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Salivary biomarkers and their efficacies as diagnostic tools for Oral Squamous Cell Carcinoma: systematic review and meta-analysis

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Key words: Systematic Review; Salivary Biomarkers; Oral squamous cell carcinoma; Screening

ABSTRACT

More than 90% of malignant tumors of the head and neck are oral squamous cell carcinomas (OSCC). Early OSCC detection using salivary biomarkers could prevent malignant transformations and enhance patient survival. A systematic search in MEDLINE and the Central Register of Controlled Trials and meta-analysis were undertaken to identify the screening potential of 6 salivary biomarkers for early OSCC detection: IL-8, IL1-β, DUSP-1 and S100P mRNAs, miR125a and miR200a microRNAs. The sensitivities of IL-8 (*0.41*; 95%CI 0.19-0.99), IL1-β (*0.26*; 95%CI 0.19-0.99), DUSP-1 (*0.61*; 95%CI 0.01-0.98), and S100P (*0.67*; 95%CI 0.32-0.99) were calculated. Specificities of the biomarkers analyzed were found to be IL-8 (*0.69*; 95%CI 0.66-0.99), IL1-β (*0.47*; 95%CI 0.46 - 0.90), DUSP-1 (*0.75*; 95%CI 0.33-1) and S100P (*0.73*; 95%CI 0.18-0.99). Early detection of OSCC was best achieved by screening for salivary mRNA DUSP-1 and S100P. Further investigation is required into miRNAs as novel biomarkers.

INTRODUCTION

Each year, more than 550,000 cases of head and neck cancers are detected worldwide, with an annual death rate approaching 300,000/year[1]. Approximately 90% of all cancers originating in the head and neck, are squamous cell carcinomas (HNSCC), typically localized in 4 common areas: the oral cavity lining, hypopharynx, larynx, and the oropharyngeal area[2]. The overall 5-year survival rate of patients with HNSCC ranges from 40-50% and is the 6th most prevalent cancer globally[3]. 90% of malignancies within the oral cavity and oropharyngeal region emerge from squamous cell typology, thereby leading to the diagnosis of oral squamous cell carcinoma[4] (OSCC). OSCC can be defined as a carcinoma with squamous differentiation, arising from mucosal epithelium. The disease presents as flat, scale-like forms, found lining the mouth and the throat, which are easily detectable due to their superficial location[5].

Currently, the gold standard for detection relies on visual clinical examination by dental health care professionals in addition to histopathological investigations of suspicious areas[6]. Globally, OSCC incidence in younger populations is increasing primarily due to elevated levels of tobacco use[7], with a notably higher male to female incidence ratio (M:F = 1.5:1) due to an increased frequency of prominent risk behaviors[8].

With respect to HNSCC, the male to female ratio ranges from 2:1 to 4:1, and concordant with OSCC, the most commonly associated risk factors are elevated consumption of both alcohol and tobacco[9].

Common risk factors for OSCC development include genetics[10-12], the presence of pre-neoplastic lesions[10-13] and immunocompromised patients[11,12]. Late diagnosis of OSCC has been identified as an important contributing factor for reduced patient survival rates (15—50% reduction in survival) highlighting the need for improved diagnostics to aid early stage identification of OSCC[14].

Recent studies demonstrate the usefulness of saliva as a source of physio-pathological biomarkers in the early detection and diagnosis of cancers occurring in the oropharyngeal area[7, 15-18].

An analysis of the literature reveals that current investigative approaches for improving oral cancer detection combine proteomic, transcriptomic and genomic techniques[13].

Analysis of oral cytokine levels can provide clues in the early detection of OSCC and/or HNSCC[19-22]. Laser-capture micro-dissection microscopy revealed that IL-8 was upregulated in associated with OSCC[23]. The saliva of patients recently diagnosed with OSCC and/or HNSCC, was found to contain significantly elevated levels of IL-8[18,23]. Additionally, several articles concur that salivary protein IL1-β can be used as a biomarker for oral cancer[19, 22, 25-28].

Some investigators have demonstrated the utility of quantifying the expression of key OSCC-associated messenger RNA (mRNA). In 2006, salivary mRNA transcript analysis was initiated in a validation cohort of 32 patients with OSCC and/ or HNSCC and 32 healthy subjects. 7 transcripts were significantly elevated in OSCC and/HNSCC patients ($p<0.05$), including Dual Specificity 1 Protein (DUSP-1) and Small calcium Protein 100 (S100P) mRNA. Combinations of these biomarkers attained an overall sensitivity and specificity

evaluation of 91%, thereby positioning them amongst the most discriminatory panels of cancer biomarkers originating from human bodily fluids[24-30].

Recent research demonstrates the utility of microRNAs (miRNAs) as a biomarker for solid tumors[11]. miRNAs are RNA transcripts of between 19-25 nucleotides found in saliva, associated with post-transcriptional regulation. miRNAs play a role in cellular growth, differentiation and apoptosis, mediation of physiological stress responses and immune function[11,31,32]. Studies have shown a differential expression of miRNAs within cancerous cells compared with normal cells[33]. Two important miRNAs implicated in OSCC are miR-125a and miR-200a, both of which were differentially expressed in saliva when comparing OSCC patients and healthy control subjects[34]. These results support the use of miRNAs (specifically miR125a and miR200a) as diagnostic tools for detection of oral cancer[34-36]. Several studies have demonstrated a statistically significant reduction in salivary levels of miR125a and miR200a in patients suffering from HNSCC as compared to healthy controls[34-36].

To date, there is no systematic review that simultaneously compares the efficacy of three most promising classes of salivary biomarkers for the detection of OSCC and/or HNSCC. The primary objective of this systematic review, therefore, is to compare the efficacy of the principal salivary biomarkers so far: cytokines IL-8 and IL1-β; mRNAs DUSP-1 and S100P and miRNAs miR-200a and miR-125a.

MATERIALS AND METHODS

Protocols and Registration:

This systematic review was created in accordance with the Preferred Reporting System for Systematic Reviews and Meta Analyses[37] (PRISMA). The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO), with reference number CRD42018095104 (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=95104)

Eligibility

Articles were incorporated into this review based upon the P.I.C.O.S model of clinical questioning for evidenced based medicine[38]. Only studies involving the following salivary biomarkers were included: IL-8, IL1-β, DUSP1, S100P, miR125a and miR200a. Eligible articles types included: clinical trials, cohort and case-control studies, comparative studies and literature reviews. The primary variable was the type of salivary biomarker. The sensitivity and specificity for early detection of OSCC and/or HNSCC were calculated. Based on previously published results, the expected outcome was predicted to be that salivary miRNAs would possess the highest overall efficacy for the detection of OSCC and/or HNSCC.

Inclusion and Exclusion Criteria:

Full text articles published from 1st January 2000 - 30th September 2017 involving adult human volunteers, aged 19-58 years old (based on highest prevalence of OSCC and/or HNSCC in the population) were included for analysis[39,40].

The following study types were excluded from this review: biographies, directories, editorials, lectures, commentaries, retracted publications, abstracts. Studies for which English translations were unavailable were also excluded.

Search Protocol

Articles were searched from two primary research databases as recommended by Cochrane - MEDLINE and the Central Register of Controlled Trials. Detailed information with respect to the search string utilized, is found in Supplementary Material - Table 3.

Data Extraction:

The titles and abstracts of the articles identified from the search results were assessed in the context of the inclusion criteria. The included articles were then screened with respect to the exclusion criteria. The following information was collected from the full text articles comprising the final selection: author(s); publication year; country; sample sizes of both cases and controls (individuals with OSCC and/or HNSCC and healthy subjects respectively); disease classification (OSCC and/or HNSCC); subject ages; time of disease diagnosis, diagnostic stage of disease; current treatment; biomarker name and classification; biomarker detection method and the main conclusions. Articles were reviewed independently by authors FIG and VV. Disputes were resolved by CCS. FIG, collected the necessary data from the chosen articles for subsequent evaluation and VV and CCS cross-checked data for suitability.

Quality Assessment Measures:

The QADAS quality assessment tool was used to appraise primary research articles (carried out by FIG and VV)[41]. Disputes were resolved by CCS. Quality assessment of review articles was carried out in accordance with CASP (Critical Appraisal Screening Process guidelines[42].

Outcome Measures:

The primary evaluated outcome was the sensitivity and specificity of detection of OSCC and/or HNSCC by salivary cytokines, mRNAs and miRNAs.

Statistical Analysis:

A meta-analysis was performed on the extracted data for all 6 salivary biomarkers. The mean sensitivity, specificity and accuracy (area under the curve (AUC) point values), along with their standard error and 95% confidence intervals were calculated from data extracted from the articles. A one-way ANOVA analysis was carried out to determine the F-distribution, and the obtained (F_{obt}) and critical (F_{crit}) F-values were calculated to p=0.05 and p=0.01. Microsoft Excel was used to create the forest plots.

RESULTS

Search Results

Initially 1112 articles were retrieved including duplicates. 1,079 studies were excluded, and, following elimination of duplicates, 578 relevant articles were obtained. 32 articles were eligible for inclusion, with a final total of 18 eventually included in the review (Fig.1).

Study Characteristics

All studies were conducted from 2000 - 2017. Data was evaluated from an aggregate of 3876 patients, with average patient participation at 117.5 persons per study. Geographically, 3 Indian, 3 European, 1 Iranian, 6 Asian, and 20 American studies were finally selected for inclusion. A complete summary of the included studies can be found in Table 1. All studies utilized the same standardized reference tests: ELISA for salivary cytokines, RT-preamp-qPCR for salivary mRNAs and microarray analyses/ miRNA stability assay analyses for salivary miRNAs.

Excluded articles were removed from this review for the following reasons: (i) not all patients received index and reference tests. (ii) Inadequate data for sensitivity and specificity of index tests. (iii) Inappropriate patient sample sizes and ages. (iv) Evaluation of index tests, aside from those specified.

Results of the quality assessment of the primary research articles included in this review, using the QADAS - 2 TOOL[41] are presented in figure 2. A detailed description of the analyzed articles is found in Supplementary Materials Table 2. None of the included articles were found to have a low risk of bias across all evaluated domains. All studies had 'low concern' with regards to applicability. Literature reviews were critically appraised for quality using the CASP TOOL assessment criteria[42] (Fig. 3). All 13 studies addressed a clearly focused question and rated positively on the article selection process.

Synthesis of results

6 articles investigated the efficacy of salivary cytokines IL-8 and 1L-1β for the early detection of OSCC and/or HNSCC. Of these, 2 articles focused solely on salivary IL-8[11,19]. The remaining 4 articles investigated the efficacy of multiple salivary biomarkers for the early detection of OSCC and/or detection. These studies included an analysis of IL1—β, DUSP1 and S100P mRNAs, and miR125a and miR200a micro-RNAs[15,24,33,43]. All authors reported sensitivity and specificity data IL-8 and IL1-β, with the exception of Cheng *et al.* who provided IL-8 concentrations in pg/ml, and Spielmann and Wong, who calculated sensitivity and specificity values for IL-8 mRNA[11,24]. 7 articles investigated the efficacy of salivary mRNA biomarkers DUSP1 and S100P for early detection and diagnosis of OSCC and/or HNSCC[15,24,29,30,33,43,44]. 3 articles analyzed salivary miRNAs miR125a and miR200a as biomarkers for head and neck malignancies. Several studies analyzed both miRNAs[32,33,43].

A total of 77 patients were enrolled in the studies investigating the efficacy of IL-8[15,24,33,43]. Average sensitivity was 0.41 (40.6 ± 0.3 % (standard error ,SE) (95% CI: 0.19-0.99; Fig. 4). Average specificity was 0.69 (68.69 ± 0.31 %; 95% CI:0.66-0.99; Fig. 4). Accuracy of IL-8 in the early detection of OSCC and/or HNSCC (equivalent to the Area Under the Curve (AUC) point value) was found to be 0.88 ± 0.18 (Table 2). A total of 61 patients were enrolled in the included studies investigating the efficacy of IL-β[15,33,43]. Average sensitivity was 0.26 (25.6 ± 0.3%; 95% CI: 0.19-0.99; Fig. 4). Average specificity was 0.47 (47.16 ± 0.3%; 95% CI: 0.46-0.90; Fig. 4). AUC of IL-8 was 0.82 ± 0.16 (Table 2).

88 patients were enrolled in the included studies investigating the efficacy of DUSP-1 mRNA[15,24,29,30,33,43,44]. Average sensitivity was 0.61 (60.9 ± 0.3% 95%

CI: 0.01-0.98; Fig. 4). Average specificity was 0.75 (74.85% ± 0.3%; 95% CI: 0.33-1.00; Fig. 4). AUC value of DUSP-1 mRNA was 0.66 ± 0.21 (Table 2). A total of 78 patients were enrolled in the studies investigating the efficacy of S100P mRNA[15,24,29,30,33,43,44]. Average sensitivity was 0.67 (67.22 ± 0.29%; 95% CI: 0.32-0.99; Fig. 4). Average detection specificity was 0.73 (73.41 ± 0.3%; 95% CI: 0.18-0.99; Fig. 4). AUC of S100P mRNA was 0.78 ± 0.21 (Table 2). A total of 74 patients were enrolled in the studies investigating the efficacy of miR125a mRNA[32,33,43] and 96 patients in the case of miR200a mRNA[32,33,43]. These studies did not evaluate the detection sensitivity or specificity therefore it was not possible to calculate the average values nor the CI. AUC value of miR125a microRNA was 0.62 ± 0.16, whilst AUC value of miR200a microRNA was 0.65 ± 0.14 (Table 2).

Statistical Evaluations of Combined Results

The data extracted from the included articles was subjected to ANOVA analysis to determine the statistical significance of any differences in sensitivity within and between the studies. The calculated F-value (F_{obt}) for both the within- and between-groups analysis was 0.83. At p=0.05, the critical F value (F_{crit}) was 3.20, and at p=0.01, the F_{crit} was 5.18. Both F_{crit} values are greater than F_{obt} (with 3 degrees of freedom between groups (dfB), and 17 degrees of freedom within groups (dfW)) (Supplementary Material Table 4). Thus, we must accept the null hypothesis, which states that there are no significant differences in the sensitivity of the salivary biomarkers for the detection of patients with HNSCC and/or OSCC malignancies at both 5% and 1% significance levels.

With regards to specificity, the F_{obt} for both the within- and between-groups analysis was -1.09. At p=0.05, the critical F value F_{crit} was 3.20, and at p=0.01, the F_{crit} was 5.18. Both F_{crit} values are greater than F_{obt} (with 3 dfB and 17 dfW) (Supplementary Material Table 4). Thus, we must accept the null hypothesis, which states that there are no significant differences in the sensitivity of the salivary biomarkers for the detection of patients with HNSCC and/or OSCC at both 5% and 1% significance levels.

As per the AUC values, the F_{obt} for both the within- and between-groups analysis was 1.22 (Supplementary Material Table 5). At p=0.05, the F_{crit} was 3.20, and at p=0.01, the F_{crit} was 5.18. Both F_{crit} values are greater than F_{obt} (with 3 dfB and 17 dfW) (Supplementary Material Table 5). Thus, we must accept the null hypothesis, which states that there are no significant differences in the accuracy of the 6 evaluated biomarkers to discriminate between OSCC and/or HNSCC, and healthy volunteers at both 5% and 1% significance levels.

DISCUSSION

The objective of this systematic review and meta-analysis was to compare the efficacy of selected salivary cytokines, mRNAs and miRNAs for the early detection of OSCC and/or HNSCC in patients presenting with clinically evident lesions, irrespective of stage of tumor progression.

The first observation was that not all studies presented detection sensitivity and specificity values. The overall interpretation of the data must therefore take this into account when recommending oral biomarkers. Of the studies presenting

this data, the highest sensitivity and specificity for the early detection of OSCC and/or HNSCC was obtained when testing for DUSP-1 and S100P mRNA. The sensitivity and specificity values for the detection of OSCC and/or HNSCC by testing for IL-8 and IL1-β were significantly lower.

Although mRNA biomarkers proved to be superior in our analyses as compared to cytokine biomarkers, in the early detection of OSCC and/or HNSCC, one must view these results in the light of the relative differences in methodology and precision between the techniques used for the generation of both datasets (PreAmp-qRT-PCR vs ELISA), in addition to the dependence on technical expertise, which could influence the quality of the results in both cases. The stage of the malignancy, and other patient factors may also affect the results of the tests for both types of biomarkers. As indicated by the AUC data analysis there was a clear difference in assay accuracy, the highest being for the cytokine markers, followed by the mRNAs and lastly the microRNAs.

The individual sensitivity and specificity analyses rank the mRNAs as better biomarkers, whilst a more holistic AUC point value analysis highlights the cytokines as being more accurate overall. The analyses are therefore open to interpretation and reasoned arguments may be made supporting the use of either cytokines or mRNA biomarkers in the early detection of OSCC and/or HNSCC. Analysis of the cytokine biomarkers revealed comparatively lower sensitivity and specificity values as compared to mRNA biomarkers, which could imply that the biological link between cytokines and OSCC and/or HNSCC may not be as strong as the link between the mRNA markers and disease. The higher AUC point estimates for the cytokine biomarkers suggests a more easily

interpretable and meaningful measure of performance in correctly distinguishing between healthy and ill subjects as compared to the mRNA biomarkers[45]. Clinicians must take these observations into account, balancing sensitivity and specificity with functionality, when selecting an appropriate test for patients suspected of OSCC and/or HNSCC.

Hilden raised concerns about overdependence on AUC point values as a deciding factor, arguing that AUC analyses present a one-dimensional perspective of an assay, hence limiting their applicability solely to the magnitude of assessment response, which, in the case of this review, would correspond to the accuracy with which the 6 analyzed biomarkers could distinguish between diseased patients and healthy comparators in early tumor detection[46]. A further shortcoming of AUC point value analyses lies in their inability to consider calculated pretest probability data, which is a pre-requisite for satisfactory evaluation within a clinical environment.

With regards to DUSP-1 and S100P, both biomarkers demonstrated high sensitivity and specificity values, coupled with average AUC point values. This last observation suggests an enhanced sensitivity to technician ability and assay execution. Our study suggests therefore that the utility of mRNA biomarkers for the early detection of OSCC and/or HNSCC in patients in a dental clinical or hospital setting would depend on the availability of expertly trained clinicians able to run and interpret the analyses.

Key strengths of this review include the large number of included articles (32 in total), facilitating and strengthening the meta-analysis. Summarization and interpretation was supported by consistency amongst contributing factors,

including, the average number of subjects recruited per study (cases and controls), similarity among patient recruitment protocols, reproducible methodology of salivary sample handling and profiling, homogeneity in the use of specialist techniques and equipment. Limitations of our study are mainly population selection bias, since the majority of examined studies were conducted in Caucasian and Asiatic populations. Additionally, studies investigating the efficacy of miRNAs lacked sensitivity and specificity values, due to the novel nature of this kind of biomarkers.

In summary, it is difficult to state conclusively which of the 6 salivary biomarkers (IL-8, IL1-β, DUSP-1, S100P, miR125a and miR200a) may be most effective for early detection of OSCC and/or HNSCC in patients aged 19-58. mRNA markers were most sensitive and specific, whilst cytokine biomarkers appeared be most accurate overall (perhaps due to simpler execution and analysis). Notwithstanding, the miRNA markers displayed the poorest overall accuracy, which is most likely due to their high dependence on expert technical ability for their execution and interpretation.

Conflict of interests: None to declare.

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Ethical approval: This systematic review and meta-analysis was carried out in accordance with the Helsinki guidelines, and approved by the Ethics Committee of CEU Cardenal Herrera University (authorization number CEI18/110).

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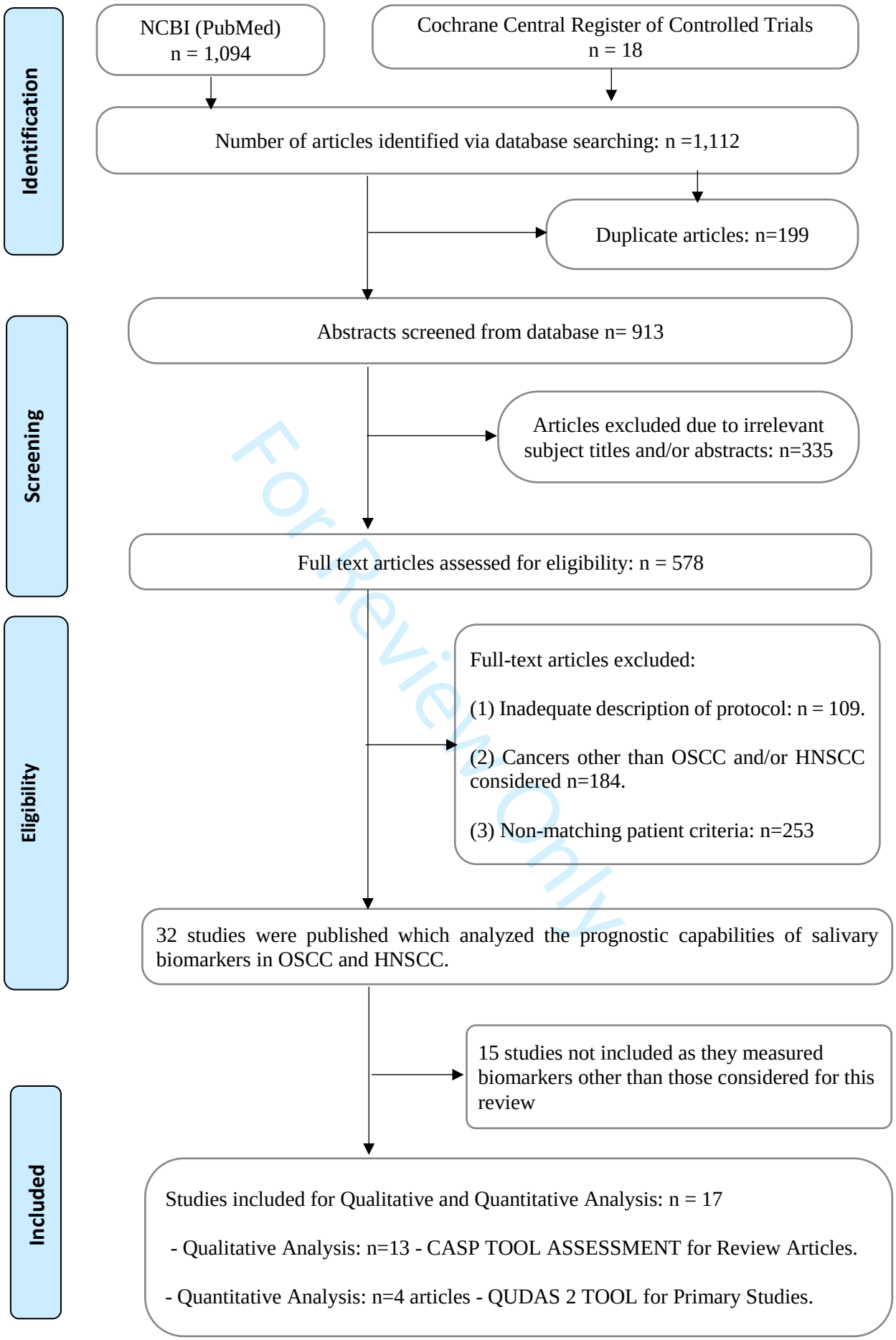
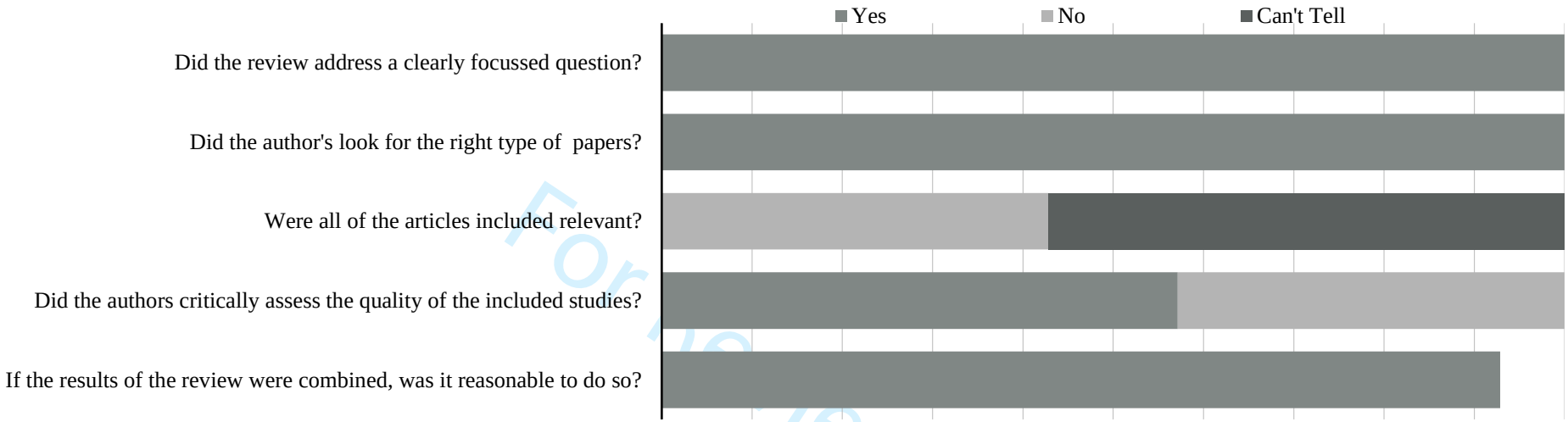


Figure 2: QUDAS - 2 TOOL for the evaluation of primary research articles:- risk of bias and applicability concerns: representation in graphical format. The graphs present a summary of author's judgements with respect to each domain as percentages across all included articles.

Figure 3 - Summary table of Critical Appraisal Skills Programme (CASP) Tool for the evaluation of literature reviews. A. Description of the validity of the search results. B. Local applicability of the search results.

A - RESULT VALIDITY



B - LOCAL APPLICABILITY

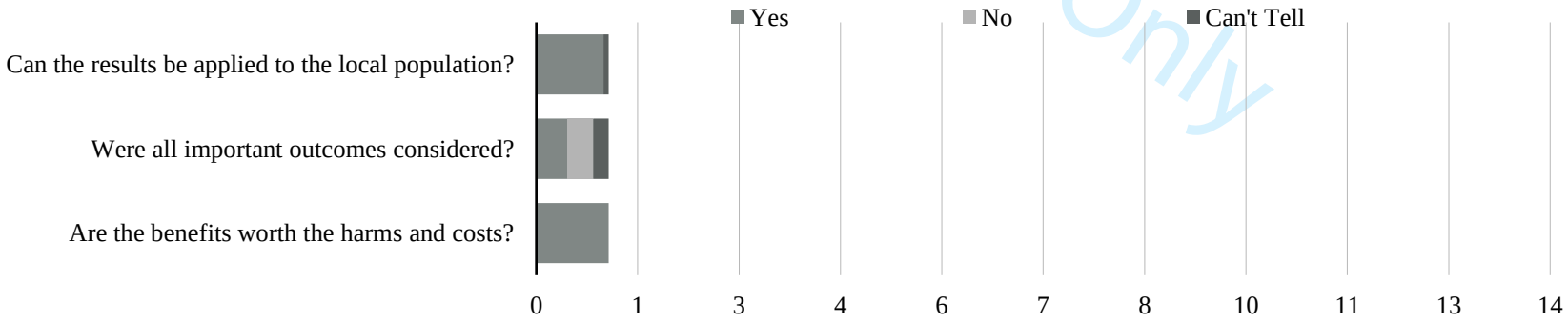
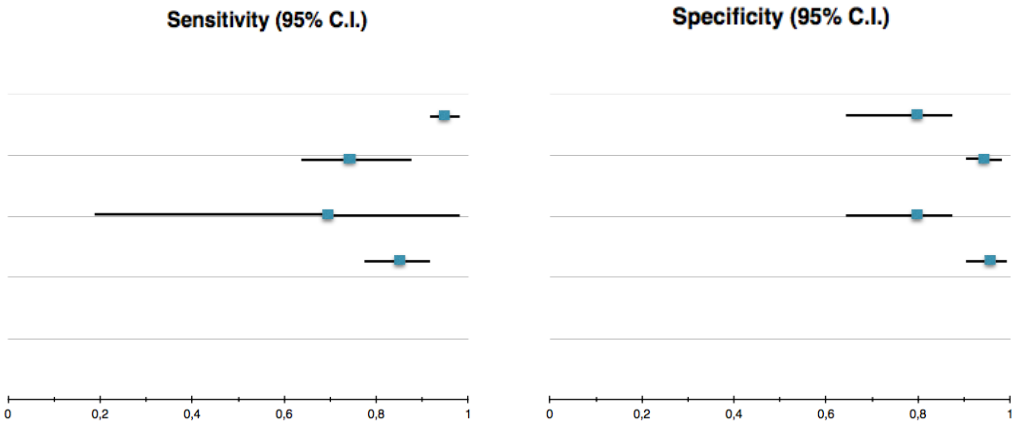


Figure 4: Stated sensitivity and specificity values with calculated 95% CI of 4 investigated salivary biomarkers for the detection of OSCC and/or HNSCC: IL-8, IL1-β, DUSP-1 and S100P

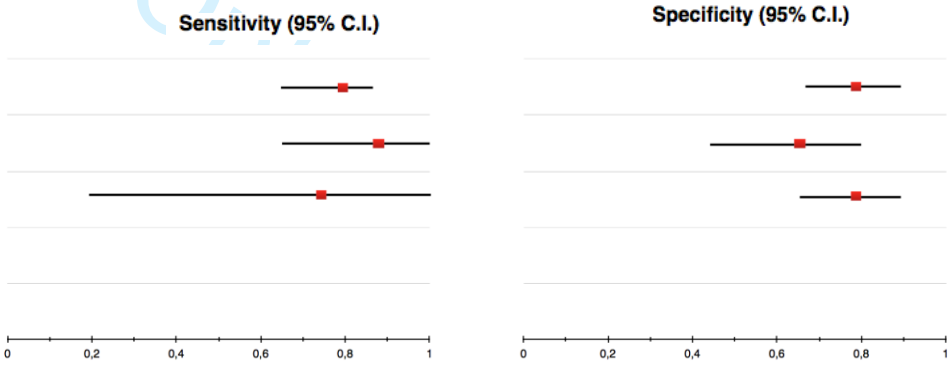
IL-8

Study	Sensitivity	95% C.I	Specificity	95% C.I
Brinkmann *et al.*	**0.97**	**(0.91 - 0.99)**	**0.80**	**(0.66 - 0.89)**
Bonne *et al.*	**0.78**	**(0.62 - 0.89)**	**0.96**	**(0.90 - 0.99)**
Yakob *et al.*	**0.75**	**(0.19 - 0.99)**	**0.80**	**(0.66 - 0.87)**
Spielmann and Wong	**0.86**	**(0.77 - 0.92)**	**0.97**	**(0.91 - 0.99)**
	(%)	**(2 d.p)**	**(%)**	**(2 d.p)**
Mean	**40.61**	**0.41**	**68.69**	**0.69**
Standard Error (S.E)	**0.31**	**-**	**0.34**	**-**



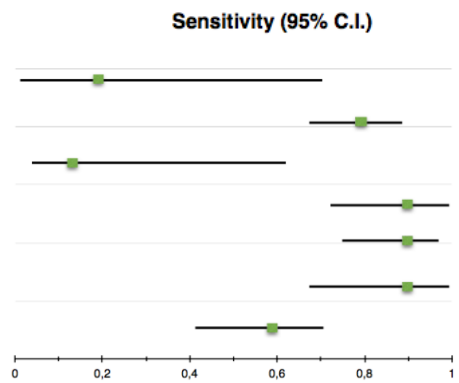
IL1-β

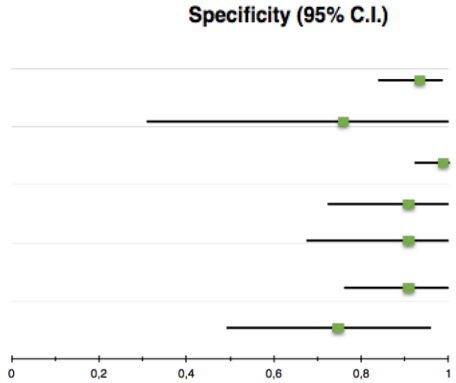
Study	Sensitivity	95% C.I	Specificity	95% C.I
Brinkmann *et al.*	**0.80**	**(0.66 - 0.89)**	**0.81**	**(0.67 - 0.90)**
Bonne *et al.*	**0.89**	**(0.65 - 0.99)**	**0.67**	**(0.46 - 0.83)**
Yakob *et al*	**0.75**	**(0.19 - 0.99)**	**0.80**	**(0.66 - 0.89)**
	(%)	**(2 d.p)**	**(%)**	**(2 d.p)**
Mean	**25.56**	**0.26**	**47.16**	**0.47**
Standard Error (S.E)	**0.31**	**-**	**0.33**	**-**



DUSP-1

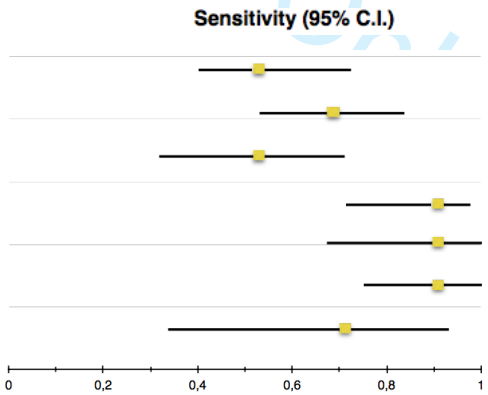
Study	Sensitivity	95% C.I	Specificity	95% C.I
Brinkmann *et al.*	0.20	(0.01 - 0.72)	0.95	(0.85 - 0.98)
Bonne *et al.*	0.80	(0.67 - 0.90)	0.77	(0.33 - 0.99)
Yakob *et al*	0.14	(0.04- 0.64)	0.98	(0.93 - 1.00)
Spielmann and Wong	0.91	(0.73 - 0.98)	0.91	(0.73 - 0.99)
Panta *et al.*	0.91	(0.76 - 0.97)	0.91	(0.68 - 0.99)
Zimmermann *et al.*	0.91	(0.68 - 0.98)	0.91	(0.76 - 0.99)
Li *et al.*	0.59	(0.42- 0.71)	0.75	(0.49 - 0.96)
	(%)	(2 d.p)	(%)	(2 d.p)
Mean	60.89	0.61	74.85	0.75
Standard Error (S.E)	0.26	-	0.30	-

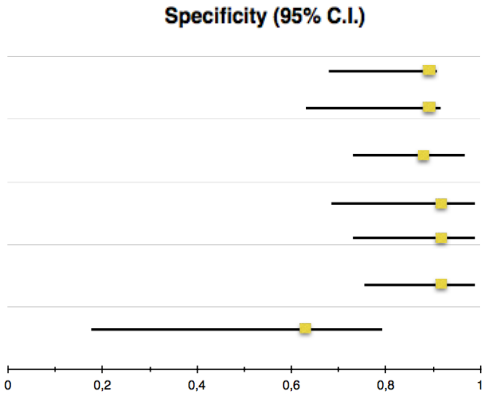




S100P

Study	Sensitivity	95% C.I	Specificity	95% C.I
Brinkmann *et al.*	0.56	(0.40 - 0.72)	0.89	(0.68 - 0.90)
Bonne *et al.*	0.71	(0.54 - 0.84)	0.89	(0.64 - 0.92)
Yakob *et al*	0.54	(0.32- 0.73)	0.88	(0.73 - 0.97)
Spielmann and Wong	0.91	(0.73 - 0.98)	0.91	(0.68 - 0.99)
Panta *et al.*	0.91	(0.68- 0.99)	0.91	(0.73 - 0.98)
Zimmermann *et al.*	0.91	(0.76 - 0.99)	0.91	(0.76 - 0.98)
Li *et al.*	0.72	(0.34- 0.93)	0.63	(0.18 - 0.79)
	(%)	(2 d.p)	(%)	(2 d.p)
Mean	67.22	0.67	73.41	0.73
Standard Error (S.E)	0.29	-	0.31	-





Author (Year)	Country	Cases of OSCC and/or HNSCC	Control	Mean age of volunteers	Salivary Biomarker *	Type	Detection Method	Principal Conclusions
Brinkmann *et al.* 2011	Serbia	35 OSCC	51	Cases: 60.9 Controls: 38.2	DUSP-1 (1, 2), IL-8 (1, 2), IL-1β (1, 6), S100P (2) IL-8 (1, 2), IL-1β (1, 6), M2BP (2, 7)	mRNA proteins	RT-PCR preamp. qPCR ELISA	Combining mRNA and protein biomarkers have the greatest potential for early detection
Li *et al.* 2004	USA	32 OSCC	32	Cases: 49.8 Controls: 49.1	DUSP-1 (1, 2), GADD45B (4), H3F3A (5), IL-8 (1,2), RGS2 (3), S100P (2), IL-1β (1, 6), SAT (5), OAZI (·)	mRNA	Microarray analysis qPCR	i) IL8, IL1B, DUSP1, HA3, OAZI, S100P and SAT were the most commonly identified mRNA biomarkers OSCC patients. ii) All 7 biomarkers demonstrated at least a 3.5 fold increase in OSCC saliva samples.
Park *et al.* 2009	USA	50 OSCC	50	Cases: 56 Controls: 52	miR125a (3), miRNA200a (2), miRNA93 (1,2)	miRNA	RT-PCR preamp. qPCR Saliva miRNA stability assay	i) miR-125a and miR-200a are differentially expressed in OSCC patients compared with healthy subjects. ii) miRNAs have the potential to be used as rapid diagnostic tools for early detection of oral cancers.
St. John *et al.* 2004	USA	32 OSCC and/or HNSCC	32	Cases: 49.3 Controls: 48.8	IL-6 (3, 6), IL-8 (1, 2)	mRNA Cytokines	RT-qPCR ELISA	i) IL-6 present in serum and IL-8 present in saliva, were both elevated in case subjects compared with healthy controls. ii) Serum IL-6 and salivary IL-8 cytokines are promising biomarkers for oral cáncer detection.

*Table 1: Summary of the descriptive characteristics of the included studies. * (1) Regulation and/or promotion of angiogenesis; (2) Regulation of metastasis; (3) Positive modulator of tumour growth progression; (4) Positive regulator of cellular apoptosis; (5) Regulator of intracellular concentrations of polyamides and their cellular exportation; (6) Positive regulator of the inflammatory response; (7) Increases cellular adhesion of tumour cells. DUSP-1, Dual Specificity Protein - 1; GADD45B, Growth arrest and DNA damage-inducible beta 45; H3F3A, H3 Histone Family 3A; IL1-β, Interleukin 1-BETA; IL-6, Interleukin 6; IL-8, Interleukin 8; M2BP, Mac-2 Binding Protein; miR-93, micro RNA 93; miR-125a, micro RNA 125a; miR200a, micro RNA 200a; OAZI, Antizyme Inhibitor 1; RGS2, Regulator of Gene Protein Signalling-2; S100P, Calcium-binding protein P*

Salivary biomarkers	AUC value	SE	Variance
Il-8 (Protein)	0.88	0.18	2.43
Il1-β (Protein)	0.82	0.16	1.58
DUSP1 (mRNA)	0.66	0.21	3.79
S100P (mRNA)	0.78	0.21	3.56
miR125a (miRNA)	0.62	0.16	1.89
miR200a (miRNA)	0.65	0.14	1.84

Table 2: Measure of variability of efficacy for salivary biomarkers Il-8, Il1-β, DUSP1, S100P , miR125a and miR200a in the detection of OSCC and/or HNSCC: mean area under curve (AUC), standard error (SE) and variance are displayed