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Review article

Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: A systematic review

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ABSTRACT

Recent evidence suggests that salivary cytokines provide information about both oral conditions and systemic diseases. This review summarizes evidence for the use of salivary cytokines as biomarkers for oral and systemic diseases. We included studies in adults and children with a focus on the latter, due to the importance of non-invasive diagnostic methods in the paediatric age group. A systematic review was performed using Medline and Web of Science covering the period of January 1996 to December 2019 according to the preferred reporting items for systematic reviews. Thirty-four studies were included in the final analysis, for a total of 2407 patients and healthy controls. Pro-inflammatory cytokines including interleukin (IL)-1β, IL-2, IL-6 and tumor necrosis factor (TNF)-α were associated with the severity of oral mucosal tissue damage in patients with cancer, and IL-1β may be an early marker of graft-versus-host disease. Salivary interferon-γ levels were correlated with oral complications and the presence of the underlying disease in HIV-infected individuals, and salivary cytokine patterns may be useful for diagnosing tuberculosis. In summary, current data illustrate that salivary cytokines are associated with oral inflammation, making them potential biomarkers for disease diagnosis and treatment efficacy. Because of the simplicity of saliva collection, this method may be useful in pediatric studies and in resource-limited settings.

1. Introduction

Human saliva is a complex fluid secreted by the salivary glands and gingiva. It contains proteins, including cytokines, as well as organic and inorganic substances that are important for maintaining oral health [1]. Saliva may also be used for diagnostic purposes, offering clear advantages over other samples, including low-cost, non-invasive collection that can be performed by individuals with limited training. This is particularly valuable in children for whom blood collection may be challenging.

Studies investigating salivary cytokines support their utility as diagnostic biomarkers for several diseases, including oral cancer and caries. Most impressively, salivary cytokines may also be correlated with and predictive of systemic diseases, and can therefore be used for diagnostic purposes beyond oral conditions [2]. Older studies mostly investigated the association of a single biomarker with local or systemic diseases [3,4]. More recent studies have used novel technologies enabling the simultaneous measurement of multiple cytokines in a small volume of saliva [5].

Several reviews have summarized the evidence on salivary cytokines as biomarkers for oral conditions [6,7]. A few studies investigated salivary cytokines in patients with psychiatric conditions [8], rheumatologic diseases such as Sjögren syndrome [9,10], cystic fibrosis [11] and sleep apnea [12]. Most studies analyzed salivary cytokines levels in association with caries, periodontitis, cancer, and infectious diseases. The aim of the current study was to systematically review the potential of salivary cytokines in oral and acute systemic diseases on diagnosis and early identification of complications, focusing on oncological and

Abbreviations: ALL, Acute lymphoblastic leukemia; EBV, Epstein–Barr virus; ELISA, Enzyme-linked immunosorbent assay; GvHD, Graft-versus-host disease; HHV, Human herpesvirus; HIV, Human immunodeficiency virus; HSCT, Hematopoietic stem cell transplant.

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infectious conditions. We included studies in adults and children with a focus on the latter. Non-invasive diagnostic sampling options are of particular interest in the paediatric age group to simplify diagnostic approaches. Conversely, chronic or congenital diseases were excluded from this systematic review.

2. Methods

This systematic review was performed according to the preferred reporting items for systematic reviews and meta-analyses criteria [13]. Studies were identified through searches of Medline and Web of Science for articles published between 1 January 1998 and 31 December 2019 using the following search terms and Boolean search operators: saliva* AND cytokine* OR biomarker* OR interleukin* AND human*. The following inclusion criteria were used: original articles of randomized controlled trials, cohort studies, and case-control studies; sufficient details reported on sample type; description of the assays used to detect cytokines; and the results of salivary cytokines. Case series, case reports, animal studies and in vitro studies were excluded. Only studies published in English were included. Finally, only papers with a focus on oncological, oral, dental, or infectious diseases were included in the analysis. The systematic review was registered at PROSPERO (CRD 42018081317).

Search results were independently screened by one reviewer and checked by a second reviewer. The following variables were extracted from the included studies: target population (adult/pediatric), number of patients, age range, and mean age, type of sample used, type of assay used for cytokine analysis, time point of collection, type of measured cytokines, mean/median cytokine concentrations, study design, publication year, and main findings (including relation to clinical aspect and impact of the findings on management). Results on biomarkers other the cytokines were not systematically extracted.

The quality assessment was performed by two authors (TD and CF) and disagreements were discussed until a consensus was reached. A modified Quality Assessment of Diagnostic Accuracy Studies tool was applied [14].

3. Results

3.1. Study characteristics

In total, 1603 articles were identified, of which 34 studies with 2407 participants were included in the final analysis (Fig. 1). Twenty-eight (82%) studies included a control group, giving a total of 995 healthy controls. All studies had a prospective study design, one study was an interventional analysis, and no studies were randomized controlled

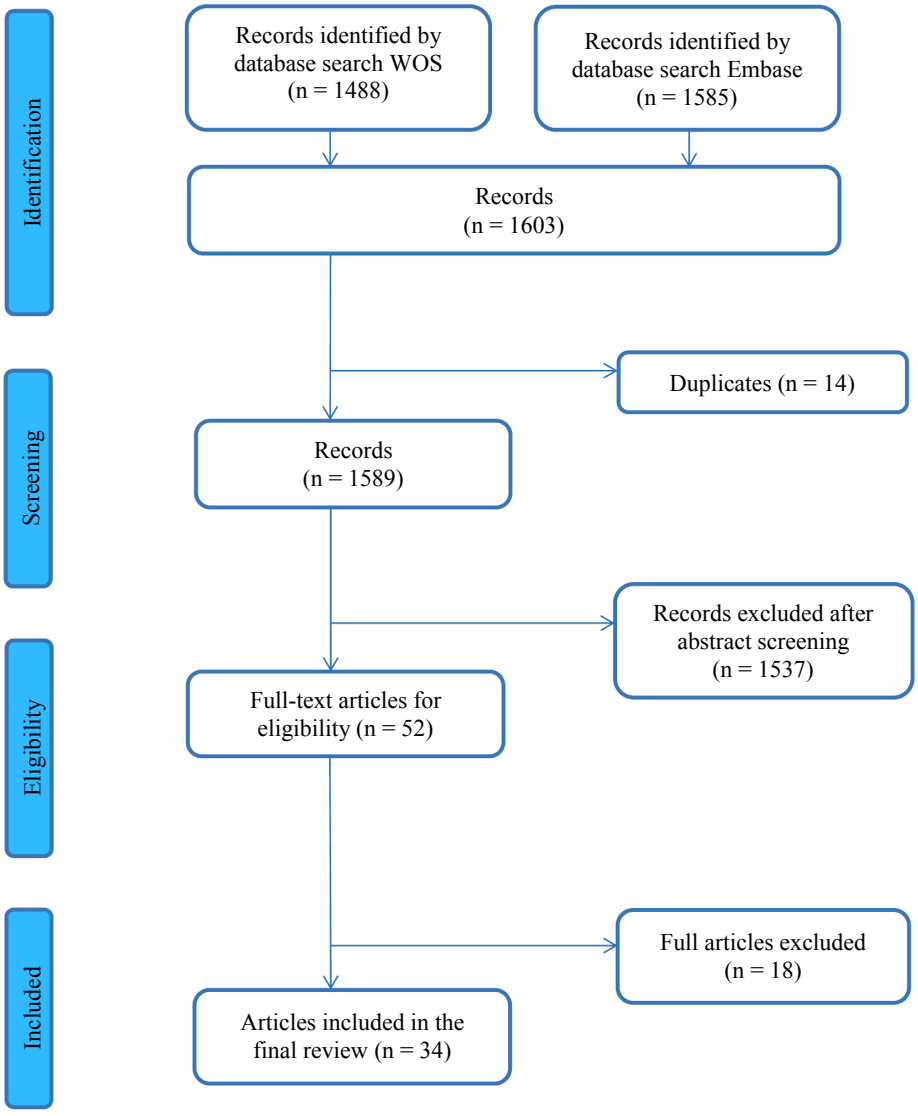

Fig. 1. Flow Diagram showing the study selection process.

trials. A certain risk of bias was present across almost all studies. Some studies had selection bias because of the large age range within the examined population. Furthermore, in some studies, samples were collected random times of day, potentially leading to additional bias. Some studies used swabs for saliva collection, which may not be representative of the whole saliva composition (Supplementary Table S1)

Eight studies analyzed salivary cytokines exclusively in children, two studies examined both children and adults, and 24 studies analyzed only adults. The selected studies were performed in 13 countries: United States of America (n = 10), India (n = 4), Poland (n = 4), Brazil (n = 3), Taiwan (n = 3), Japan (n = 2), South Africa (n = 2) Italy (n = 1), China (n = 1), Estonia (n = 1), Finland (n = 1), Turkey (n = 1), and Venezuela (n = 1) (Table 1).

Twenty-four studies analyzed cytokines in saliva only, eight studies analyzed cytokines in saliva and blood or serum, one study examined cytokines in saliva and buccal epithelia and one study assessed cytokines in saliva, blood, and tissue. The tests used to measure cytokines in the included studies were enzyme-linked immunosorbent assay (ELISA) (n = 22), bead-based xMAP technology (n = 7), both ELISA and bead-based xMAP technology (n = 3), and bead-based flow cytometry technology (n = 2). A single cytokine was investigated in seven studies, whereas the remaining studies investigated 2–69 cytokines or other biomarkers. The most investigated cytokines were interleukin (IL)-6 (23/34, 67%), tumor necrosis factor (TNF)-α (15/34, 44%), and IL-10 (14/34, 41%). Further details on the cytokines measured in the studies are summarized in Table 2.

3.2. Non-oral oncological disorders

We identified seven studies analyzing salivary cytokines in patients with non-oral oncological disorders, including 609 patients with leukemia who had received hematopoietic stem cell transplants (HSCTs). Two of these studies exclusively analyzed children. All seven studies investigated salivary cytokines as possible biomarkers of chemotherapy-induced local adverse effects such as mucositis or systemic disease. The most important findings from each study are summarized as follows (also see Table 2).

Krzaczek et al. investigated IL-2, IL-6, IL-10, TNF-α, and interferon (IFN)-γ levels in saliva and blood in 44 children with acute lymphoblastic leukemia (ALL) during treatment and 1 year after treatment in comparison to 40 controls [15]. Salivary TNF-α levels were higher (median, 0.5 pg/ml) 1 year after treatment in patients than in controls (median 0.4 pg/ml). Although the authors concluded that the difference was significant, the measured concentrations were low. For IL-10, the concentrations were higher in the control group (median, 19.1 pg/ml) than in patients during treatment (median, 8.3 pg/ml) and at 1 year after treatment (median, 10.2 pg/ml). Mucositis was generally more common in patients with ALL during treatment, but the correlations between mucositis and salivary cytokine levels were not analyzed.

Pels et al. compared salivary IL-2 concentrations and the rates of mucositis during chemotherapy in 78 children with ALL and healthy controls at three time points before chemotherapy, in the first week of treatment and 6–18 months after chemotherapy [16]. Salivary IL-2 concentrations increased during chemotherapy, and this increase was associated with the severity of mucositis.

Morales et al. investigated IL-1, IL-6, and TNF-α content in saliva and blood in 21 children and adolescents with ALL and healthy controls 1 h before and 12 and 96 h after chemotherapy [17]. Cytokine concentrations were generally higher in saliva than in blood, and their levels were higher in children with ALL than in controls. IL-6 concentrations in saliva and blood significantly increased from <25 pg/ml before chemotherapy to >60 pg/ml at 96 h after treatment. Interestingly, the levels of IL-1 and TNF-α, two other pro-inflammatory cytokines, were not different in blood or saliva between before and after chemotherapy. An association of cytokine concentrations with mucositis was not reported.

Fall-Dickson et al. analyzed TNF-α concentrations in saliva, blood, and buccal epithelial tissue in 25 adults receiving HSCT at baseline and 9 days after conditioning chemotherapy [18]. Buccal samples exhibited significantly higher TNF-α RNA expression on day 9, and its expression was correlated with the severity of mucositis. The mean salivary TNF-α concentrations were also higher on day 9 (11 pg/ml) than at baseline (4 pg/ml), but this difference was not considered significant. Conversely, TNF-α concentrations in blood were low at baseline and on day 9 after chemotherapy (3 pg/ml).

Resende et al. analyzed IL-10 concentrations in the blood and saliva of 58 pediatric and adult patients receiving HSCTs who developed acute graft-versus-host disease (GvHD) [19]. Weekly blood and saliva samples were obtained from 7 days before to 100 days after transplantation. Salivary IL-10 concentrations were not associated with the risk of GvHD. However, IL-10 concentrations in blood were associated with the occurrence of GvHD, as its mean levels were 504 pg/ml in patients with GvHD and 276 pg/ml in those without GvHD. Further research in the same population revealed an increase of salivary IL-1β concentrations before GvHD was diagnosed and in its blood concentrations at the time of GvHD diagnosis [20]. However, salivary IL-1β levels were low, reaching 5 pg/ml at 5 weeks after the GvHD diagnosis.

Koizumi et al. analyzed 27 cytokines in saliva, blood, and oral mucosal transudate samples from patients with non-small cell lung cancer and healthy controls [21]. A panel of 12 cytokines differentiated patients with lung cancer from healthy controls. In particular, the concentrations of IL-10 (mean, 4.37 pg/ml) and IP-10 (mean, 1765 pg/ml) were significantly elevated in the saliva of patients with lung cancer, and these cytokines detected non-small cell lung cancer with a sensitivity of 61% and specificity of 81%. Both study groups included comparable numbers of current or former smokers.

3.3. Systemic infectious diseases

Six studies analyzed salivary cytokines in patients with systemic infectious diseases, including studies assessing HIV infection (n = 3) and tuberculosis in adults (n = 2) and one study investigating human herpesvirus (HHV)-6/Epstein–Barr virus (EBV) prevalence in children with seizures. The studies included a total of 564 patients.

Spear et al. compared the concentrations of six cytokines in saliva and blood with HIV viral loads in 60 HIV-infected patients and 10 uninfected controls [22]. Overall, IL-1β, IFN-γ and IL-10 concentrations were significantly higher in saliva than in plasma, whereas TNF receptor 2 levels were significantly higher in plasma than in saliva. IL-1β and IFN-γ concentrations in saliva and blood were not correlated. Salivary IFN-γ levels were significantly higher in HIV-infected patients than in uninfected controls, whereas the opposite finding was noted for salivary IL-10 concentrations. No correlation was observed between salivary cytokine levels and HIV viral loads in saliva. Information on oral infections or inflammation was not available in this study.

Vastardis et al. analyzed the T helper cell 1 (Th1)-type cytokines IL-2, IL-12 and IFN-γ and the Th2-type cytokines IL-4 and IL-10 in 39 HIV-infected individuals with varying degrees of periodontal disease [23]. There was no significant difference in the severity of periodontitis according to the cytokine concentrations.

Another study by Black et al. investigated salivary cytokine levels in parotid saliva from 52 HIV-infected individuals with and without oral candidiasis/hairy leukoplakia and 22 healthy controls [24]. In HIV-infected patients with oral candidiasis or oral hairy leukoplakia, salivary IFN-γ content was elevated compared with that in HIV-infected individuals without oral lesions or healthy controls. Interestingly, IL-2 levels were only elevated in HIV-infected patients with oral candidiasis. No differences were noted between HIV-infected individuals and healthy controls without oral changes.

Jacobs et al. examined 33 salivary biomarkers in 104 individuals with suspected tuberculosis [5]. The levels of 10 biomarkers

Table 1
Summary of studies include in the systematic review ordered by topic, target condition and year of publication. Publications including children have been shaded in grey.

First author [reference]	Year of Publication	Country	Total n/ paediatric n	Target condition	Underlying disease (n)	Age range (years)	Sample	Assay	Cytokines	Main outcomes
Non-oral oncological disorders										
Krzaczek [15]	2019	PL	84/84	Mucositis	21 patients with ALL in maintenance (A) 23 patients ALL in the first year after treatment (B) 40 healthy children	A) 4–18 B) 5–17 4–15	Stimulated saliva, blood	ELISA	IL-2, IL-6, IL-10, TNF-α, IFN-γ	TNF-α elevated in group A. IL-10 elevated in the control group
Pels [16]	2015	PL	156/156	Mucositis	78 children with ALL 78 healthy children	2–18	Unstimulated saliva	ELISA	IL-2	IL-2 elevated after chemotherapy
Morales-Rojas [17]	2012	VE	42/42	Mucositis	21 children with high risk ALL 21 healthy children	4–19	Unstimulated saliva, serum	ELISA	IL-1, IL-6, TNF-α	IL-6 and TNF-α elevated in patients with mucositis Increase only detectable 96 h after the administration of chemotherapy
Fall-Dickson [18]	2007	US	25/0	Mucositis	25 adults after HSCT ns	32–68	Unstimulated saliva, blood and buccal brush biopsy	ELISA	TNF-α	Buccal TNF-α elevated at day 9 after chemotherapy in patients with mucositis
Resende [19]	2010	BR	116/ns	GvHD	58 allogeneic HSCT recipients 58 allogeneic HSCT donors	5–56 6–69	Unstimulated saliva, blood	ELISA	IL-10 IL-10 polymorphisms (−1082)	IL-10 elevated in blood at week 5 after HSCT in patients with GvHD IL-10 polymorphisms of recipient and donor not associated with GVHD
Resende [20]	2013	BR	116/ns	GvHD	58 allogeneic HSCT recipients 58 allogeneic HSCT donors	5–56 6–69	Unstimulated saliva, blood	ELISA	IL-1β IL-1β polymorphisms (+3954)	IL-1β increased progressively before diagnosis of GvHD
Koizumi [21]	2018	JP	70/0	Lung cancer	35 adults with non-small cell lung cancer 35 healthy adults, non-smokers	42–80 42–69	Unstimulated saliva	Bead-based xMAP	IL-1ra, IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL13, IL-1, IL-17A, IP-10, TNF-α, IFN-γ *	IL-10 and IP-10 elevated in patients with head and neck cancer
Infectious diseases										
Spear [22]	2005	US	88/0	HIV	70 adults with HIV (10 with CD4 levels < 200 mm^{-3}; 30 with 200–500 mm^{-3}; 30 with > 500 mm^{-3})	26–58	Unstimulated saliva, serum	ELISA	IL-1β, IL- 6, IL-10, TNF-α, IFN-γ, TNF-α receptor 2	IFN-γ elevated in seronegative patients No correlation between salivary cytokine levels and HIV viral loads
Vastardis [23]	2003	US	39/0	HIV	35 male adults with HIV	25–54	Unstimulated saliva	ELISA	IL-2, IL-4, IL-10, IL-12, IFN-γ	No significant relationship between periodontal disease and cytokine concentrations
Black [24]	2000	US	74/ns	HIV	52 adults with HIV (39 with normal oral health 8 with candidiasis; 5 with oral hairy leukoplakia) 22 HIV-uninfected with normal oral health	ns	Unstimulated saliva from parotid	ELISA	IL-1, IL-2, IL-4, IL-5, IL-10, TGF-β, TNF-α, IFN-γ	IL-1α and IFN-γ higher in patients with HIV and oral disease than in with HIV-infected patients without oral disease IL-10 was highest in healthy controls
Jacobs [5]	2016	SA	51/ns	TB	18 patients with TB (4 with HIV) 33 patients with other respiratory diseases (12 with HIV)	ns	Stimulated saliva	Bead-based xMAP	69 markers including IFN-γ, IFN-α, IP-10, TNF-α, IL-1β †	Five salivary markers were identified as diagnostic panel for TB independently of the HIV status
Jacobs [25]	2016	SA	104/ns	TB	32 patients with TB 72 with other respiratory diseases	ns	Unstimulated saliva	Bead-based xMAP	33 markers including IFN-γ, IL-1, IL-2, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17A, TNF, IP-10	Seven salivary markers were identified for diagnosing TB and monitoring treatment
Bartolini [26]	2018	US	212/212	HHV-6/EBV	115 patients with seizures 51 patients with fever and no seizures 46 healthy controls	1 month to 18 years 7–18	Unstimulated saliva	Bead-based flow cytometry	IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12p70	IL-8 and IL-1β elevated in patients with seizures IL-1β correlated with HHV-6 viral load
Oral and dental diseases										

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Table 1 (*continued*)

First author [reference]	Year of Publication	Country	Total n/ paediatric n	Target condition	Underlying disease (n)	Age range (years)	Sample	Assay	Cytokines	Main outcomes
Sharma et al. [27]	2017	IN	50/50	Caries	25 patients with early childhood caries 25 healthy controls	1–6	Unstimulated saliva	ELISA	IL-6, IL-8, TNF-α	IL-6, IL-8 and TNF-α levels elevated in patients with caries
Cogulu et al. [28]	2015	TR	108/108	Caries	37 patients with high patients caries 37 with moderate caries 34 individuals without caries	6–12	Unstimulated saliva, blood	ELISA	IL-1β, IL-1ra, IL-10	*Streptococcus mutans* level in saliva positively correlated with IL-1β
Menon et al. [29]	2016	IN	22/22	Caries	22 patients with caries	3–6	Unstimulated saliva	ELISA	IL-6	IL-6 level elevated in patients with caries Reduction with dental treatment
Gornowicz et al. [30]	2012	PL	36/0	Caries	26 patients with caries 10 healthy controls	18	Unstimulated saliva	ELISA	IL-6, IL-8, TNF-α	TNF-α and IL-8 elevated in patients with caries
Wu et al. [31]	2018	TW	57/0	Periodontitis	30 patients with periodontitis 27 healthy controls	32–56 24–57	Unstimulated saliva	ELISA and bead-based xMAP	IL-6, IL-8, IL-1β, IL-1ra, TNF-α [§]	Salivary IL-1β, MMP-8 and MMP-9 elevated in patients with periodontitis
Lee [32]	2018	TW	54/0	Periodontitis	34 patients with periodontitis 20 healthy controls	35–60 28–65	Unstimulated saliva	ELISA and bead-based xMAP	IL-6, IL-8, IL-1β, IL-1ra [‖]	Salivary IL-1β and MMP-8 elevated in patients with periodontitis and decreased after treatment
Teles et al. [33]	2009	US	118/0	Periodontitis	74 patients with periodontitis 44 healthy controls	18–65 18–65	Unstimulated saliva	Bead-based xMAP	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF	No statistical difference in cytokine levels between both groups
Belstrom et al. [34]	2017	FI	29/0	Gingivitis	29 healthy non-smokers	22–9	Stimulated saliva	Bead-based xMAP	IL-1β, IL-1ra, VEGF, IL-8, MCP-1	Decrease of all salivary cytokines
Santos et al. [35]	2016	BR	71/71	Gingivitis	37 patients with paraparesis 34 patients with tetraparesis	6–17	Unstimulated saliva	Bead-based xMAP	IL-1β, IL-6, IL-8 TNF-α	IL-1β, IL-6, IL-8 and TNF-α elevated in patients with gingivitis
Liskmann et al. [36]	2006	EE	30/0	Overdentures	12 patients with *peri*-implant disease (*peri*-implantitis or mucositis) 18 healthy controls	ns	Unstimulated saliva	ELISA	IL-6, IL-10	IL-6 and IL-10 elevated in patients with *peri*-implant disease because of local inflammatory stimulus and immunological response
Leigh et al. [37]	2002	US	17/0	Stomatitis	9 with *Candida* infection 8 patients without *Candida* infection	39–76 45–79	Unstimulated saliva	ELISA	IFN-γ, IL-12, IL-4, IL-10	No difference between the groups
Oral and neck cancer										
Deepthi [38]	2019	IN	90/0	Oral and neck cancer	30 patients with oral squamous cell carcinoma 30 patients with leukoplakia 30 healthy controls	ns	Unstimulated saliva	ELISA	TNF-α	TNF-α elevated in patients with oral cancer Lower in patients with leukoplakia and healthy controls
Lee et al. [39]	2018	TW	65/ns	Oral and neck cancer	41 patients with oral cancer	ns	Unstimulated saliva	Bead-based xMAP	IL-6, IL-8, IL-10, IL-1β, IFN-γ, TNF-α [¶]	IL-1β, IL-6, IL-8 and TNF-α elevated in patients with cancer
Zhang et al. [40]	2016	CN	62/ns	Oral and neck cancer	40 patients with oral cancer 20 healthy controls	27–70 ns	Unstimulated saliva	ELISA	IL-6 and sIgA	IL-6 elevated in patients with cancer
Polz-Dacewicz et al. [41]	2016	PL	118/ns	Oral and neck cancer	78 patients with histopathologically confirmed oropharyngeal squamous cell carcinoma 40 healthy	40–78	Unstimulated saliva, serum	ELISA	IL-10, TNF-α, TGF-β, VEGF	All tested cytokines elevated in patients with cancer EBV associated with high IL-10, HPV associated with high TGF-β TNF-α and VEGF correlated with the histological tumour grade
Russo et al. [42]	2016	US	16/ns	Oral and neck cancer	16 subjects in the initial cohort (12 received radiotherapy and six underwent only surgery) ns	ns 42–70	Stimulated saliva	ELISA and bead-based xMAP	EGF, GRO-α, IL-1α, IL-1β, IL-6, IL-8, TNF-α, VEGF	Seven cytokines elevated after treatment except
Bossi et al. [43]	2016	IT	75/ns	Oral and neck cancer	50 patients with locally advanced head and neck cancer 10 healthy adults 10 patients with other cancers	ns	Unstimulated saliva	Bead-based flow cytometry	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α, TNF-β, osteopontin, VEGF	Chemoradiotherapy increased IL-1β, IL-6 and TNF-α levels in patients with head and neck cancer Correlation with the severity of mucositis

(*continued on next page*)

Table 1 (*continued*)

First author [reference]	Year of Publication	Country	Total n/ paediatric n	Target condition	Underlying disease (n)	Age range (years)	Sample	Assay	Cytokines	Main outcomes
Korostoff et al. [44]	2011	US	74/ns	Oral and neck cancer	18 patients with squamous cell carcinoma of the tongue 56 control patient (14 healthy, 14 smoker, 14 drinker, 14 smoker and drinker)	35–80 45–75	Unstimulated saliva	ELISA	IL-1, IL-6, IL-8, VEGF, TNF-α	All 5 cytokines were elevated in the endophytic tumour type IL-8 level correlated with the presence of metastasis and poor prognosis
Sharma et al. [45]	2011	IN	60/0	Oral and neck cancer	20 patients with oral leukoplakia and periodontitis 20 with only periodontitis 20 healthy controls	31–65 ns	Unstimulated saliva	ELISA	Il-6 in saliva	IL-6 elevated in patients with leukoplakia, correlated with severity Tobacco consumption increased IL-6
Katakura et al. [46]	2007	JP	39/ns	Oral and neck cancer	19 patients with oral cancer 20 healthy controls	ns	Unstimulated saliva	ELISA	4 biomarkers in saliva: IL-1B, IL-6, IL-8 and osteopontin	All four cytokines elevated in patients with oral cancer, especially IL-6
Rhodus et al. [47]	2005	US	39/ns	Oral and neck cancer	13 patients with oral lichen planus (OLP); 13 patients with oral squamous cell cancer (OSCC) 13 age- and sex-matched controls	OLP:57 OSCC 59 58	Unstimulated saliva and histology of tissue for dysplasia	ELISA	IL-1, IL-6, IL-8, TNF-α	IL-6 and IL-8 elevated in patients with malignant transformation No difference of the four cytokines between smokers and non-smokers

* CCL1, FGF2, CSF3, CSF2, CXCL10, CCL2, CCL3, PDGF-BB, RANTES, VEGF (Koizumi T, clinical research report).

†, NCAM, transthyretin, MIP-4, antithrombin-III, GDF-15, ADAMTS13, A2M, haptoglobin, CRP, SAP, PCT, ferritin, TPA, fibrinogen, SAA, vitronectin, ECM1, vitamin D binding protein, sFas, granzyme A, sFasL, sCD137, granzyme B, perforin, myoglobin, P-selectin, lipocalin-2, TPO, SCF, BCA-1, ENA-78, TSLP, CCL-1, SDF-1α, MIP-1β, VEGF, sCD40L, Apo A-1, Apo CIII, complement component 3, CFH, PAI-1, BDNF, cathepsin D, MPO, MMP-2, MMP-9, HCC-1, PEDF, complement C4, IL-17F, IL-22, IL-33, IL-21, IL-23, IL-25, IL-31, IL-28A, IL-16 (Jacobs R *PLOS One*).

‡ CXCL1, IL-1α, MDC, TNF-α, IFN-ɣ, IP-10, VEGF, MCP-1, MIP-1β, fractalkine, granzyme A, sFasL, sFasL, sCD137, A2M, haptoglobin, CRP, SAP, PCT, TPA, fibrinogen, SAA (Jacobs R *Cytokine*).

§ CRP, lactoferrin, MMP-8, MMP-9, PDGF-BB, VEGF (Wu Y-C, *Journal of the Formosan Medical Association*).

‖ CRP, lactoferrin, MMP-8, MMP-9, PDGF-BB, VEGF (Lee CH, *J Peridont Res*). ¶ MPI-1β, eotaxin, G-CSF, GRO, VEGF, EGF, IL-1α (Lee LT, *Int J Oral Maxillofac Surg*). ELISA, enzyme-linked immunosorbent assay; IL, interleukin; TNF, tumour necrosis factor; IFN, interferon; HSCT, haematopoietic stem cell transplant; ns, not specified; GvHD, graft-versus-host disease; HIV, human immunodeficiency virus; TB, tuberculosis; TGF, transforming growth factor; IP-10, interferon gamma-induced protein 10; HHV, human herpesvirus; EBV, Epstein–Barr virus; IL-1ra, IL-1 receptor antagonist; GM-CSF, granulocyte–macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; MCP, monocyte chemoattractant protein; sIgA, soluble immunoglobulin A; GRO, growth-regulated oncogene. Studies with children.

significantly differed between patients with tuberculosis disease and those with other respiratory diseases, and seven salivary markers comprised an optimal discriminative biosignature, including the cytokine monocyte chemoattractant protein (MCP)-1 and six other markers (C-reactive protein, ferritin, serum amyloid P, alpha-2-microglobulin, and tissue plasminogen activator). In another study of patients with tuberculosis, including 51 individuals with tuberculosis disease and 12 patients with concomitant HIV infection, the combination of five biomarkers (IL-1β, IL-23, extracellular matrix protein 1, hemofiltrate CC chemokine-1 and fibrinogen) identified patients with tuberculosis with 89% sensitivity and 90% specificity [25].

The only study in this review that focused on infectious diseases other than tuberculosis and HIV was performed by Bartolini et al., who compared the levels of six salivary cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF) in children with fever and seizures (n = 32) or fever alone (n = 30) [26]. Patients with seizures had higher concentrations of IL-8 (mean, 1158.07 pg/ml) and IL-1β (mean, 185.76 pg/ml) than controls. No differences were found for the other cytokines. In both groups, 25 children tested positive for HHV-6B DNA in saliva, and the viral load was positively correlated with IL-1β concentrations, but not IL-8 concentrations. Interestingly, neither fever duration nor the peak temperature was correlated with the levels of any of the cytokines measured.

3.4. Oral and dental diseases

We identified 12 studies analyzing salivary cytokine levels in 659 patients with dental diseases (caries, periodontitis, gingivitis, overdenture, and stomatitis). Four studies involved children. Eleven studies analyzed cytokine levels in unstimulated saliva, and one examined their levels in stimulated saliva. Most studies investigated both pro-inflammatory (e.g., IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokines (e.g., IL-1 receptor antagonist [IL-1ra], IL-10).

Sharma et al. compared salivary IL-6, IL-8 and TNF-α levels in 25 children with early caries and 25 healthy controls [27]. The levels of all three cytokines were associated with the severity of dental caries, and the IL-8 concentration was highest in patients with early childhood caries (mean, 1614 pg/ml), followed by TNF-α (mean, 759 pg/ml) and IL-6 (mean, 29 pg/ml). The concentrations of all three cytokines decreased 2 weeks after dental intervention including excavation of the carious lesions and restoration.

Cogulu et al. investigated the correlations of *Streptococcus mutans* infection in blood with IL-1β, IL-1ra and IL-10 levels in saliva and blood of 108 children with caries of different severities [28]. Cytokine levels were not correlated with dental caries, but they were associated with the presence of *S. mutans*, which has been implicated as a causative agent of dental caries. Salivary IL-1β concentrations were higher (mean, 103 pg/ml) in patients with high *S. mutans* counts than in those lower *S. mutans* counts (mean, 77 pg/ml). A negative correlation was observed between IL and 1ra levels and the severity of infection (mean concentration, 155 pg/ml for high *S. mutans* counts vs. 350 pg/ml for low *S. mutans* counts).

Similar results to those reported by Sharma et al. were recorded in a study conducted by Menon et al., who examined IL-6 concentrations in 22 children with early childhood caries before and 3 months after treatment [29]. Treatment and oral rehabilitation were associated with decreased salivary IL-6 concentrations (mean, 100 pg/ml vs. 21 pg/ml).

Findings in early childhood caries were also replicated in older individuals in a study by Gornowicz et al., who assessed salivary IL-6, IL-8 and TNF-α concentrations in 26 adolescents with caries and 10 healthy controls [30]. The mean levels of all three cytokines were significantly higher in patients with caries (IL-6, 18.5 pg/ml; IL-8, 1,489 pg/ml; TNF-α, 36.50 pg/ml) than in controls.

Wu et al. studied several salivary cytokines in 30 patients with periodontitis and 27 healthy controls [31] IL-1β (288 pg/ml vs. 71.5 pg/ml) and matrix metalloproteinase (MMP)-9 levels (263 pg/ml vs. 65 pg/ml) were significantly higher in patients with periodontitis than in controls. The levels of other analyzed cytokines, including IL-6, IL-8, IL-1RA, TNF-α, vascular endothelial growth factor (VEGF) and CRP, did not differ between the two groups. A similar study by Lee et al. examining 11 biomarkers in 34 patients with periodontitis and 20 healthy controls identified IL-1β and MMP-8 elevation in patients with periodontitis, which was reversed 4–6 weeks after non-surgical treatment. [32]. Contrarily, Teles et al. investigated 10 salivary cytokines in 74 patients with chronic periodontitis and 44 healthy controls without any intervention and observed no differences between the groups [33].

An interesting interventional study by Belstrom et al. analyzed IL-1β, IL-1ra, IL-8, MCP-1, and VEGF concentrations in 28 healthy non-smokers, finding that discontinuing dental care for 10 days induced gingival inflammation [34]. Cytokine levels were assessed 4, 7, 10, and 24 days after the intervention started. The levels of all five cytokines were lower at the initiation of inflammation, and their levels were correlated with the integrity of gingival tissue, returning to baseline at the end of the observation period.

Research in 71 children with gingival inflammation and cerebral palsy by Santos et al. found positive correlations of salivary IL-1β, IL-6, IL-8 and TNF-α levels with the oral inflammatory status [35]. Liskmann et al. investigated salivary IL-6 and IL-10 content in 12 toothless patients with overdenture and 18 healthy controls [36]. Salivary IL-6 levels were significantly higher in patients with peri-implant infection than in healthy controls. IL-10 was only detectable in patients with peri-implant disease, which involves serious inflammation of the dental implant and leads to the loss of the dental implant in the further course of inflammation.

A study examining a distinct combination of cytokines by Leigh et al. explored the Th1-type salivary cytokines IFN-γ, IL-12 and IL-2 and Th2-type cytokines IL-4 and IL-10 in nine patients with stomatitis and eight healthy controls and observed no differences in their levels between the groups [37].

3.5. Oral and neck cancer

Nine studies investigated salivary cytokines in 578 adult patients with oral and neck cancer. Two studies additionally examined cytokines in fresh or frozen tissue. All studies focused on cytokines as biomarkers of malignant transformation or indicators of treatment response.

Deepthi et al. investigated salivary TNF-α levels in 30 patients with oral squamous cell carcinoma, 30 patients with leukoplakia and 30 healthy controls. TNF-α concentrations were higher in patients with oral cancer (mean, 43.75 pg/ml) than in those with leukoplakia (mean, 21.825 pg/ml) [38]. In the control group, the mean salivary TNF-α concentration was 4.65 pg/ml.

Lee et al. investigated oral squamous cell cancer using 14 biomarkers in saliva and serum for early detection [39]. IL-1β, IL-6, IL-8 and TNF-α levels were increased in patients with oral squamous cell cancer, suggesting their potential as early biomarkers. Zhang et al. investigated salivary IL-6 levels in 40 patients with oral cancer and 20 healthy controls [40]. IL-6 concentrations were higher in patients with oral cancer than in controls (4.6 pg/ml vs. 1.3 pg/ml). The authors stated that the differences were significant, but the measured concentrations were generally considered low.

Polz-Dacewicz et al. analyzed IL-10, TNF-α, TGF-β, and VEGF levels in serum and saliva from 78 patients with confirmed oropharyngeal squamous cell carcinoma and healthy controls [41]. The concentrations of all four cytokines were higher in saliva than in serum and significantly higher in patients with oral cancer than in controls. Human papillomavirus (HPV) and EBV DNA were detected in tumor tissue 31 and 51% of patients, respectively, versus 3 and 20% of controls, respectively. Patients with detectable EBV DNA had the highest IL-10 concentrations, whereas those with detectable HPV DNA had the highest TGF-β concentrations. In addition, TNF-α and VEGF levels were correlated with the histological tumor grade and tumor size. In particular, the median VEGF concentration was 4321 pg/ml (range 1566–7791) in patients with

Table 2
Summary of cytokines analysed in more than two studies.

First author	Year of Ppublication	Time point	IL-1	IL-1α	IL-1B	IL-1ra	Il-2	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12	IL-13	IL-15	IL-17a	IL-21	IL-33	TNF-α	TNF-β	IFN-γ	VEGF-α
Krzaczek ∞	2019	T0																				
Pels ∞	2015	T0																				
		T1																				
		T2																				
Morales-Rojas ∞	2011	T0																				
		T1																				
		T2																				
Fall-Dickson	2007	T0																				
		T1																				
Resende	2010	T0																				
		T1																				
Resende	2013	T0																				
		T1																				
Koizumi	2018	T0																				
Spear	2005	T0																				
Vastardis	2003	T0																				
Black	2000	T0																				
Jacobs	2016	T0																				
		T1																				
		T2 *																				
Jacobs.	2016	T0																				
		T1																				
		T2																				
Bartolini ∞	2018	T0																				
Sharma ∞	2017	T0																				
		T1																				
Cogulu ∞	2015	T0																				
Menon ∞	2016	T0																				
		T1																				
Gornowicz	2012	T0																				
Wu	2018	T0																				
Lee	2018	T0																				
		T1																				
Teles	2009	T0																				
Belstrom	2017	T0																				
		T1																				
		T2																				
		T3																				
		T4																				
		T5																				
Santos ∞	2016	T0																				
Liskmann	2006	T0																				
Leigh	2002	T0																				
Deephti	2019	T0																				
Lee	2018	T1																				
Zhang	2016	T0																				
Polz-Dacewicz	2016	T0																				
		T1																				
		T2																				
Russo	2016	T0																				
		T1																				
Bossi	2016	T0																				
		T1																				
		T2																				
Korostoff	2011	T0																				
Sharma	2011	T0																				
Katakura	2007	T0																				
Rhodus	2005	T0																				

Not analysed
Slightly elevated
Elevated
Slightly decreased
Decreased
No changes

∞ studies including children.
IL, interleukin; IL-1ra, IL-1 receptor antagonist; TNF, tumour necrosis factor; IFN, interferon; VEGF, vascular epithelial growth factor.

cancer, compared with 280 pg/ml in healthy controls.

Russo et al. evaluated eight salivary cytokines including growth-regulated oncogene (GRO)-α, IL-1α, IL-1β, IL-6, IL-8, TNF-α and VEGF in 16 patients with head and neck cancer before and 12 months after surgery and radiotherapy [42]. The levels of all cytokines were significantly increased after treatment initiation independently of the type of treatment received. This was particularly true for GRO-α and IL-6. IL-6 and IL-8 levels concurrently increased, suggesting a common regulatory mechanism. The authors stated that the treatment response was not correlated with reductions of salivary cytokine concentrations at 12 months after treatment.

Bossi et al. assessed 13 salivary cytokines in 55 patients with head

and neck cancer, 10 patients with other cancers and 10 healthy controls [43]. Cytokines concentrations increased during the treatment course (measured in weekly intervals); in particular, IL-1β (mean, 1501.0 pg/ml), IL-6 (mean, 80.5 pg/ml) and TNF-α levels (68.6 pg/ml) at week 3 were correlated with the severity of mucositis.

Korostoff et al. investigated IL-1α, IL-6, IL-8, VEGF and TNF-α levels in 18 patients with endophytic and exophytic squamous cell carcinoma of the tongue and 56 healthy controls [44]. The levels of all five cytokines were elevated in patients with endophytic squamous cell carcinoma, and higher concentrations were correlated with decreased survival rates. In addition, IL-8 concentrations (mean, 2895 pg/ml) were correlated with presence of metastasis and poor prognosis.

Sharma et al. investigated the correlation of salivary IL-6 levels with malignant progression in 60 patients [45]. IL-6 levels increased with the severity of oral dysplasia, suggesting its utility as a marker for the leukoplakia grade. In addition, an association between tobacco consumption and increased salivary IL-6 levels was observed.

Katakura et al. compared IL-1β, IL-6, IL-8 and osteopontin concentrations in 19 patients with oral and neck cancer and 20 healthy individuals [46]. The levels of all four cytokines were elevated in patients with oral cancer as follows: IL-1β, 159 pg/ml vs. 14 pg/ml; IL-6, 67 pg/ml vs. 0 pg/ml; and IL-8, 720 pg/ml vs. 250 pg/ml. Osteopontin levels didn't differ significantly between healthy patients' and cancer patients.

Rhodus et al. compared salivary IL-1α, IL-6, IL-8 and TNF-α levels in 26 patients with either oral lichen planus or squamous cell carcinoma and healthy controls [47]. TNF-α and IL-1α levels were significantly higher in patients with oral lichen planus than in controls, and IL-6 and IL-8 levels were significantly higher in patients with oral squamous cell carcinoma. The authors concluded that these cytokines may be useful as surrogate markers of oral malignant transformation and/or treatment response.

4. Discussion

We reviewed the current evidence on salivary cytokines as biomarkers for oral and systemic diseases. Although the analysis of salivary cytokines is simple and applicable for many fields, our review revealed considerable heterogeneity in study design, patient age, the analysed cytokines, underlying diseases, and ethnic origin. Interestingly, the few studies that compared salivary cytokine levels with serum or blood levels simultaneously reported higher concentrations of some cytokines in saliva, but correlations between the concentrations in the two compartments were not demonstrated, suggesting local cytokine production [17,22].

Almost one third of the studies identified included either exclusively children or had a mixed children and adult study design. Studies in children were mainly performed in patients with ALL and salivary cytokines investigated as biomarkers for mucositis or GvHD. Few paediatric studies also investigated salivary cytokines as markers of caries and gingivitis. In the adult studies salivary cytokines were analysed for risk of mucositis or GvHD in HSCT patients. In addition, several studies used salivary cytokines as diagnostic makers of infectious diseases or complications thereof (HIV and TB). Studies investigating salivary cytokines as diagnostic markers for oral and neck cancers were only found in adults.

Salivary cytokines were, perhaps unsurprisingly, less commonly investigated in patients with systemic diseases. Studies in oncology analyzed salivary cytokines to assess the severity of mucositis and revealed that pro-inflammatory cytokines including IL-1β, IL-2, IL-6 and TNF-α were associated with the severity of oral mucosal tissue damage [16–18]. The early detection of mucositis and diagnostic screening without the need for an in-depth oral examination would be useful because oral examination is often challenging and esophageal lesions may not be visible to the examiner. Salivary cytokines may help clarify the timing of early therapeutic intervention before patient quality of life is significantly impaired. As mucositis progresses, patient quality of life decreases considerably and, in severe cases of mucositis, mortality is increased.

The approach of investigating cytokines as early predictors of the development of GvHD in patients who received HSCTs is also promising. Markers for accurately diagnosing and predicting the risk of acute GvHD are urgently needed because its clinical diagnosis is challenging and GvHD is a major cause of morbidity and mortality in HSCT recipients [48]. The current limited evidence suggests that serum, but not salivary, IL-10 was associated with GvHD and that salivary IL-1β might be predictive of GvHD development [19,20]. However, cautious interpretation of these results is warranted, and the findings require conformation in additional studies, especially because salivary IL-1β concentrations were near the lower limit of detection.

Salivary cytokines as diagnostic tools in infectious diseases have mainly been investigated using two approaches: for the quantification and early detection of oral complications associated with infection (such as HIV) and as diagnostic tools for the infection itself (tuberculosis and HIV). Salivary IFN-γ levels have been reported to be correlated with both oral complications and the presence of the underlying disease in HIV-infected individuals. This is in accordance with the findings for blood cytokines in HIV-infected patients, in whom IFN-γ concentrations were elevated early in the course of infection whereas controlled infection was linked to mild IFN-γ elevation [49]. Therefore, salivary cytokine analysis in HIV-infected individuals may facilitate the detection of oral complications and relapse of the underlying disease. Regarding tuberculosis, the few available studies suggest that salivary cytokines could be used to diagnose the disease. This promising approach can be performed using easily obtained samples, which is particularly useful for low-research settings and for pediatric patients [50–52]. However, the available studies were single-center trials, and confirmation in other settings is required.

Because inflammation plays an important role in tumorigenesis [53], the measurement of pro-inflammatory cytokines as early predictors of local malignant transformation and treatment monitoring is attractive. Tumors consist of different cell types that can release cytokines that may directly influence cells or induce cancer-triggering inflammation. Several cytokines including IL-1α, IL-1β, IL-6, IL-8 and TNF-α have been found to be associated with oral dysplasia or cancer [40–42,46,47]. For example, IL-6 is a potent pro-inflammatory cytokine that in combination with TNF-α and IL-1 can induce acute inflammation [54]. IL-6 has also been found to inhibit dendritic cell differentiation, inducing immune tolerance in tumors and aiding the formation of metastases [55]. This was confirmed in studies investigating serum IL-6, which identified this cytokine as a prognostic marker for head and neck cancer, squamous cell carcinoma, melanoma, gastric cancer, renal cancer, ovarian cancer, and breast cancer [56–59].

Another cytokine that is often investigated in correlation with cancer progression is IL-1β. IL-1β is a key inflammatory cytokine released during inflammasome activation that can act alone or in conjunction with other cytokines [60]. Interestingly, studies investigating oral caries and periodontitis reported similar findings as those in oral, head, and neck cancer; namely, IL-1ra, IL-1β, IL-6, IL-8 and TNF-α levels were elevated in pediatric patients with caries and acute inflammation of the oral mucosa [27–29,35]. Importantly, cytokine concentrations decreased to baseline after treatment [32,34], and their levels were depressed in more chronic inflammatory processes [33].

Most studies used unstimulated saliva for analysis, as it has the advantage of being free of oral stimulations that potentially change the composition and concentrations of the detected cytokines. However, the volume obtained using this method may be low, particularly in patients with hyposalivation. Other studies used stimulated saliva samples obtained after the patient had chewed paraffin gum. This method can be applied in children older than 4 years. As much of the stimulated saliva is produced by the parotid gland, the obtained fluid is more viscous.

The time of day of sample collection is also important for comparing the results of different studies. Most studies obtained samples in the

morning hours because saliva production exhibits a circadian rhythm. Pre-analytic procedures are also important because saliva has high proteolytic activity, which could lead to cytokine degradation prior to analysis [61]. The manufacturers' recommendations for multiplex bead-based assays suggest that centrifugation and the addition of a protease inhibitor could prevent protein degradation [62].

The main limitation of the review was the considerable heterogeneity of the studies, including different collection methods, analytical backgrounds, and the time of sample collection, which precluded the performance of a meta-analysis. Another limitation was that most studies did not report the influence of age on cytokine expression. Only one study in children investigated the effects of age, observing positive correlations of age with IL-1β and IL-8 cytokines. Age-dependent expression in blood has been reported for many cytokines in children and adults, and it is highly likely that this is also true for salivary cytokines [63–66]. In addition, the influence of smoking, dietary composition, and hygiene standards were not analyzed in the different studies. Only the study by Belstrom and colleagues [34] investigated the influence of poor oral hygiene on salivary cytokines.

In conclusion, saliva is a biological fluid that offers several opportunities for diagnosing and monitoring oral and acute systemic diseases. Because of to the simplicity of saliva collection, this method may be particularly useful for studies performed in children and in settings with limited resources. Current data clearly indicate that cytokines are associated with oral inflammation, and they may serve as early markers for the diagnosis of infectious diseases and their oral and systemic complications.

Author contributions

TD and CF reviewed the papers. TD and NR, illustrated and edited the manuscript. CF, NF, and AF reviewed the paper. All authors approved the submitted manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

[1] J. Liu, Y. Duan, Saliva: a potential media for disease diagnostics and monitoring, Oral. Oncol. 48 (7) (2012) 569–577.
[2] J.M. Yoshizawa, et al., Salivary biomarkers: toward future clinical and diagnostic utilities, Clin. Microbiol. Rev. 26 (4) (2013) 781–791.
[3] Y.S. Cheng, T. Rees, J. Wright, A review of research on salivary biomarkers for oral cancer detection, Clin. Transl. Med. 3 (1) (2014) 3.
[4] D. Dakovic, et al., Salivary interleukin-8 levels in children suffering from type 1 diabetes mellitus, J. Clin. Pediatr. Dent. 37 (4) (2013) 377–380.
[5] R. Jacobs, et al., Diagnostic potential of novel salivary host biomarkers as candidates for the immunological diagnosis of tuberculosis disease and monitoring of tuberculosis treatment response, PLoS ONE 11 (8) (2016), e0160546.
[6] V.R. Dikova, S. Principe, J.V. Bagan, Salivary inflammatory proteins in patients with oral potentially malignant disorders, J. Clin. Exp. Dent. 11 (7) (2019) e659–e664.
[7] H.A. Sahibzada, et al., Salivary IL-8, IL-6 and TNF-alpha as potential diagnostic biomarkers for oral cancer, Diagnostics (Basel) 7 (2) (2017).
[8] C.C. Cloak, et al., Psychiatric symptoms, salivary cortisol and cytokine levels in young marijuana users, J. Neuroimmune Pharmacol. 10 (2) (2015) 380–390.
[9] E.H. Kang, et al., Salivary cytokine profiles in primary Sjogren's syndrome differ from those in non-Sjogren sicca in terms of TNF-alpha levels and Th-1/Th-2 ratios, Clin. Exp. Rheumatol. 29 (6) (2011) 970–976.
[10] X. Chen, et al., Elevated cytokine levels in tears and saliva of patients with primary Sjogren's syndrome correlate with clinical ocular and oral manifestations, Sci. Rep. 9 (1) (2019) 7319.
[11] S. Nie, et al., Correlations of salivary biomarkers with clinical assessments in patients with cystic fibrosis, PLoS ONE 10 (8) (2015), e0135237.
[12] N. Nizam, et al., Salivary cytokines and the association between obstructive sleep apnea syndrome and periodontal disease, J. Periodontol. 85 (7) (2014) e251–e258.
[13] D. Moher, et al., Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, Int. J. Surg. 8 (5) (2010) 336–341.
[14] P.F. Whiting, et al., QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies, Ann. Intern. Med. 155 (8) (2011) 529–536.
[15] P.M. Krzaczek, et al., Salivary and serum concentrations of selected pro- and antiinflammatory cytokines in relation to oral lesions among children undergoing maintenance therapy of acute lymphoblastic leukemia, Contemp. Oncol. (Pozn) 23 (2) (2019) 81–86.
[16] E. Pels, Comparison of saliva interleukin-2 concentration to the condition of gums in children with acute lymphoblastic leukaemia during anti-tumour treatment, Cancer Chemother. Pharmacol. 76 (1) (2015) 205–210.
[17] T. Morales-Rojas, et al., Proinflammatory cytokines during the initial phase of oral mucositis in patients with acute lymphoblastic leukaemia, Int. J. Paediatr. Dent. 22 (3) (2012) 191–196.
[18] J.M. Fall-Dickson, et al., Oral mucositis-related oropharyngeal pain and correlative tumor necrosis factor-alpha expression in adult oncology patients undergoing hematopoietic stem cell transplantation, Clin. Ther. 29 (Suppl) (2007) 2547–2561.
[19] R.G. Resende, et al., Investigation of functional IL-10 gene polymorphism and IL-10 levels in acute graft-versus-host disease, J. Clin. Immunol. 30 (3) (2010) 465–473.
[20] R.G. Resende, et al., Association between IL1B (+3954) polymorphisms and IL-1beta levels in blood and saliva, together with acute graft-versus-host disease, J. Interferon Cytokine Res. 33 (7) (2013) 392–397.
[21] T. Koizumi, V. Shetty, M. Yamaguchi, Salivary cytokine panel indicative of non-small cell lung cancer, J. Int. Med. Res. 46 (9) (2018) 3570–3582.
[22] G.T. Spear, et al., Relationship of HIV RNA and cytokines in saliva from HIV-infected individuals, FEMS Immunol. Med. Microbiol. 45 (2) (2005) 129–136.
[23] S. Vastardis, et al., Influence of periodontal disease on Th1/Th2-type cytokines in saliva of HIV-positive individuals, Oral Microbiol. Immunol. 18 (2) (2003) 88–91.
[24] K.P. Black, et al., Cytokine profiles in parotid saliva from HIV-1-infected individuals: changes associated with opportunistic infections in the oral cavity, Oral Microbiol. Immunol. 15 (2) (2000) 74–81.
[25] R. Jacobs, et al., Host biomarkers detected in saliva show promise as markers for the diagnosis of pulmonary tuberculosis disease and monitoring of the response to tuberculosis treatment, Cytokine 81 (2016) 50–56.
[26] L. Bartolini, et al., Detection of HHV-6 and EBV and cytokine levels in saliva from children with seizures: results of a multi-center cross-sectional study, Front. Neurol. 9 (2018) 834.
[27] V. Sharma, et al., Diagnostic potential of inflammatory biomarkers in early childhood caries – A case control study, Clin. Chim. Acta 471 (2017) 158–163.
[28] D. Cogulu, et al., Associations of interleukin (IL)-1beta, IL-1 receptor antagonist, and IL-10 with dental caries, J. Oral Sci. 57 (1) (2015) 31–36.
[29] M.M. Menon, et al., Evaluation of salivary interleukin-6 in children with early childhood caries after treatment, Contemp. Clin. Dent. 7 (2) (2016) 198–202.
[30] A. Gornowicz, et al., Pro-inflammatory cytokines in saliva of adolescents with dental caries disease, Ann. Agric. Environ. Med. 19 (4) (2012) 711–716.
[31] Y.C. Wu, et al., Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population, J. Formos. Med. Assoc. 117 (9) (2018) 841–848.
[32] C.H. Lee, et al., The potential of salivary biomarkers for predicting the sensitivity and monitoring the response to nonsurgical periodontal therapy: A preliminary assessment, J. Periodontal Res. 53 (4) (2018) 545–554.
[33] R.P. Teles, et al., Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study, J. Periodontal Res. 44 (3) (2009) 411–417.
[34] D. Belstrom, et al., Salivary cytokine levels in early gingival inflammation, J. Oral Microbiol. 9 (1) (2017) 1364101.
[35] M. Santos, et al., Inflammatory markers in saliva as indicators of gingival inflammation in cerebral palsy children with and without cervical motor control, Int. J. Paediatr. Dent. 27 (5) (2017) 364–371.
[36] S. Liskmann, et al., Correlations between clinical parameters and interleukin-6 and interleukin-10 levels in saliva from totally edentulous patients with peri-implant disease, Int. J. Oral Maxillofac. Implants 21 (4) (2006) 543–550.
[37] J.E. Leigh, et al., Salivary cytokine profiles in the immunocompetent individual with Candida-associated denture stomatitis, Oral Microbiol. Immunol. 17 (5) (2002) 311–314.
[38] G, D., S.R.K. Nandan, P.G. Kulkarni, Salivary tumour necrosis factor-alpha as a biomarker in oral leukoplakia and oral squamous cell carcinoma, Asian Pac. J. Cancer Prev. 20(7) (2019) 2087–2093.
[39] L.T. Lee, et al., Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma, Int. J. Oral Maxillofac. Surg. 47 (6) (2018) 699–707.

[40] S. Zhang, et al., Variation and significance of secretory immunoglobulin A, interleukin 6 and dendritic cells in oral cancer, Oncol. Lett. 13 (4) (2017) 2297–2303.
[41] M. Polz-Dacewicz, et al., Salivary and serum IL-10, TNF-alpha, TGF-beta, VEGF levels in oropharyngeal squamous cell carcinoma and correlation with HPV and EBV infections, Infect. Agent Cancer 11 (2016) 45.
[42] N. Russo, et al., Cytokines in saliva increase in head and neck cancer patients after treatment, Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. 122 (4) (2016) 483–490 e1.
[43] P. Bossi, et al., Salivary cytokine levels and oral mucositis in head and neck cancer patients treated with chemotherapy and radiation therapy, Int. J. Radiat. Oncol. Biol. Phys. 96 (5) (2016) 959–966.
[44] A. Korostoff, et al., The role of salivary cytokine biomarkers in tongue cancer invasion and mortality, Oral Oncol. 47 (4) (2011) 282–287.
[45] M. Sharma, et al., Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis, Clin. Oral Investig. 15 (5) (2011) 705–714.
[46] A. Katakura, et al., Comparison of salivary cytokine levels in oral cancer patients and healthy subjects, Bull. Tokyo Dent. Coll. 48 (4) (2007) 199–203.
[47] N.L. Rhodus, et al., The feasibility of monitoring NF-kappaB associated cytokines: TNF-alpha, IL-1alpha, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus, Mol. Carcinog. 44 (2) (2005) 77–82.
[48] M.O. Jamil, S. Mineishi, State-of-the-art acute and chronic GVHD treatment, Int. J. Hematol. 101 (5) (2015) 452–466.
[49] S.R. Roff, E.N. Noon-Song, J.K. Yamamoto, The significance of interferon-gamma in HIV-1 pathogenesis, therapy, and prophylaxis, Front. Immunol. 4 (2014) 498.
[50] T. Connell, et al., Interferon-gamma release assays for the diagnosis of tuberculosis, Pediatr. Infect. Dis. J. 28 (8) (2009) 758–759.
[51] N.R. Meier, et al., A systematic review on novel mycobacterium tuberculosis antigens and their discriminatory potential for the diagnosis of latent and active tuberculosis, Front. Immunol. 9 (2018) 2476.
[52] N. Ritz, N. Curtis, Novel concepts in the epidemiology, diagnosis and prevention of childhood tuberculosis, Swiss Med. Wkly. 144 (2014), w14000.
[53] M. Karin, Nuclear factor-kappaB in cancer development and progression, Nature 441 (7092) (2006) 431–436.
[54] G. Ramadori, B. Christ, Cytokines and the hepatic acute-phase response, Semin. Liver Dis. 19 (2) (1999) 141–155.
[55] S.J. Park, et al., IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation, J. Immunol. 173 (6) (2004) 3844–3854.
[56] C.W. Wu, et al., Serum interleukin-6 levels reflect disease status of gastric cancer, Am. J. Gastroenterol. 91 (7) (1996) 1417–1422.
[57] T. Tsukamoto, et al., Interleukin-6 in renal cell carcinoma, J. Urol. 148 (6) (1992) 1778–1781, discussion 1781–2.
[58] M. Plante, et al., Interleukin-6 level in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer, Cancer 73 (7) (1994) 1882–1888.
[59] H. De Schutter, et al., The prognostic value of the hypoxia markers CA IX and GLUT 1 and the cytokines VEGF and IL 6 in head and neck squamous cell carcinoma treated by radiotherapy +/- chemotherapy, BMC Cancer 5 (2005) 42.
[60] M. Lamkanfi, V.M. Dixit, Mechanisms and functions of inflammasomes, Cell 157 (5) (2014) 1013–1022.
[61] K.R. Bhattarai, H.R. Kim, H.J. Chae, Compliance with saliva collection protocol in healthy volunteers: strategies for managing risk and errors, Int. J. Med. Sci. 15 (8) (2018) 823–831.
[62] Luminex™ Assays Support: How to I process saliva samples for the Lumiex assay platform. [cited 2020 17 April]; Available from: https://www.thermofisher.com/ch/en/home/technical-resources/technical-reference-library/antibodies-immunoassays-support-center/luminex-assays-support/luminex-assays-support-getting-started.html.
[63] M.L. Decker, et al., Cytokine profiling in healthy children shows association of age with cytokine concentrations, Sci. Rep. 7 (1) (2017) 17842.
[64] M.L. Decker, M.P. Grobusch, N. Ritz, Influence of age and other factors on cytokine expression profiles in healthy children-a systematic review, Front. Pediatr. 5 (2017) 255.
[65] H.O. Kim, et al., Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays, J. Transl. Med. 9 (2011) 113.
[66] R.P. Stowe, et al., Plasma cytokine levels in a population-based study: relation to age and ethnicity, J. Gerontol. A Biol. Sci. Med. Sci. 65 (4) (2010) 429–433.