28- 0.96) and pooled specificity of 0.31 (CI 0.03- 0.79) with +PLR 0.99 (0.49 – 2.02) and -NLR 0.99 (0.18 – 5.48) and a DOR of 1.05 (0.07 – 16.14) with an overall diagnostic accuracy (AUC) of 0.49. **Conclusion:** This study findings provide evidence on ability of DNA ploidy for various OPMDs for early screening and diagnosis. DNA ploidy can be taken for secondary level of prevention for OPMD under early diagnosis and prompt treatment.

Keywords: Accuracy- computed tomography- diagnosis- meta-analysis- oral cancer

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Introduction

Oral squamous cell carcinoma (OSCC) is often diagnosed at a late stage and therefore early detection and timely intervention are crucial for decreasing morbidity and mortality of OSCC [1, 2]. A portion of OSCC preceded by oral potentially malignant disorders (OPMD), mainly being leukoplakia, and presence of dysplasia is often considered as a discriminator for malignant potential of OPMD [3]. Delayed detection is primary reason for high morbidity and mortality rates, and this strongly supports the need to perk up early detection of oral cancers [3]. The gold standard for oral cancer diagnosis is still a biopsy, which is not suited for screening purposes due to its invasive nature, high cost, and need for specially trained medical personal and equipment [4, 5].

Recent studies have reported the potential use of DNA ploidy analysis to predict the behaviour of various OPMDs [6]. If any correlation between DNA ploidy and the histological grade of dysplasia can be demonstrated, it might be used as an adjunctive aid for pathologists to arrive at a consensus in diagnosing the grade of epithelial dysplasia [7].

Abnormal nuclear DNA content, DNA aneuploidy, is an indicator of numerical chromosomal changes and its emergence is often a critical step in carcinogenesis [8]. DNA aneuploidy can be measured in a relatively robust and sensitive assay, though lately its reputation as a marker of progression has been questioned [9]. DNA ploidy status can be measured either by flow cytometry (FCM-DNA) or image cytometry (DNA- ICM). Studies have shown that identifying DNA aneuploidy in squamous epithelium can lead to an earlier detection and diagnosis of OSCC by up to two years [8]. Since this non-invasive procedure

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is well tolerated by patients, OSCC could be detected at an earlier stage, which could significantly increase the chance of recovery and thereby reduce the burden on the healthcare system [9]. Although DNA aneuploidy is an accepted biomarker for malignancy, the effectiveness of the procedure is still controversially discussed [10]. Considering various studies, pooled sensitivities and specificities of 55–100%, resulted in the fact that there is limited evidence [11, 12].

Understanding the correct diagnosis will help clinicians to make the correct diagnosis and choose the best treatment. Diagnostic accuracy includes sensitivity, specificity, and summary receiver operating characteristic (SROC) analysis [13].

Sensitivity and specificity describe the test's ability to accurately identify patients and non-patients, respectively. These are independent of the prevalence of the disease, the pathway of disease in the population at a given time, and summary of receiver operating characteristics (SROC) analysis is used to assess diagnostic power [14, 15].

Till date, no studies have provided a comprehensive, quantitative and diagnostic accuracy analysis of DNA ploidy for oral potentially malignant disorders (OPMDs). Therefore, we updated our research for existing scientific evidences and conducted this review with the aim to compare the diagnostic accuracy of DNA ploidy compared to biopsy followed by histopathological investigation in patients with OPMDs.

Materials and Methods

Protocol and Registration

The systematic review and meta-analysis protocol was registered at the international prospective register of systematic reviews (PROSPERO- CRD42024524656) and performed in accordance with the PRISMA- DTA checklist [16].

Study Design

The following focused research question in the Participants (P), Index test (I), reference standard (R) and target condition (T) format was proposed "Is there a difference in the diagnostic accuracy of DNA ploidy (Index Test) compared to biopsy followed by histopathological investigation (gold standard) for OPMDs?

Eligibility Criteria

studies were selected based on following criteria's

Inclusion Criteria

(1) Study Design

Observational studies, retrospective study, prospective study, cross-sectional study comparing the diagnostic accuracy of DNA ploidy with biopsy

(2) Participant characteristics

patients diagnosed with OPMDs above 18 years and up.

(3) Outcome measurements

Diagnostic accuracy including sensitivity, specificity, accuracy, determined using different methods irrespective of the methods of quantifying the outcomes.

(4) Articles written in English language

(5) Articles from January 2000 – April 2024 and available as free available full text articles

Exclusion Criteria

(1) Non-clinical studies, in-vitro studies, and animal studies. Studies reporting about a single diagnostic tool were also excluded.

(2) Studies done on individuals less than 18 years of age.

(3) Studies not fully available in the database.

(4) Article reporting only abstracts were also excluded.

(5) Studies not reporting primary outcomes of accuracy, sensitivity, and specificity as well as where primary outcomes are not possible to calculate from the given raw data.

Search protocol and study selection

A comprehensive electronic search was performed till April 2024 for the studies published within the last 24 years (from 2000 to 2024) using the following databases: PubMed and EBSCOhost to retrieve articles in the English language. The searches in the clinical trials database, cross-referencing and grey literature were conducted using Google Scholar, Greylist, and OpenGrey. In addition to the electronic search, a hand search was also made, and reference lists of the selected articles were screened.

Search Strategy

Appropriate key words and Medical Subject Heading (MeSH) terms were selected and combined with Boolean operators like AND. The search strategy used was as follows: (DNA ploidy AND biopsy AND sensitivity AND specificity AND OPMDs AND diagnosis).

Search Strategy according to PICT Format:

	Strategy
Population	(("pre-cancerous lesion"[MeSH Terms] OR "oral lichen planus" OR "oral leukoplakia" OR ("oral submucous fibrosis"[MeSH Terms] OR "erythroplakia" OR ("oral dysplasia"[MeSH Terms] OR ("oral potentially malignant disorders" OR ("malignant transformation "[MeSH Terms]
Index test	("DNA ploidy"[MeSH Terms] OR "image cytometry" AND "DNA aneuploidy" AND "DNA diploidy" OR "dysplasia" OR "oral cancer" OR "biological marker"[MeSH Terms] OR ("biological marker" AND "diagnostic efficacy")
Reference condition	("oral biopsy" OR "histopathological investigation"[MeSH Terms] OR "malignancy" AND "cytology" OR "ploidy"
Target condition	("sensitivity" OR "specificity" OR "diagnostic odds ratio"[MeSH Terms] OR ("positive likelihood ratio" AND "negative likelihood ratio" OR "positive predictive value" OR ("negative predictive value" AND "summary receiver operating characteristics" AND "diagnostic accuracy")

Screening process

Two review authors did the search and screening, in accordance with the previously agreed process. The article selection process was divided into two phases. During phase one, two reviewers examined the titles and abstracts of each paper. Articles that met the inclusion criteria were excluded. In Phase 2, selected entire articles were independently evaluated and screened by the same reviewers. Any disagreements were settled through conversation. When two reviewers could not reach an agreement, a third reviewer was consulted to make the ultimate decision. All three authors agreed on the final selection.

For included studies, study details were extracted under following headings: authors, study year, sample size, study design, various OPMDs assessed, sensitivity, specificity and conclusion. Sensitivity and specificity data were compiled from each study and values like true positive, true negative, false positive and false negatives were calculated manually for the studies using the below formula's where the data was not provided by authors. The corresponding authors were contacted via email where further information was needed.

a) False positive = (1-specificity) x (1- diseased cases/ total sample)

b) True negative = specificity x (1- diseased cases/ total sample)

c) True positive = sensitivity x diseased cases/ total sample

d) False negative = (1- sensitivity) x diseased cases/ total sample

Assessment of methodological quality

Risk of bias was assessed through quality assessment of diagnostic accuracy studies - 2 (QUADAS-2) tool [17]. The QUADAS-2 is a modified instrument created to evaluate nature of symptomatic examinations through its four domains: patient selection, index test, reference standard, flow and timing of patient. Every domain had flagging inquiries with choices of "Yes", "No" or "Unclear". The general risk of bias was evaluated as high: whenever responded to 'No' to any question, Low: whenever addressed 'Yes' to all inquiries and Unclear: whenever addressed 'Unclear' to all inquiries or joined by any 'Yes'. Risk of bias summary and applicability concern was graphically plotted using Review Manager (RevMan) software version 5.3.

Statistical analysis

Crude information was utilized to work out responsiveness and explicitness for each biomarker with their assessment technique. For by and large exactness, we determined pooled responsiveness, pooled explicitness with 95% certainty stretch, region under outline recipient working trademark. (Understanding of AUC values were as per the following: esteem above 80% were considered as brilliant, somewhere in the range of 70% and 80% as great, somewhere in the range of 60% and 69% as fair and beneath 60% as unfortunate results for a symptomatic test [18].

Data synthesis

To evaluate the effect of heterogeneity, Higgins I2 test was utilized. This test addresses the extent of fluctuation because of heterogeneity instead of because of inspecting blunder [19]. As per I2 test measurement the heterogeneity could be.

Additional analysis

Additional analysis was performed with positive likelihood ratio (PLR) and negative likelihood ratio (NLR) using DerSimonian-Laird's estimator considering random effect model. Positive likelihood ratio (PLR) in range of 2-5, 5-10 and >10 represents small, moderate and large increase in probability of disease when test is positive while Negative likelihood ratio (NLR) in range of 0.2-0.5, 0.2-0.1 and <0.1 represents small, moderate and large decrease in probability of disease when test is negative [20].

Results

Study Selection

After copies evaluation, reference rundown of all included examinations was screened. Of which 121 examinations were barred. After this full text articles were evaluated for qualification and articles that didn't meet consideration rules were barred. Only nine studies fitted into inclusion criteria and were subjected to qualitative analysis and seven studies for meta-analysis as shown in Figure 1.

Study Characteristics

A summary of descriptive characteristics of all included nine studies [21-29] is provided in (Table 1). Data was evaluated from nine studies [21-29] from aggregate of 2167 patients diagnosed with various OPMDs (oral leukoplakia (OL), oral lichen planus (OLP), erythroplakia (OE), oral erythron-leukoplakia and other oral dysplastic lesions. Among the included studies, one study was from Turkey [21], two studies [22, 24] from United Kingdom, one study [23] from Netherlands, two studies [25, 27] from India, one study [26] from China, one study from Switzerland [28] and one study [29] from Canada. Four studies [21, 24 , 25 , 29] had retrospective study design, five studies [22, 23, 26-28] had prospective study design. For various oral potentially malignant disorders (OPMDs), the index test used was DNA ploidy compared to the reference standard (biopsy followed by histopathological investigation). All the included studies had an overall sensitivity ranging from 33 – 97% with mean sensitivity of 73.5% while overall specificity ranged from 9.1 – 100% with mean specificity being 75.9%. It was concluded that DNA ploidy overall had a greater diagnostic accuracy compared to conventional modalities and could be used as reliable and valid diagnostic tool. DNA ploidy is a highly valuable, non- invasive, patient friendly method and with high sensitivity and specificity that can be used to screen lesions with high malignant lesions.

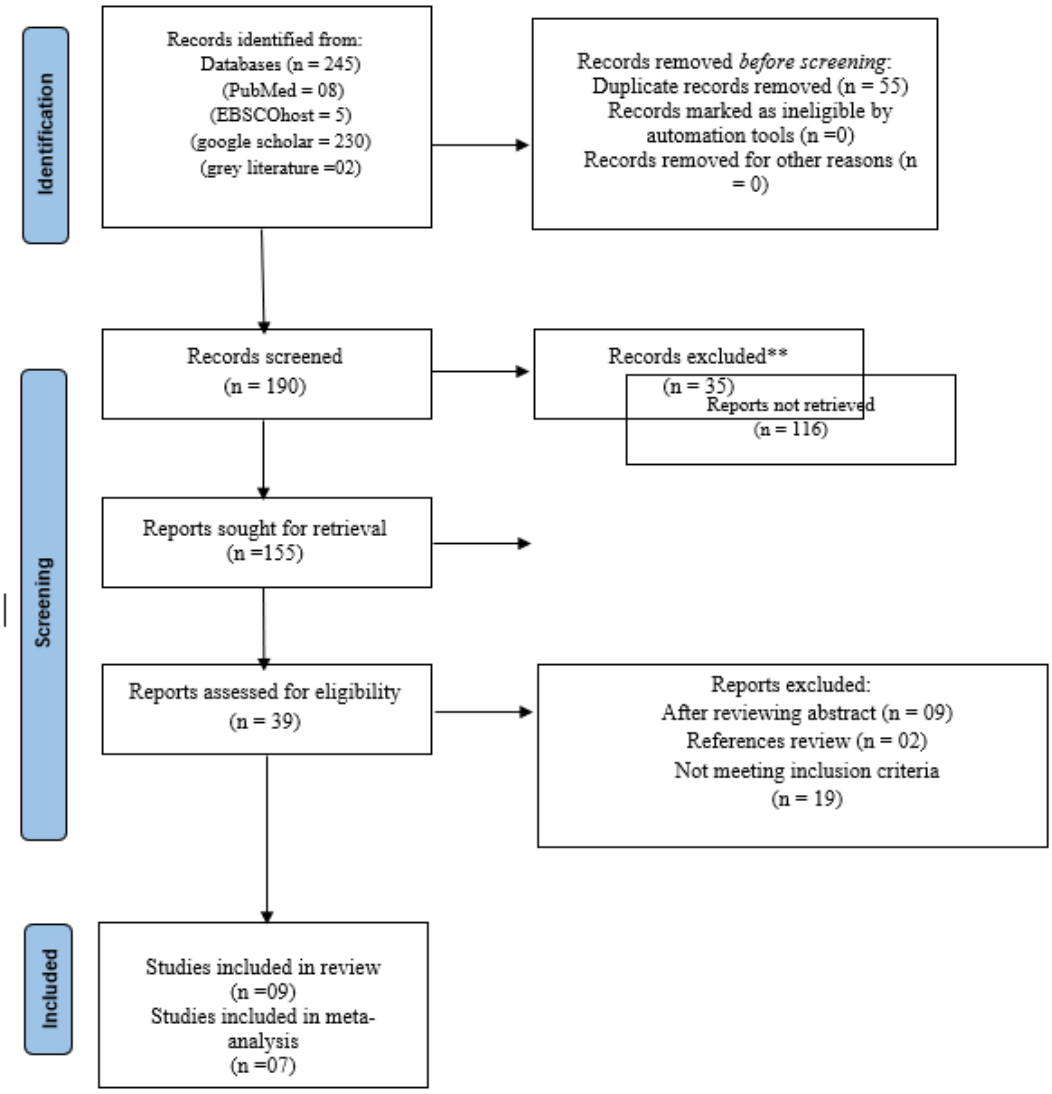

Figure 1. PRISMA 2020 Flow Diagram

Risk of Bias within Studies

Eight studies [21-27, 29] was classified as low ROB for all four domains. Patient selection was considered as high ROB in one study [28], which was mainly due to method of patient enrollment, nature of study design and implementing inappropriate exclusion.

The index test was considered to be at low ROB in all included studies. Low ROB was reported with respect to index test domain in all study due to absence of insufficient details reported as to whether results of index test was interpreted without prior knowledge of reference standard results, lack of pre-specification of a test-positive threshold and statement of conflict of interest. Similarly, the reference standard and flow and timing domain was considered at low risk in all studies as depicted in Figure 2 and 3.

Synthesis of Results

The meta-analysis was conducted for evaluating the overall diagnostic accuracy of DNA ploidy for patients

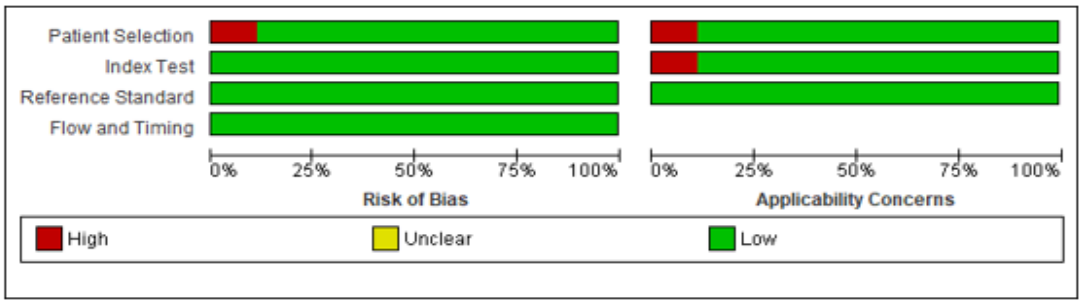

Figure 2. Risk of Bias Graph: presented as percentages across all included studies

Table 1. Descriptive Study Details of Included Studies

Authors, year of study	Country	Sample size	Study Design	OPMD assessed	Sensitivity (%)	Specificity (%)	Conclusion
Pektas et al., 2006 [21]	Turkey	44	Retrospective study	OLP, OL, erythroplakia, erythro-leukoplakia,	90.9	9.1	It is a good alternative to biopsy with high sensitivity and specificity
Torres – Rendon et al., 2009 [22]	United Kingdom	86	Prospective study	Oral dysplastic lesions	33	88	Dysplastic lesions with high malignant transformation rate can be diagnosed easily with DNA ploidy
Bremmer et al., 2011 [23]	Netherlands	62	Prospective study	OL	54	60	It has good prognostic indicator of disease
Sperandio et al., 2013 [24]	United Kingdom	273	Retrospective study	Dysplastic lesions	65.2	75	It has diagnostic efficacy equal to biopsy
Dineshkumar et al., 2019 [25]	India	40	Retrospective study	OL	80	100	Highly efficient tool with good diagnostic value
Li et al., 2020 [26]	China	401	Prospective study	OL, OLP, erythroplakia	61.5	77.5	It can be used as non-invasive tool for OPMD screening
Sathasivam et al., 2021 [27]	India	90	Prospective study	OL, OLP, erythroplakia, erythron-leukoplakia	58.1	79.5	DNA ploidy overall has good diagnostic efficacy
Bechstedt et al., 2022 [28]	Switzerland	602	Prospective study	OLP, OL,	93.5	98	It is a highly sensitive method with good patient acceptance
Liu et al., 2024 [29]	Canada	569	Retrospective study	Oral pre-cancerous lesion	97	96	It could be an effective screening method for lesions with high malignant risk

OE, oral erythroplakia; OL, oral leukoplakia; OLP, oral lichen planus

with OPMDs. Summary statistics measure was calculated in terms of pooled sensitivity, specificity, positive and negative likelihood ratio (PLR & NLR), diagnostic odd's ratio (DOR) and area under the curve (AUC).

As shown in Figure 4-5, data was evaluated from seven studies [22-28] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.71 (CI 0.28- 0.96) and pooled specificity was 0.31 (CI 0.03- 0.79) with I2

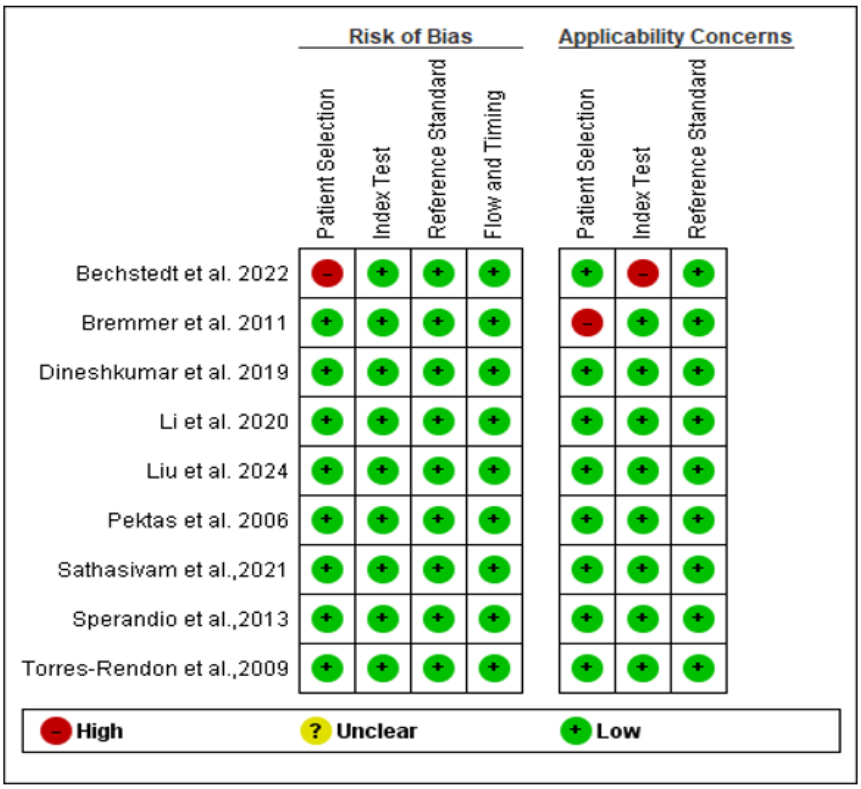

Figure 3. Risk of Bias Summary: for each included study

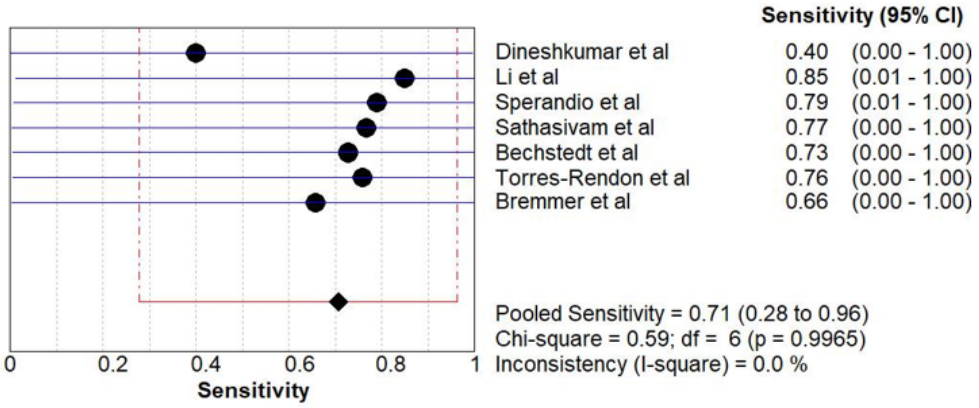

Figure 4. Pooled Sensitivity of DNA Ploidy for Patients with OPMD

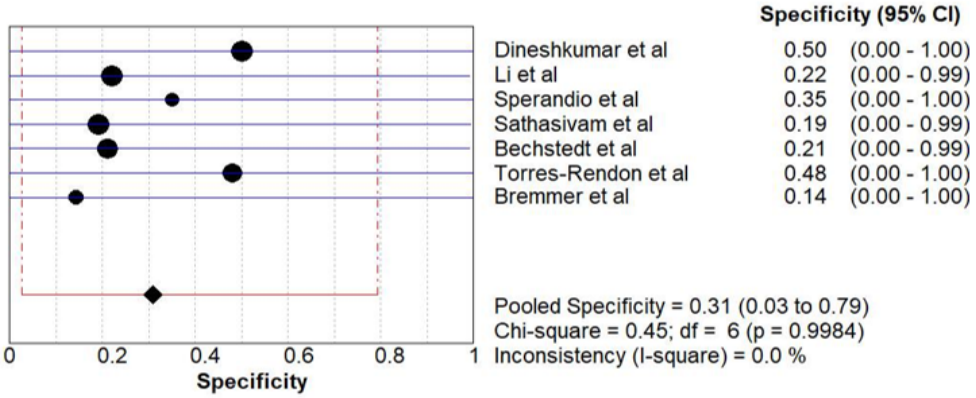

Figure 5. Pooled Specificity of DNA Ploidy for Patients with OPMD

being 0%.

As shown in Figure 6. the area under the curve (AUC) was plotted with sensitivity and 1-specificity and standard error. An overall accuracy of (AUC) 0.49 was seen for DNA ploidy indicating that the DNA ploidy had an overall moderate to low diagnostic efficacy in diagnosing the condition.

Additional analysis

Likelihood ratio was estimated which signifies the ability of the index test to predict the test results (positive / negative) when the disease condition in actual is present or absent. As shown in Figure 7 - 8, pooled positive likelihood ratio (PLR) 0.99 (0.49 – 2.02) and

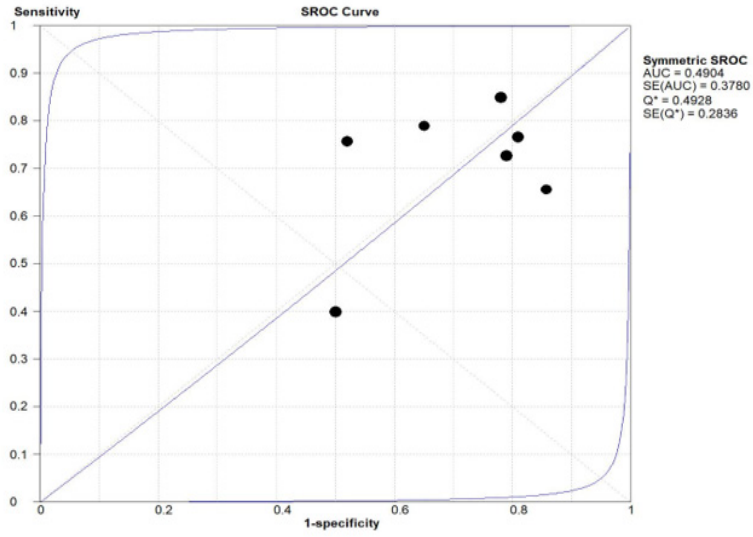

Figure 6. Overall Accuracy of DNA Ploidy for Patients with OPMD

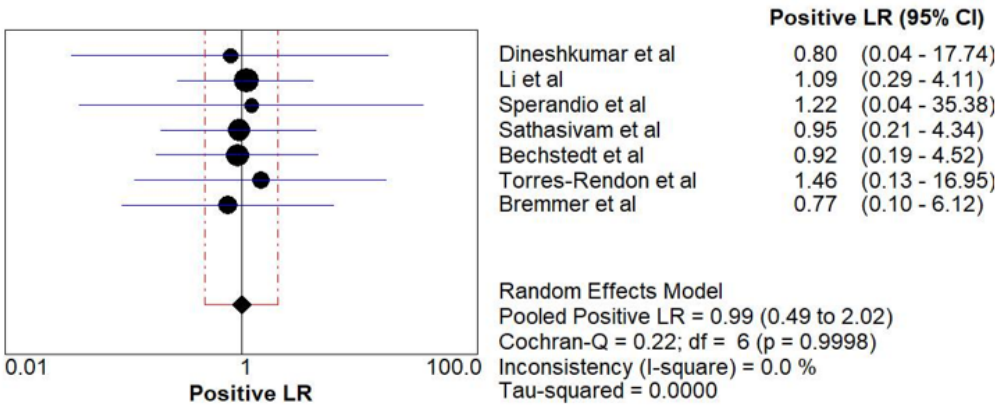

Figure 7. Pooled +LR of DNA Ploidy for Patients with OPMD

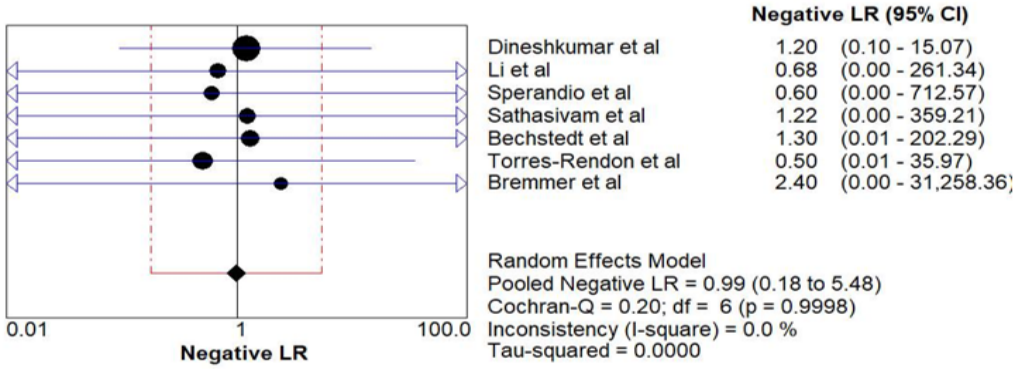

Figure 8. Pooled -LR of DNA Ploidy for Patients with OPMD

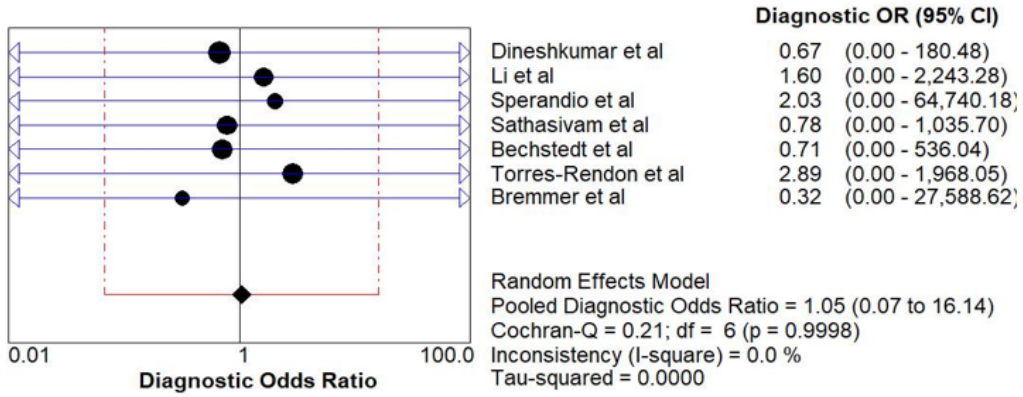

Figure 9. Pooled (DOR) of DNA Ploidy for Patients with OPMD

negative likelihood ratio (NLR) 0.99 (0.18 – 5.48) was estimated. Pooled +PLR suggested that CECT is 0.99 times more likely to have a positive detection of presence of OPMDs than someone without OPMDs while pooled -NLR suggested that DNA ploidy is 0.99 times as likely to have a negative OPMDs detection as someone without OPMDs.

As shown in Figure 9. the pooled Diagnostic Odds Ratio (DOR) is 1.05 (0.07 – 16.14) suggesting that overall ability of index test in correctly diagnosing the target condition is fair to moderate.

Discussion

Surgical biopsy remains the most definitive and reliable method for diagnosing oral lesions. However, the high prevalence of oral abnormalities of 5% to 15% detected as a result of oral screening programs and the difficulty of routinely subjecting large numbers of patients to a surgical biopsy makes the procedure impractical for the assessment of early lesions and recurrences in oral cancer [14, 15].

Ye et al. [30] conducted meta-analysis on comparing the diagnostic accuracy of brush biopsy and DNA image cytometry for pre-cancerous and cancerous lesions. Thirteen studies were included with data on aggregate of 1981 lesions. It was found that SROC of brush biopsy and DNA image cytometry was 0.88 and 0.98. The overall sensitivity, specificity and DOR of DNA image cytometry was 89%, 99% and 446 while of brush biopsy it was 86%, 81% and 20.36 respectively. From the results of the study, it was concluded that DNA image cytometry has greater diagnostic accuracy and can taken as an alternate adjunct to oral brush biopsy in detecting oral pre-cancer and cancerous lesions.

Datta et al. [9] conducted a systematic review to assess the effectiveness of DNA-image cytometry for oral potentially malignant disorders. 11 studies were included in analysis. The included studies reported an overall pooled sensitivity of (16-96%) and specificity of (90-100%). It was concluded that DNA-image cytometry can be used as a marker of malignancy and an excellent oral cancer screening tool.

Annapoorani et al. [31] carried out a systematic review to assess DNA ploidy status as a prognostic marker in various OPMDs. 30 studies (24-retrospective studies, 06 – prospective studies) were included in review. Various OPMDs were oral leukoplakia (OL), oral lichen planus (OLP), erythroplakia, proliferative verrucous leukoplakia was included. From the results of studies, it was concluded that DNA ploidy can be used as an useful prognostic biomarker for tracking the malignant transformation of lesions.

This systematic review was conducted to assess and evaluate the diagnostic accuracy of DNA ploidy for oral potentially malignant disorders (OPMDs). Databases were searched till April 2024 for studies evaluating the diagnostic potential of DNA ploidy for OPMDs in terms of pooled sensitivity, specificity, SROC, AUC and DOR. Nine studies [21-29] (Four studies [21, 24, 25, 29] had retrospective study design, five studies [22, 23, 26-28] had prospective study design). Various OPMDs (oral leukoplakia (OL), oral lichen planus (OLP), erythroplakia (OE), oral erythron-leukoplakia and other oral dysplastic lesions were included in studies. All the included studies had an overall sensitivity ranging from 33 – 97% with mean sensitivity of 73.5% while overall specificity ranged from 9.1 – 100% with mean specificity being 75.9%. From the results of the review, it was concluded that DNA ploidy overall had a greater diagnostic accuracy compared to conventional modalities and could be used as reliable and valid diagnostic tool.

Meta-analysis revealed an overall pooled sensitivity of 0.71 (CI 0.28- 0.96) and pooled specificity of 0.31 (CI 0.03- 0.79) with +PLR 0.99 (0.49 – 2.02) and -NLR 0.99 (0.18 – 5.48) and a DOR of 1.05 (0.07 – 16.14) with an overall diagnostic accuracy (AUC) of 0.49 suggesting that DNA ploidy was moderate to fair ability in diagnosing the target condition. Furthermore, standardized diagnostic accuracy studies with strict reporting using STARD (standards for reporting of diagnostic accuracy studies) guidelines or longitudinal studies with larger follow up period should be carried out to validate our study findings.

The adherence to the PRISMA guidelines, the thorough unrestricted literature search, utilization of reliable methodology with regard to the qualitative synthesis of data, the quality assessment of evidence with the QUADAS -2 tool strengthens this review. The quality assessment of all the included studies showed low risk of bias whereas overall quality was high, specifying lack of potential and inevitable sources of bias with limited variability and reporting deficiencies.

A systematic review is a transparent and repeatable procedure for identifying, selecting and critically assessing published or unpublished data to address a well-defined research question. Meta-analyses, a statistical analysis that incorporates numerical data from related studies, are frequently paired with systematic reviews. The best evidence is generally regarded as systematic reviews and meta-analyses. However, the calibre of the included studies has an impact on how strong the evidence is from a systematic review and meta-analysis. In the current systematic review, sufficient studies with a brief observation period and a known risk of bias were included. As a result, the presently available evidence is sufficient to make therapeutic recommendations in response to the current systematic review's focus question

Limitation

Firstly, the studies included had varying designs, with both retrospective and prospective approaches, leading to potential heterogeneity in the results. Additionally, the sample sizes across studies were inconsistent, which may affect the generalizability of the findings. Moreover, while DNA ploidy analysis shows promise as a diagnostic tool, its moderate diagnostic accuracy suggests that further standardized studies with longer follow-up periods are needed to validate its utility. Finally, the review is limited by the available studies up to April 2024, and newer research may influence future conclusions.

In conclusion, it was found that DNA ploidy has an overall moderate to fair diagnostic ability and is a valid and reliable tool in diagnosing the target condition. Our findings provide evidence on ability of DNA ploidy for various OPMDs for early screening and diagnosis. Thus, we can conclude DNA ploidy for secondary level of prevention for OPMD under early diagnosis and prompt treatment. However, further standardized accuracy studies are indicated to validate the overall diagnostic accuracy of DNA ploidy.

Author Contribution Statement

All authors contributed equally in this study.

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Novelty Expression

This research investigates the diagnostic accuracy

of DNA ploidy analysis in identifying oral potentially malignant disorders (OPMDs). Given the challenges associated with traditional biopsy methods, including invasiveness and patient discomfort, DNA ploidy analysis emerges as a non-invasive alternative with the potential for early detection of malignancies. The study's findings suggest that DNA ploidy exhibits promising sensitivity and specificity, making it a valuable adjunct in diagnosing OPMDs.

The significance of this study lies in its contribution to improving early cancer detection strategies, which is crucial for enhancing patient outcomes. By analyzing various OPMDs, the research aims to establish a clear correlation between DNA ploidy status and malignant transformation risk. This insight could lead to more targeted monitoring and timely interventions, thereby reducing the incidence and mortality associated with oral cancers.

Furthermore, the research highlights the need for further validation of DNA ploidy as a diagnostic tool. Despite demonstrating moderate diagnostic efficacy, the variability in sensitivity and specificity across studies underscores the necessity for standardized protocols and larger-scale investigations. Ultimately, the integration of DNA ploidy analysis into clinical practice could reshape the landscape of oral cancer screening, promoting more effective management of at-risk patients.

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