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"A systematic review of salivary biomarkers of acute pain, infection, and inflammation"

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Abstract

Background and Aims: Pain, infection, and inflammation have become a serious concern for public health worldwide. Hence, accurate, reliable, and valid methods of pain, infection, and inflammation assessment are essential to have an accurate diagnosis and effective treatment management. Currently, self-reporting assessment approaches are used in clinical practice to measure pain intensity. Also, for infection and inflammation, blood or tissue samples are used. However, these methods are not suitable for all kind of patients (e.g., children, old people); therefore, reliable, objective and non-invasive methods are needed. Monitoring the concentration of biomolecules in human bio-fluid can be an ideal choice. Therefore this review aims to discover the biomolecules related to acute or nociceptive pain, infection, and inflammation in saliva.

Methods: Two different systematic reviews and a literature review in line with the three objectives were completed. Electronic searches from PubMed (MEDLINE), EMBASE, and Web of Science were conducted in May and June 2020. No limitation was applied to study type, article type, and language. Papers published in or after the year 2000 until May 2020 were screened and selected.

Results: For the acute pain systematic review, nine studies were obtained, and three different potential salivary biomarkers of acute pain (cortisol, salivary alpha-amylase, and substance P) were detected, which all had a positive correlation with acute pain intensity. For the infection and inflammation systematic review, 50 studies were obtained, and 28 different biomarkers of inflammation and infection (mainly oral inflammation diseases) were studied. The inflammation-related-biomarkers investigated; Interleukin-1 (IL-1), Interleukin 1 beta (IL-1β), Interleukin-1 alpha (IL-1α), Interleukin-6 (IL-6), Interleukin 4 (IL-4), Interleukin-17 (IL-17), Interleukin-17A (IL-17A), Interleukin-23 (IL-23), Interleukin-10(IL-10), Interleukin-18 (IL-18), Interleukin 8 (IL-8), Colony-stimulating factor-1 (CSF-1), Tumor necrosis factor-alpha (TNF-α), ,Soluble Tumor Necrosis Factor Receptor-2 (sTNFR-2), Macrophage Protein inflammation -1 alpha (MIP-1a), Monocyte chemoattractant protein-1 (MCP-1), Interferon-gamma (IFN-γ), Extracellular matrix protein 1(ECM-1) ,C-reactive protein (CRP), Matrix metalloproteinase-8 (MMP-8), Matrix metalloproteinase-9 (MMP-9), Matrix metalloproteinase 7 (MMP-7) ,Salivary Immunoglobulin A (IgA), Immunoglobulin-A (IgA), Immunoglobulin-M (IgM), Immunoglobulin-G (IgG), Procalcitonin, Annexin-1, Salivary SP-D (surfactant protein D) Hepatitis B surface antigen, Calprotectin, and β-glucuronidase. The levels of these biomarkers were increased, decreased, or not changed in different diseases. Also, the concentration of most of these biomarkers varied in relation to age and gender.

Conclusions: The results from this systematic review support potential salivary biomarkers for diagnosing and monitoring acute pain, infection, and inflammation disease. Cortisol and sAA can be potential biomarkers of acute pain. IL-1β and IL-6 can be a potential biomarker of OLP and periodontitis. TNF-α has the potential to be a biomarker in OLP. CPR can be a potential biomarker in inflammation and infection disease. MMP-8 is one of the critical biomolecules in periodontitis. Further research is required to ascertain salivary biomarkers' use as a method to quantify acute pain intensity, infection, and inflammation disease.

Keywords: infection, inflammation, biomarker, acute pain, assessment, saliva, systematic review

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List of Abbreviations

sAA	Salivary alpha amylase
SP	Substance P
IL-1	Interleukin-1
IL-1β	Interleukin 1 beta
IL-1 α	Interleukin-1 α
IL-6	Interleukin-6
IL-4	Interleukin 4
IL-17	Interleukin-17
IL-17A	Interleukin-17A
IL-23	Interleukin-23
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-8	Interleukin 8
CSF-1	Colony-stimulating factor-1
TNF-α	Tumor necrosis factor-α
GH	Growth hormone
HIV	Human immunodeficiency virus
sTNFR-2	Soluble Tumor Necrosis Factor Receptor-2
MIP-1a	Macrophage Protein inflammation -1 alpha
MCP-1	Monocyte chemo attractant protein-1
IFN-γ	Interferon gamma
ECM-1	Extracellular matrix protein 1

CPR	C-reactive protein
MMP-8	Matrix metalloproteinase-8
MMP-9	Matrix metalloproteinase-9
MMP-7	Matrix metalloproteinase 7
sIgA	Salivary immunoglobulin A
IgA	Immunoglobulin-A
IgM	Immunoglobulin-M
IgG	Immunoglobulin-
ProCT	Procalcitonin
ANXA-1	Annexin-1
Salivary SP-D	Salivary SP-D (surfactant protein D)
ORD	Other respiratory disease
HbsAg	Hepatitis B surface antigen
HbsAb	Hepatitis B surface antibody
HbcAb,	Hepatitis B core antibody
BPS	Behavioural Pain Scale
HIV	Human immunodeficiency viruses
ELISA	Enzyme-linked immunosorbent assay
TNF	Tumor necrosis factor
VAS	Visual analogue scale
NRS	Numerical rating scale
VDS	Verbal descriptor scale
CPT	Cold pressor task
ICC	Immunocytochemistry
PCR	Polymerase chain reaction

SD	Standard deviation
OLP	Oral lichen planus
RA	Rheumatoid arthritis
OA	Osteoarthritis
ANS	Autonomic nervous system
HPA	Hypothalamic– pituitary–adrenal
SNS	Sympathetic nervous system
AMI	Acute myocardial infarction
SSS	Stimulated sublingual saliva
TSST	Trier Social Stress Test
CVD	Cardiovascular disease
TB	Tuberculosis

1. Introduction

1.1 Pain definition and an overview of pain assessment

According to the International Association for the Study of Pain (IASP), pain is defined as an “unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” (Bogduk etal., 1994). Pain is a complex phenomenon that can occur for various reasons and varies in position, strength, and discomfort (McGrath, 1994). Furthermore, pain is categorised as acute and chronic. Acute pain is associated with trauma or injury, while chronic pain lasts more than three months. (Bogduk et al., 1994).

Pain and nociception are two different processes, pain is considered to be an experience while nociception is the physical mechanism which generates that experience (Sneddon, 2018). Experiencing pain can be different in every individual and is affected by several factors, such as psychological, genetic, physical and social factors (McGrath, 1994). Nowadays, acute pain or nociceptive pain is one of the key concerns for public health worldwide and is a major indication of disease processes (Wadensten et al., 2011). Hence, controlling and managing pain is vital to enhance function and reduce complications in clinical settings considerably and help clinicians understand pain mechanisms better and diagnose and treat patients accurately (Vallano et al., 2006, Cervo et al., 2007, Herr, 2011, Dix et al., 2004).

Currently, the gold standard for pain diagnosis in clinical settings self-reporting. This usually comes in two different forms, a scale which is mainly used for acute pain and a questionnaire which is mainly used for chronic pain (Breivik et al., 2008). However, behavioural methods such as the Behavioural Pain Scale (BPS) is used in patients who cannot self-report pain. Therefore, a more reliable and cost-effective approach is needed to further understand the identify and quantify of acute pain intensity experienced by the patient (Herr et al., 2011).

Nociceptive pain pathways encompass perception, nociceptive transduction, modulation, and transmission. As shown in Figure 1, to date, three standard tools to assess acute pain intensity; the visual analogue scale (VAS), the numerical rating scale (NRS), the verbal descriptor scale (VDS) (Haefeli and Elfering, 2006). Nowadays, NRS, VDS, and VAS have been proven to be valid, reliable, sensitive, and good pain intensity indicators in clinical settings (Haefeli and Elfering, 2006, Breivik et al., 2008). However, there are limitations associated with the use of these scales; for instance, these methods are unsuitable for patients who are in comma and individuals who experience cognitive impairment (Bendinger and Plunkett, 2016). Also, the self-reporting method is not suitable for infants and young children as children can deny pain (Breivik et al., 2008). Therefore, due to the lack of evidence in clinical settings, more reliable and accurate methods are required to measure acute pain (Shannon and Bucknall, 2003).

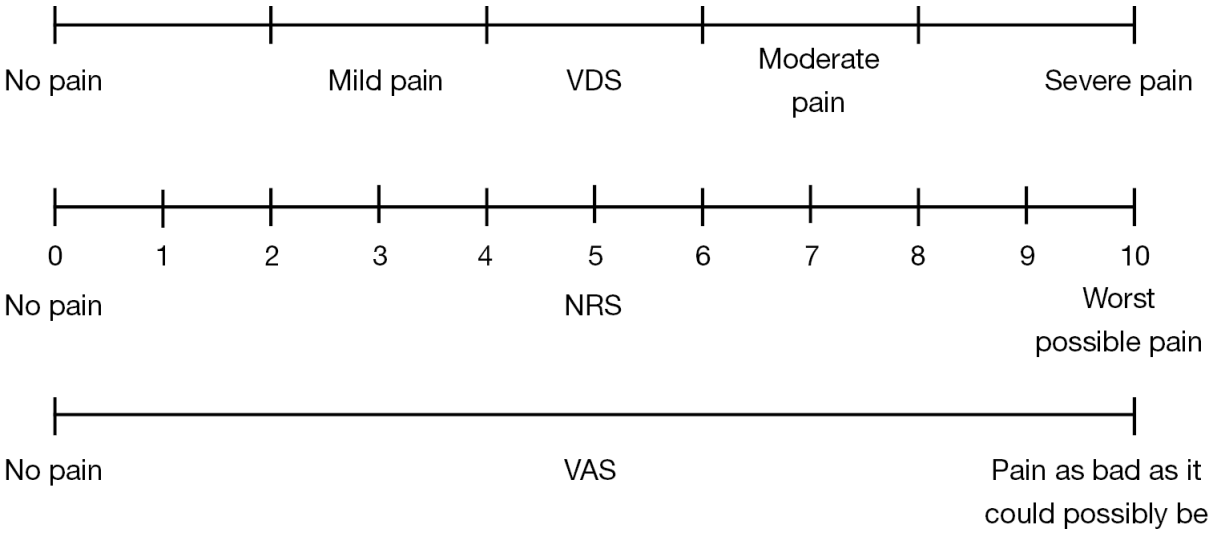

Figure 1: *Demonstrates the three different ways of assessing pain intensity (Woo et al., 2015).*

For a pain assessment method, it is crucial to be as painless and hazardless as possible. According to the literature, making use of bio-fluids (such as saliva) is one of the most suitable An easy-access method that is both reliable and validated in previous studies is to measure acute pain by monitoring the concentration of certain biomolecules in saliva, which are involved in the nociceptive pain pathways (Sobas et

al., 2016). Examples of these biomolecules are several neurotransmitters and cortisol (Sobas et al., 2016). Neurotransmitters are signalling molecules of the nervous system, which are responsible for transmitting information among neurons via chemical synapses. These biomolecules are selected to further understand both objective and quantitative assessment of pain as these biomolecules provide information with regards to changes in the nervous system (Ghanavatinejad et al., 2019).

1.2 Overview and assessment of Infection and inflammation

Infectious and inflammatory diseases are that involve many different clinical illnesses and can be categorised into an infection, acute, and chronic inflammation (Signore and Glaudemans, 2011). Inflammations can possibly affect multiple organs (systemic) or can be restrained in a specific organ, which is usually degenerating and might need life-long treatment (Signore and Glaudemans, 2011).

Infection is ‘the invasion of a host organism's bodily tissues by disease-causing organisms, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce’ (Signore, 2013). Infection is caused by microorganisms such as prions, viruses, bacteria, viroids, and larger organisms (e.g. fungi and parasites) (Signore, 2013). Currently, blood or tissue samples are needed to diagnose a systemic infectious disease. Collecting tissue and blood samples are effective, however, these methods are invasive, expensive, and also need extensive time to obtain the results (Yoshizawa et al., 2013). Therefore, an alternative diagnostic method should be taken into consideration.

A number of infectious agents can already be detected through a saliva test. Such examples are the HIV (Reynolds and Muwonga, 2004) and the hepatitis (A, B, and C) viruses (Amado et al., 2006). Therefore, using saliva as a medium for monitoring and detecting infectious agents has become an advance in the early stage management of infectious diseases (Yoshizawa et al., 2013).

Moreover, inflammation is defined as “the response of the immune system to harmful stimuli such as irradiation, pathogens, toxic compounds, or damaged cells” (Medzhitov, 2010). This immune response puts effort to fight the harmful stimuli and start the healing process (Ferrero-Miliani et al., 2007). Therefore, inflammation is known as a defence mechanism that is essential for human health (Nathan and Ding, 2010). In the case of acute inflammation, the human body minimises the impending injury or infection by cellular and molecular events, which help the tissue homeostasis to repair. However, uncontrolled acute inflammation can turn into chronic inflammation, which can be linked to different types of chronic inflammatory diseases (Zhou et al., 2016).

Hence, it is vital to diagnose inflammation at an early stage to ease further complications in clinical settings. Currently, inflammation measurement methods involve blood sampling, which is not suitable for all patients (e.g., children and old people) (Prasad et al., 2016).

1.3 Biomarker

Biomarker refers to “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention” (Biomarkers Definitions Working, 2001). In other words, biomarkers are able to provide data about the current physiological states of living organisms within the body (Ilyin et al., 2004). Also, biomarkers can help to further understand and predict the causes, diagnosis, and prognosis of diseases by measuring the levels of these biomarkers in the blood, saliva, and other body fluids (Mayeux, 2004). Biomarkers are classified into two major types; exposure and disease biomarkers. Biomarkers of exposure are used in prediction only. However, biomarkers of disease are used in the screening, diagnosing, and monitoring a disease progression over time (Mayeux, 2004).

Moreover, as shown in Figure 2, biomarkers can vary and exist in different forms, including, metabolites, RNA, microbes, DNA, lipids, proteins, cytokines, chemokines, and enzymes (Yoshizawa et al., 2013). Alterations in the concentration of biomarkers can be related with the progression and regression of a specific disease (Wagner et al., 2004).

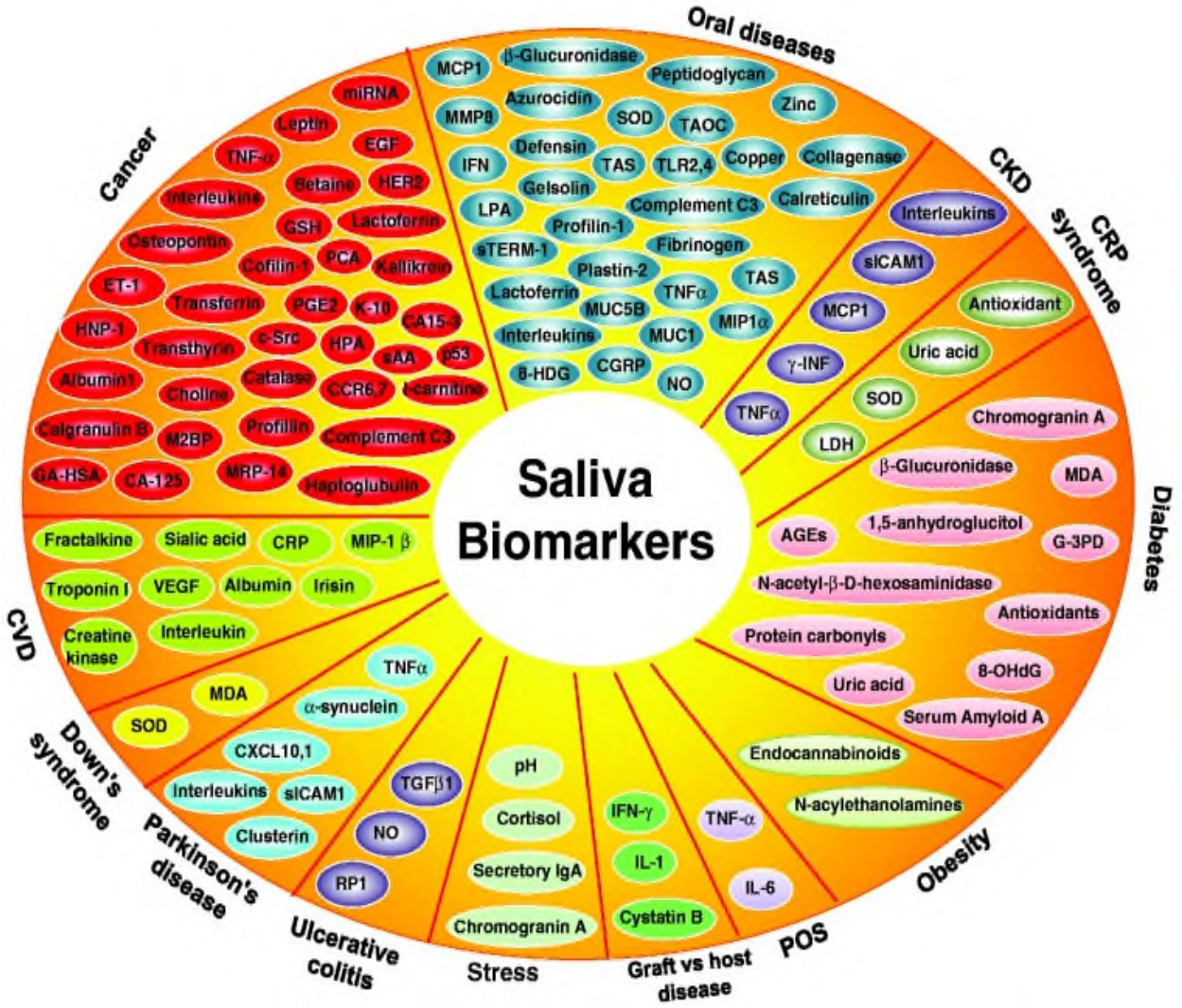

Figure 2: *Represents salivary biomarkers for different disease (Prasad et al., 2016).*

1.4 Use of Saliva for diagnosis of health and disease conditions

Saliva is a complex mixture that is derived from three parts of major salivary glands. As shown in Figure 3, these glands are named parotid, sublingual, and submandibular gland, which contribute about 90% of the total fluid secretion. Saliva also contains

nasal and bronchial secretions, gingival crevicular fluid, oral mucosal cells, serum filtrate, microbiota, and food debris (Yoshizawa et al., 2013). Although parotid glands are the largest, they can only produce about 20% of total saliva under the unstimulated resting state. In addition, the submandibular glands produce about 65% of the total saliva in the unstimulated resting state (Humphrey and Williamson, 2001). Moreover, the salivary glands are composed of acini (secretory unit) that are made up of acinar cells (serous or mucous). The saliva is composed mainly of water (99%), protein (0.3%), and inorganic substance (0.2%). The salivary proteins are constantly secreted via an acinar cell and synthesised by salivary glands (Proctor, 2016, Yoshizawa et al., 2013).

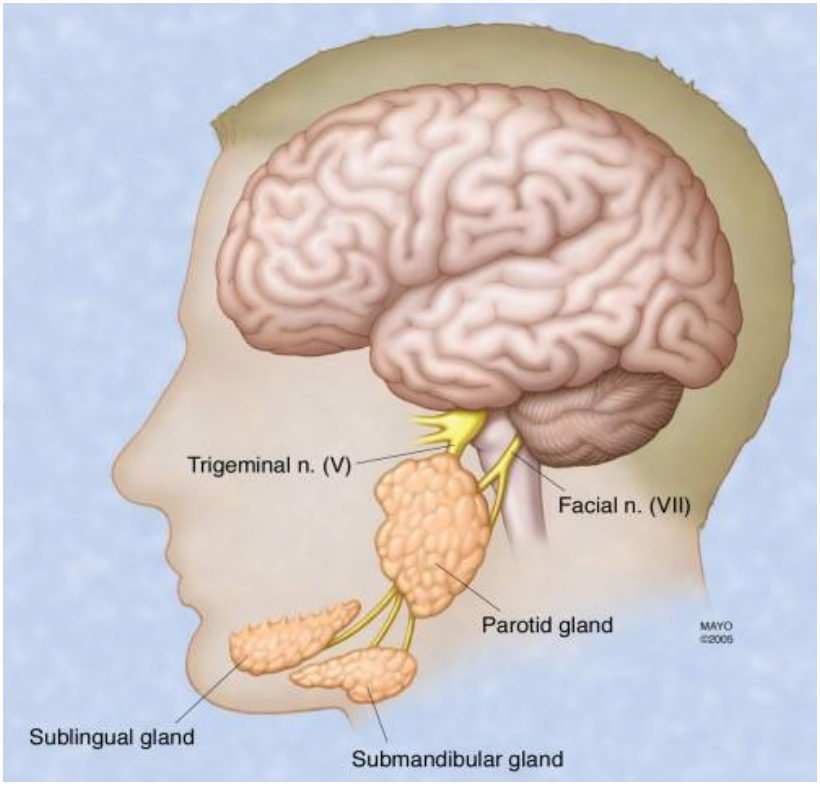

Figure 3: *Represents the major salivary glands (the parotid, the submandibular, and sublingual glands) (Yoshizawa et al., 2013).*

Nowadays, saliva can be used as a diagnostic tool in clinical settings as it provides valid information about diseases and suggests distinguishing advantages over blood. For instance, the salivary diagnostic methods are developed in monitoring cancer (Hu et al., 2008), autoimmune diseases (Hu et al., 2007), viral infection (Emmons, 1997) and bacterial infection (Adam et al., 1999).

Most of the substances found in blood are also present in saliva, as a result, 40% of the proteins which have been recommended to be biomarkers of diseases can also be found in saliva (Loo et al., 2010). Thus, saliva is rewarded to be a tool in the diagnosis of diseases as it has the ability to reflect the physiological body state. Moreover, saliva collection has some advantages over blood collection. It is simple process and non-invasive, it reduces the discomfort which is associated with blood, cerebrospinal fluid, or interstitial collection procedures often used in pain research (Fischer et al., 1998). Also, non-invasive collection dramatically diminishes anxiety and discomfort in patients (D'Mello and Dickenson, 2008, Jasim et al., 2018, Kalman and Grahn, 2004). In addition, saliva collection is safe to handle, easy to ship and store and is economical (Yoshizawa et al., 2013). For the physician, saliva is safer than the venepuncture as it could infect the healthcare providers to blood-borne or infectious diseases. Consequently, saliva has the potential to be a tool in diagnosis and prognosis of pain (Lee and Wong, 2009, Segal and Wong, 2008) as well as infection and inflammation (Prasad et al., 2016). Therefore this systematic review is aimed to find potential biomarkers of pain, infection, and inflammation.

There are several immunological methods used to detect the salivary biomarkers. ELISA (quantifying biological substances), is the standard analytical method which is used in most cases (Lorenzo-Pouso et al., 2018).

1.5 Aim and Hypotheses

To the best of our knowledge, the diagnostic value of salivary biomarkers for pain, infection, and inflammation have not been systematically assessed with all the

currently available data yet. The aim of this work is to run systematic literature reviews 1) to find potential biomarkers of acute pain in saliva; 2) to find potential biomarkers of infection and inflammation in saliva; 3) to investigate whether sex, age, smoking or stress affect the salivary biomarker's concentration levels.

The hypotheses are that 1) there are biomolecules in saliva that exhibit a change in the presence of acute pain, and that 2) there are biomolecules in saliva that exhibit change in the presence of infection and inflammation.

2. Material and methods

Two different systematic reviews and a literature review in line with the three objectives were completed. Electronic searches were conducted from PubMed (MEDLINE), EMBASE, and Web of Science. Initially, studies obtained from the search were imported to Endnote X9 (Clarivate, Philadelphia USA), and duplicate studies were removed. The studies were reviewed through screening of the titles and abstracts in Rayyan software (Ouzzani et al., 2016). Further details on the selection of studies are shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Figure 4 and 5).

Moreover, the data were extracted in the form of a table in Microsoft Word (Microsoft Corporation, USA). The data were recorded following this format:

- The name of biomarker
- The type of biomarker molecule
- The type of disease
- The number of participants
- The method of detection
- The results of the study
- The name of the author and the year of publication

2.1 Salivary biomarkers of nociceptive pain keywords, inclusion and exclusion criteria

For the potential salivary biomarkers of nociceptive pain, “marker” OR “biomarker” AND “saliva” AND “nociception” OR “pain” keywords were used.

2.1.1 Inclusion criteria

- Studies that focus on analysing the biomarkers of acute pain in human saliva.
- Clinical studies, including clinical trials (randomised controlled trial and non-randomised controlled trials) and observational studies (cohort, case-control, case series, and cross-sectional).
- Papers published in or after the year 2000 until May 2020.

- No limitation was applied to study type, article type, and language.

2.1.2 Exclusion criteria

- Non-human (animals) studies were excluded.
- Studies that focus on chronic pain or other diseases.
- Studies that do not analyse biomarkers of acute pain in human saliva.

2.2 Salivary biomarkers of infection and inflammation keywords, inclusion and exclusion criteria

For the potential salivary biomarkers of infection and inflammation, “saliva” AND, “biomarker”, OR, “marker”, AND, “infection”, OR, “inflammation” keywords were used.

2.2.1 Inclusion criteria

- Studies that focus on analysing biomarkers of infection and inflammation in human saliva.
- Clinical studies, including clinical trials (randomised controlled trial and non-randomised controlled trials) and observational studies (cohort, case control, case series and cross-sectional).
- Papers published in or after the year 2000 until June 2020.
- No limitation was applied to study type, article type, and language.

2.2.2 Exclusion criteria

- Non-human (animals) studies were excluded.
- Studies that do not analyse biomarkers of infection and inflammation.

3. Results:

3.1 Identification and selection of studies of nociceptive pain

The procedure for screening and selecting the studies for the potential biomarkers of nociceptive pain is summarised in Figure 4. Four hundred sixty-eight were identified by the chosen databases searched. Four hundred nineteen articles were identified after removing the duplicates in Endnote X9 (Clarivate, Philadelphia USA). After screening the titles and abstracts of the studies retrieved, twenty articles were chosen for full-text reviewing. Eleven articles were excluded as they were focused on salivary biomarkers of chronic pain. Finally, nine studies were included in this systematic review.

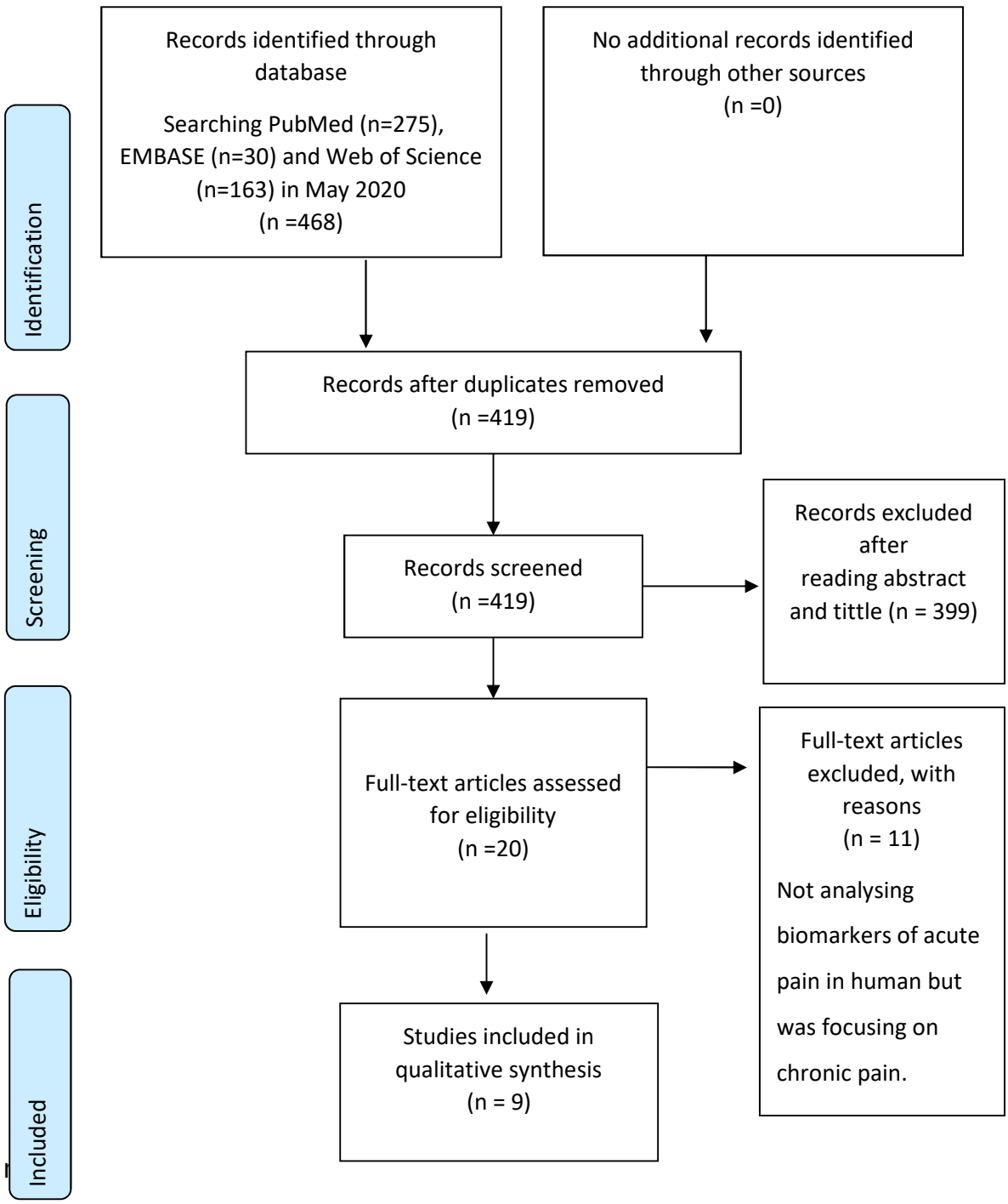

***Figure 4**: PRISMA Flow Diagram of salivary biomarkers of nociception pain*.

3.2 Identification and selection of studies of infection and inflammation

The procedure for screening and selecting studies for potential biomarkers of infection and inflammation is summarised in Figure 5. Two thousand and seventy, nine articles were identified from the chosen databases. After removing the duplicates in Endnote X9 (Clarivate, Philadelphia USA), one thousand eight hundred four articles were identified. After screening the titles and abstracts via the Rayyan software (Ouzzani et al., 2016), ninety-four articles were extracted for full-text studying. Forty-two articles were excluded as twenty-eight studies were focused on blood samples, five were focused on urine samples, seven were focused on cardiovascular disease (CVD), and two studies were surveys. Finally, fifty studies were included in this review.

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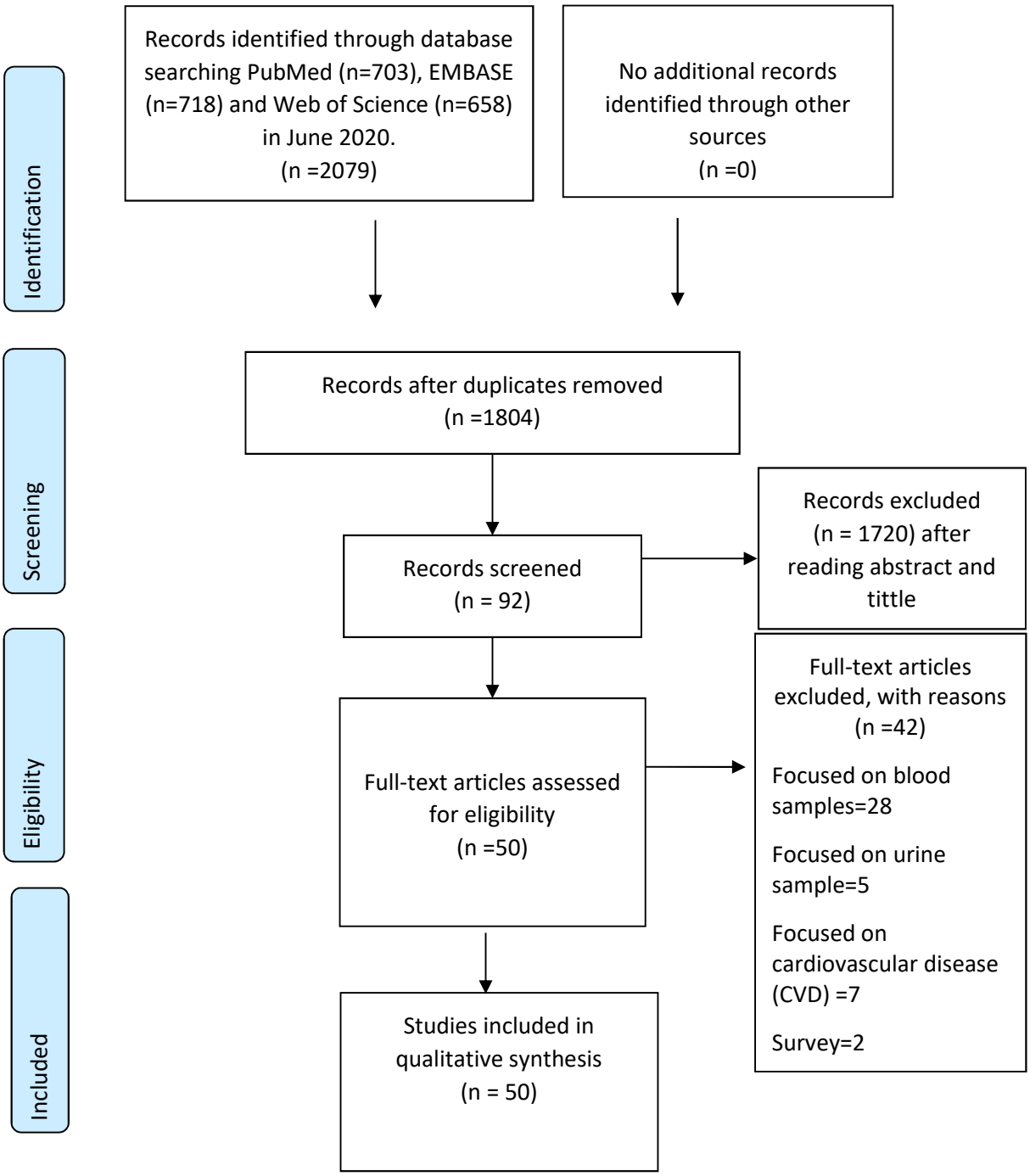

Figure 5: PRISMA Flow Diagram of salivary biomarkers infection and inflammation**.**

3.3 Salivary biomolecules related to acute pain

A total of 890 participants were recruited in 9 different studies included in this review. Participants were in different age groups and sex. Two studies only included males (Muller, 2011, Shen et al., 2012). One study recruited only children aged 6 to 18 years old (11.67±3.79) (Jenkins et al., 2018). Studies were focused on different types of acute pain. Three studies were focused on experimental pain and induced acute pain by using the cold pressor task (CPT) (Koenig et al., 2017, Goodin et al., 2012, Jenkins et al., 2018), and electric skin stimulation (Muller, 2011). Three studies investigated acute dental pain (Haug and Marthinussen, 2019, Ahmadi-Motamayel et al., 2013, Ahmad M et al., 2014), one study was about acute myocardial infarction chest pain (Shen et al., 2012), and one study was focused on acute heat pain perception (Wittwer et al., 2016). Three different potential salivary biomarkers of acute pain (cortisol, sAA, and SP) were detected in the participant's samples. These biomarkers had a positive correlation with acute pain. More details are provided in Table 1

Table 1. Potential salivary biomarkers of acute pain. Results presented as mean±SD where appropriate.

Biomarkers	Type of molecule	Type of pain (disease)	Participants	Pain intensity assessment	Method detection	Findings	Reference
Cortisol	Hormone	CPT	30 healthy participants 30 control subjects	VAS	Cortisol assays	Cortisol concentrations increased over time	(Koenig et al., 2017)
		Acute dental pain	42 patients, 39 control subjects	NRS	Cortisol assays	Elevated in patients (0.39 ± 0.88 mg/dl), ($p < .05$)	(Haug and Marthinussen , 2019)
		Induced pain with painful electric skin stimuli.	32 healthy participants 32 control subject	VAS	ELISA	Higher salivary cortisol (AUC) areas under the response curve (-69 ± 124 nmol/l) Vs control (29 ± 139 nmol/l) P = 0.004	(Muller, 2011)
		CPT	10 healthy participant	NRS	Cortisol assays	Increased	(Goodin et al., 2012a)
sAA	Enzyme/ Protein	Severe acute dental pain	36 patients no control group	VAS	Biochemical kit and spectrophotometer.	Level of sAA had a positive correlation with VAS pain scale	(Ahmadi-Motamayel et al., 2013)
		CPT	73 children with leukemia cancer randomly assigned into distraction, reappraisal, or reassurance	NRS	Enzyme kinetic assay	Level of sAA in reassurance group increased after CPT Vs children in the distraction (p = .021)	(Jenkins et al., 2018)

		Acute myocardial infarction (AMI) in chest pain	85 patients 388 control subjects	Not measured	Alpha-amylase Salivary Assay Kit	Increased sAA activity in patients (7.94± 3.37U/mL) Vs Control group (6.85± 2.07 U/mL), P<0.001.	(Shen et al., 2012)
		Acute heat pain	27 healthy participants	VAS	Biochemical Analyses	Level of sAA correlated positively with pain intensity in responses to acute heat pain stimuli.	(Wittwer et al., 2016)
SP	Peptide	Acute dental pain	30 patients, 35 control subjects	VAS	ELISA	Elevated in patients (869.4 ± 30.7 pg/ml) Vs control group (462.9 ± 39.6 pg/ml).	(Ahmad M et al., 2014),

Key: (CPT): Cold pressor task, (NRS): Numeric rating scale, (ELISA): enzyme-linked immunosorbent assay, (VAS): visual analogue scale.

3.4 Salivary biomolecules related to infection and inflammation

A total of 3,773 participants were recruited in 50 different studies included in this review. Thirty-four different biomarkers were studied as potential biomarkers of infection and inflammation in saliva (Table 2).

3.4.1 Salivary infection related-biomarkers

There were six potential biomarkers of infection studied in six studies. The infection-related biomarkers studied were ECM-1, IL-17A, IgA, IgM, IgG, and CPR.

The concentration levels of ECM-1 and IL-17A were increased in Tuberculosis (Jacobs et al., 2016a). Also, CPR was increased in Tuberculosis (Jacobs et al., 2016b),

One study reported that CPR was elevated in neonatal sepsis (Omran et al., 2018).

Another study reported, IgA was elevated in HIV infection (Mandal et al., 2016).

Oba et al. (2000) reported that IgA and IgM were elevated in hepatovirus infection patients.

Formenty et al, (2006) reported that IgG was elevated in Ebola virus hemorrhagic fever patients.

3.4.2 Salivary inflammation related-biomarkers

There were thirty-four potential biomarkers of inflammation (mainly oral inflammation diseases) investigated in fifty studies. The inflammation-related biomarkers studied were IL-1 ,IL-1 α, IL-1β, IL-6, IL-4, IL-17, IL-17A, IL-23, IL-10, IL-18, IL-8, CSF-1, TNF-α, sTNFR-2, MIP-1a ,MCP-1, IFN-γ, CPR, MMP-8, MMP-9, MMP-7, IgA, ProCT, Calprotectin, ANXA1, Mucin 4, Salivary SP-D and β-glucuronidase..

Moreover, higher CRP concentration levels were reported in 6 different studies investigating periodontitis and oral lichen planus (OLP) disease (Christodoulides et al., 2005, Noack et al., 2001, Jayaprakash et al., 2014, Shojaee et al., 2013, Metgud and Bajaj, 2016, Christodoulides et al., 2007). However, Aurer et al. (2005), reported a lower level of CPR in periodontitis.

MMP-8 concentration level was increased in 12 different studies investigating periodontitis and gingivitis disease (Miller et al., 2006, Johnson et al., 2016, Gursoy et al., 2010, Ebersole et al., 2013, Ebersole et al., 2015, Kushlinskii et al., 2011, Gupta et al., 2015, Rangbulla et al., 2017, Martinez et al., 2017, Ramseier et al., 2009, Mirrielees et al., 2010, Christodoulides et al., 2007). However, Scannapieco et al. (2007), reported no significant differences in MMP-8 level in periodontitis disease.

Only one study reported an increased level of MMP-9 in periodontitis disease (Ramseier et al., 2009). Nisha et al. (2018) reported an increased level of MIP-1a and MCP-1 in periodontitis. Hassan et al. (2018) reported a higher level of ANXA-1 in periodontitis. Lundmark et al. (2017) reported a decreased level of Mucin-4 in periodontitis and a higher level of MMP-7 in periodontitis. Lamster et al. (2003) and Prabhahar et al. (2014) reported a higher level of β-glucuronidase in periodontitis. However, three studies reported no significant changes in (IL-10) level in periodontitis (Ramseier et al., 2009, Vastardis et al., 2003, Teles et al., 2009)

Christodoulides et al. (2007) reported an increased level of IL-1 in periodontitis, hence,vRhodus et al. (2005) reported an increased level of IL-1-α in OLP disease.

Another potential biomarker of inflammation studied was IL-1β in periodontitis and gingivitis. Six studies reported an increased level of IL-1β in periodontitis (Miller et al., 2006, Ebersole et al., 2013, Rangbulla et al., 2017, Mirrielees et al., 2010, Hassan et al., 2018). However, Ramseier et al. (2009) reported no significant difference in IL-1β levels in periodontitis and gingivitis (Ramseier et al., 2009).

In four studies, higher IL-6 concentration was reported in gingivitis, periodontitis and OLP disease (Ebersole et al., 2013, Zhang et al., 2008, Juretic et al., 2013, Rhodus et al., 2005). However, 3 studies reported no significant differences in IL-6 level in periodontitis (Gursoy et al., 2009,Teles et al., 2009).

Martinez et al. (2017) reported an increase in the level of CSF-1 in periodontitis.

IL-8 was reported higher in concentration in OLP disease and asthma in 3 studies (Zhang et al., 2008, Rhodus et al., 2005, Zamora-Mendoza et al., 2019).

TNF-α was reported higher in concentration in OLP disease in 6 studies (Pezelj-Ribaric et al., 2004, Zhang et al., 2008, Juretic et al., 2013, Rhodus et al., 2005, Ghallab et al., 2010, Frodge et al., 2008). However, 4 studies reported no significant differences in TNF-α level in periodontitis (Mirrielees et al., 2010, Aurer et al., 2005, Gursoy et al., 2009, Teles et al., 2009).

Calprotectin level was increased in 2 studies in gingivitis (Yucel et al., 2020, Ramseier et al., 2009). Also, Majster et al. (2019) reported higher calprotectin level in inflammatory bowel disease (IBD).

IFN-γ level was increased in OLP disease in two studies (Malekzadeh et al., 2015, Ghallab et al., 2010); however, Liu et al. (2009) reported a lower level of IFN-γ in OLP disease.

IL-4 was increased in OLP disease in 2 studies (Malekzadeh et al., 2015, Liu et al., 2014); however, Teles et al. (2009) reported no differences in IL-4 in periodontitis.

Wang et al. (2015) reported an increased level of IL-17 in OLP disease, and IL-23 was not changed.

More details are provided in Table 2.

Table 2. Potential salivary biomarkers of infection and inflammation. Results presented as mean±SD where appropriate.

Biomarker	Type of molecule	Disease	Participants	Method Detection	Findings	References
ECM-1	Protein	Tuberculosis	18 patients, 33 ORD	luminex multiplex immunoassay	Increased-levels in TB patient's Vs ORD (14.6±14.4 pg/ml) (22.3 ±12.4 pg/ml. and p=0.046.	(Jacobs et al., 2016a)
CPR	Protein	Periodontitis	15 patients, 15 control subjects	ELISA	Elevated in the patient 2001 pg mL^{-1} vs control 225 pg mL^{-1}	(Christodoulides et al., 2005)
		Periodontitis	109 patients, 65 control subjects	Radial immunodiffusion assay	Elevated in the patient group (4.06± 5.55 mg/l) Vs control group (1.70± 1.91mg/l), p=0.011	(Noack et al., 2001)
		Periodontitis	40 patients, 20 control subjects	CLIA	Elevated in the patients (2.49 ± 0.47 ng/mL) Vs controls (0.56 ± 0.20 ng/mL)	(Jayaprakash et al., 2014)
		Periodontitis	60 patients, 30 control subjects	ELISA	Elevated in the patients (5332.62 ± 5051.63 pg/mL) Vs controls (3108.51±3574.47 pg/mL) and p=0.045.	(Shojaee et al., 2013)
		OLP disease	20 patients, 20 control subjects	Immunoturbidimetry	Elevated in the patients (0.23± 0.05 mg/l) Vs controls (0.13± 0.18 mg/l)	(Metgud and Bajaj, 2016)
		Neonatal sepsis	35 patients, 35 control subjects	ELISA	Elevated in the patient group (12.0± 4.6 ng/l) Vs control group (2.8± 1.2 ng/l) p<0.001	(Omran et al., 2018)

		Periodontitis	28 patients, 28 control subjects	ELISA	Elevated in the patient group	(Christodoulides et al., 2007)
		Tuberculosis	32 patients, 72 ORD	ELISA	Levels increased in TB vs ORD p<0.0001.	(Jacobs et al., 2016b)
		Periodontitis	10 patients, 14 control subjects	ELISA	Lower level in patients	(Aurer et al., 2005)
MMP-8	Enzyme/Protein	Periodontitis	28 patients, 29 control subjects	ELISA	Elevated in the patient group (408.6 ± 423.3 ng/mL) Vs control group (95.1± 80.1 ng/mL), p=0.0005.	(Miller et al., 2006)
		Periodontitis	10 patients, 10 control subjects	Luminex assay	Increased levels in patients (129.8±891.9) Vs control group (51.9±102.4), p=0.011	(Johnson et al., 2016)
		Periodontitis	84 patients, 81 control subjects	ELISA and IFMA	Increased in the patient group (1001.6± 72.2 ng/mL Vs control group (80.3 ± 46.6), p=0.044.	(Gursoy et al., 2010)
		Periodontitis	50 patients, 30 control subjects	ELISA	Increased levels in patients (283.47± 203.47 pg/mL) compared to control group (52.63 ± 40.62 pg/mL), p<0.0001.	(Ebersole et al., 2013)
		Periodontitis	144 patients, 65 control subjects	ELISA	Increased levels in patients (348.76±202.1 ng/mL) Vs control 190.91 ±143.89 ng/mL)	(Ebersole et al., 2015)
		Periodontitis	82 patients, 63 control subjects	EIA	Increased levels in patient group (298.37±234.81 pg/mL) Vs control group (249.0±187.7 pg/mL), p>0.05.	(Kushlinskii et al., 2011)

		Periodontitis	20 patients, 20 control subjects	ELISA	Increased levels in patients (348.26±202.1 pg/mL) compared to the control group (190.91±143.89 pg/mL) and p<0.05.	(Gupta et al., 2015)
		Periodontitis	30 patients, 20 control subjects	ELISA	Increased levels in patients (672.18±411.0 pg/mL) Vs Control: (57.95±31.64 pg/mL) and p<.001.	(Rangbulla et al., 2017)
		Periodontitis	29 patients, 7control subjects	ELISA	Increased levels in patients (333±66.7 pg/mL Vs Control (66.7±33.3 pg/mL) and p<.05.	(Martinez et al., 2017)
		Gingivitis/ Periodontitis	81 patients, 18 control subjects	ELISA+PCR	Increased level Vs control group (p <0.001)	(Ramseier et al., 2009)
		RA and Periodontal Disease	115 patients, 35 control subjects	ELISA	Increased levels in patients (p≤0.002).	(Mirrielees et al., 2010)
		Periodontitis	28 patients, 28 control subjects	ELISA	Elevated in the patient group	(Christodoulides et al., 2007)
		Periodontitis	40 patients, 40 control subjects	ELISA	No significant difference was found between groups	(Scannapieco et al., 2007)
MMP-9	Enzyme/Protein	Gingivitis/ Periodontitis	81 patients, 18 control subjects	ELISA+PCR	Increased level vs control group (p =0.001)	(Ramseier et al., 2009)
IL-1β	Cytokines	Periodontitis	84 patients, 81 control subjects	ELISA	Increased levels in patients (753.7±1022.4 ng/mL) Vs control group (212.8 ± 167.4 ng/mL), p=0.009.	(Miller et al., 2006)
		Periodontitis	50 patients, 30 control subjects	ELISA	Increased levels in patients (90.94± 85.22 pg/mL) Vs control group (7.24 ± 7.69 pg/mL), p<0.0004.	(Ebersole et al., 2013)

		Periodontitis	30 patients, 20 control subjects	ELISA	Increased levels in patients (530.76± 343.85 pg/mL) Vs control (89.93±25.48 pg/mL), p<0.001.	(Rangbulla et al., 2017)
		RA and Periodontal disease	115 patients, 35 control subjects	ELISA	Increased levels in patients (p=0.002).	(Mirrielees et al., 2010)
		Gingivitis/Periodontitis in pregnancy	78 patients, 69 control subjects	ELISA	Increased levels in patients p< 0.05)	(Hassan et al., 2018)
		Gingivitis/ Periodontitis	81 patients, 18 control subjects	ELISA	No significant difference was found between groups	(Ramseier et al., 2009)
IL-1	Cytokines	Periodontitis	28 patients, 28 control subjects	ELISA	Elevated in the patient group	(Christodoulides et al., 2007)
IL-1-α	Cytokines	OLP disease	13 patients, 13 control subjects	ELISA	Increased levels in patients (148.12± 21.30 pg/mL) Vs controls (2.26 ± 0.72 pg/mL) and p<0.0001.	(Rhodus et al., 2005)
IL-6	Cytokines	Periodontitis	50 patients, 30 control subjects	ELISA	Elevated levels in patients (35.57± 48.17 pg/mL) Vs control group (3.30 ± 2.32 pg/mL), p<0.0001.	(Ebersole et al., 2013)
		Gingivitis/ Periodontitis	81 patients, 18 control subjects	ELISA	No significant difference was found between groups	(Ramseier et al., 2009)
		OLP disease	30 patients, 30 control subjects	ELISA	Elevated levels in patients (48.79±8.53 pg/mL) Vs controls (29.90±4.68 pg/mL) and P=0.00.	(Zhang et al., 2008)
		OLP disease	19 patients, 19 control subjects	ELISA	Elevated levels in patients (0.431± 0.217 pg/mL) Vs controls (0.002± 0.002 pg/mL) and p=0.01.	(Juretic et al., 2013)

		OLP disease	13 patients, 13 control subjects	ELISA	Elevated levels in patients (148.12± 21.30 pg/mL) Vs controls (2.26 ± 0.72 pg/mL) and p<0.0001.	(Rhodus et al., 2005)
		Periodontitis	84 patients, 81 control subjects	ELISA	No significant difference between groups was found	(Gursoy et al., 2009)
		Periodontitis	74 patients, 44 control subjects	Luminex Assay	No significant difference was found between groups	(Teles et al., 2009)
s-IgA	Protein/Antibodies	Periodontitis	30 patients, 20 control subjects	ELISA	Elevated levels in patients (196.28±54.61 pg/mL) vs controls (81.23 ±24.61 pg/mL) and p<.001.	(Rangbulla et al., 2017)
		HIV infection	28 patients, 28 control subjects children	ELISA	Level decreased in patients (81.6 ± 6.2 µg/ml) Vs controls (145.5 ± 17.8 µg/ml)	(Mandal et al., 2016)
IgA	Protein/Antibodies	Hepatovirus	24 patients, 18 control subjects	ELISA	Positive	(Oba et al., 2000)
IgM	Protein/Antibodies	Hepatovirus	24 patients, 18 control subjects	ELISA	Positive	(Oba et al., 2000)
IgG	Protein/Antibodies	Ebola virus hemorrhagic fever	24 patients, 10 control subjects	ELISA and (RT-PCR).	Positive	(Formenty et al., 2006)
HbsAg, HbsAb, HbcAb,	Protein/Antibodies/Antigen	Hepatitis B virus	22 patients	ICC and PCR	Positive	(Chen et al., 2009)
CSF-1	Cytokines	Periodontitis	29 patients, 7control subjects	ELISA	Increased levels in patients (1,671.0±1,199.9 pg/ml) vs controls (4,454.7 ± 190.8 pg/ml), p=0.001.	(Martinez et al., 2017)
TNF- α	Cytokines	RA and Periodontal disease	115 patients, 35 control subjects	Luminex human cytokine/chemokine	No change	(Mirrielees et al., 2010)

				multiplex kits		
		OLP disease	40 patients, 20 control subjects	ELISA	Increased levels in patients ($p<0.0001$).	(Pezelj-Ribaric et al., 2004)
		OLP disease	30 patients, 30 control subjects	ELISA	Increased levels in patients (29.92 ±9.99 pg/mL) vs controls (6.16±1.93 pg/mL) and $p=0.01$.	(Zhang et al., 2008)
		OLP disease	19 patients, 19 control subjects	ELISA	Increased levels in patients (0.601±0.178 pg/mL) Vs controls (0.013±0.033pg/mL) and $p<0.001$.	(Juretic et al., 2013)
		OLP disease	13 patients, 13 control subjects	ELISA	Increased levels in patients (74.23± 38.34 pg/mL) Vs controls (3.36 ± 2.07 pg/mL), and $P<0.0001$.	(Rhodus et al., 2005)
		OLP disease	20 patients, 20 control subjects	ELISA	Increased levels in patients (44.485± 16.81 pg/ml) Vs controls (1.405±0.25 pg/ml), ($p<0.0001$)	Ghallab et al., 2010)
		OLP disease	60 patients, 40 control subjects	ELISA	Levels decreased in patient group (21.057± 11.860 pg/ml) vs control group (24.185± 7.455 pg/ml)	(Liu et al., 2014)
		Periodontitis	35 patients, 39 control subjects	Luminex human cytokine and ELISA	Increased levels in patients (mean: 4.33 pg/ml) Vs controls (mean: 2.03 pg/ml) $p = 0.02$),	(Frodge et al., 2008)
		Periodontitis	10 patients, 14 control subjects	ELISA	No difference was detected	(Aurer et al., 2005)
		Periodontitis	84 patients, 81 control subjects	ELISA	No significant difference was found between groups	(Gursoy et al., 2009)
		Periodontitis	74 patients, 44 control subjects	Luminex Assay	No significant difference was found between groups	(Teles et al., 2009)

sTNFR-2	Cytokines	OLP disease	20 patients, 20 control subjects	ELISA	Increased levels in patients (350.4± 330.89 pg/ml) Vs controls (45± 13.82 pg/ml), (p<0.01)	Ghallab et al., 2010)
IL-18	Cytokines	OLP disease	103 patients, 48 control subjects	ELISA	Increased levels in patients (21.32±8.26 pg/mL) Vs controls (2.29±5.11 pg/mL) p<0.05).	(Zhang et al., 2012)
IL-8	Cytokines	OLP disease	30 patients, 30 control subjects	ELISA	Increased levels in patients (1737.49±1073.54 pg/mL) Vs controls (641.46 ± 172.91 pg/mL) and p=0.01.	(Zhang et al., 2008)
		OLP disease	13 patients, 13 control subjects	ELISA	Increased levels in patients (293.64± 86.84 pg/mL) Vs controls (1355.88 ± 28.37 pg/mL) and p <0.0001.	(Rhodus et al., 2005)
		Asthma	35 patients, 30 control subjects Children	Immunoassay and SERS	Increased levels in patients (3056.9 pg/ml) Vs controls (1070.87 pg/ml), p=0.004	(Zamora-Mendoza et al., 2019)
		Periodontitis	74 patients, 44 control subjects	Luminex Assay	No significant difference was found between groups	(Teles et al., 2009)
ProCT	Protein	Periodontitis with RA and OA	33 patients, 50 control subjects	ELISA	Increased levels in patients, p = 0.048.	(Redman et al., 2016)
Calprotectin	Protein	Gingivitis in children with cystic fibrosis	10 patients, 10 control subjects	ELISA	Increased levels in patients, p= 0.017	(Yucel et al., 2020)
		Gingivitis/ Periodontitis	81 patients, 18 control subjects	ELISA+PCR	Increased levels in patients p=0.023	(Ramseier et al., 2009)
		Inflammatory bowel disease	32 patients, 15 control subjects	ELISA	Increased levels in patients, p=0.001	(Majster et al., 2019)
MIP-1a	Cytokines/ Chemokines	Gingivitis/ Periodontitis	50 patients, 25 control subjects	ELISA	Increased levels in patients and p<0.001.	(Nisha et al., 2018)

MCP-1	Cytokines/ Chemokines	Gingivitis/ Periodontitis	50 patients, 25 control subjects	ELISA	Increased levels in patients and p<0.001.	(Nisha et al., 2018)
Salivary exRNA	RNA Nucleic acid	Gingivitis	30 patients	Affymetrix's expression microarrays	Associated with gingivitis	(Kaczor-Urbanowicz et al., 2018
ANXA-1	Protein	Gingivitis/ Periodontitis in pregnancy	78 patients, 69 control subjects	ELISA	Increased level vs controls (p<0.0001).	(Hassan et al., 2018)
Mucin 4	Protein	Periodontitis	37 patients, 39 control subjects	ELISA	Levels decreased in patients compared to controls. (p<0.01)	(Lundmark et al., 2017)
MMP-7	Enzyme/Protein	Periodontitis	37 patients, 39 control subjects	ELISA	Increased levels in patients compared to controls (p<0.05).	(Lundmark et al., 2017)
IFN-γ	Cytokines	OLP disease	63 patients, 63 control subjects	ELISA	Increased levels in patients compared to controls (7.74 ± 0.09 pg/ml)	(Malekzadeh et al., 2015)
		OLP disease	20 patients, 20 control subjects	ELISA	Increased levels in patients (23.953± 5.33 pg/ml) Vs controls (6.41±2.53 pg/ml), (p<0.0001).	(Ghallab et al., 2010)
		Periodontitis with HIV positive	39 patients, no control subjects	ELISA	No significant difference was found between groups	(Vastardis et al., 2003)
		Periodontitis	74 patients, 44 control subjects	Luminex Assay	No significant difference was found between groups	(Teles et al., 2009)
		OLP disease	60 patients, 40 control subjects	ELISA	Lower levels in OLP patients	(Liu et al., 2009)
IL-4	Cytokines	OLP disease	63 patients, 63 control subjects	ELISA	Increased levels in patients compared to controls (3.876 ± 0.05 pg/ml), (p=0.042).	(Malekzadeh et al., 2015)
		OLP disease	60 patients, 40 control subjects	ELISA	Levesl increased in patients (20.545± 63.482 pg/ml) Vs controls (15.917± 62.897 pg/ml).	(Liu et al., 2014)
				ELISA		

		Periodontitis with HIV positive Periodontitis	39 patients, no control subjects 74 patients, 44 control subjects	Luminex Assay	No significant difference was found between groups No significant difference was found between groups	(Vastardis et al., 2003) (Teles et al., 2009)
IL-17	Cytokines	OLP disease	35 patients, 15 control subjects	ELISA	Increased levels in patients (46.15 ± 7.78 pg/m) vs controls (42.13 ± 12.02 pg/m)	(Wang et al., 2015)
IL-17A	Cytokines	Tuberculosis	18 patients, 33 ORD	Luminex multiplex immunoassay	Increased levels in TB patients vs ORD patients, p=0.028	(Jacobs et al., 2016a)
IL-23	Cytokines	OLP disease Tuberculosis	35 patients, 15 control subjects 18 patients, 33 ORD	ELISA luminex multiplex immunoassay	No significant difference was found between groups Increased levels in TB patient's vs ORD, p=0.0023	(Wang et al., 2015) (Jacobs et al., 2016a)
β-glucuronidase	Enzyme	Periodontitis Periodontitis	50 patients, 20 control subjects 380 patients	Saliva b-Glucuronidase Assay Saliva b-Glucuronidase Assay	Level increased (105.48±2.52 mg/l) vs control (42.1±2.53 mg/l) p<0.001 Highly significant correlations	(Prabhahar et al., 2014) (Lamster et al., 2003)
IL-10	Cytokines	Asthma Gingivitis/ Periodontitis Periodontitis Periodontitis with HIV positive	35 patients, 30 control subjects Children 81 patients, 18 control subjects 74 patients, 44 control subjects 39 patients, no control subjects	SERS and ELISA Luminex assay ELISA ELISA	Levels increased in patients (24.60 pg/ml) Vs controls (15.82 pg/ml), p=0.008 No significant difference was found between groups No significant difference was found between groups No significant difference was found between groups	(Zamora-Mendoza et al., 2019) (Ramseier et al., 2009) (Teles et al., 2009) (Vastardis et al., 2003)
Salivary SP-D	Protein	Asthma	21 patients, 19 control subjects	ELISA	Salivary SP-D levels were higher in asthmatic children	Okazaki et al., 2017)

			Children		than in healthy controls.	

Key: *(ORD): other respiratory disease, (ELISA): enzyme-linked immunosorbent assay, (CLIA): chemiluminescence immunoassay, (RT-PCR): reverse-transcriptase polymerase chain reaction, (SERS): Surface Enhanced Raman Spectroscopy, (OLP): oral lichen planus, (RA): rheumatoid arthritis.*

3.5 Summary

Overall, cortisol, sAA, and SP were studied in nine studies in acute pain-related biomarkers.

Also, IL-1, IL-1α, IL-1β, IL-6, IL-4, IL-17, IL-17A, IL-23, IL-10, IL-18, IL-8, CSF-1, TNF-α, sTNFR-2, MIP-1α, MCP-1, IFN-γ, CPR, MMP-8, MMP-9, MMP-7, IgA, ProCT, Calprotectin, ANXA1, Mucin 4, Salivary SP-D and β-glucuronidase were studied in forty-four studies in inflammation-related biomarkers. The majority of these studies investigated oral diseases. Moreover, ECM-1, IL-17A, IgA, IgM, IgG, and CPR were studied in six studies about infection-related biomarkers.

The method of biomarkers detection for the majority of these studies was ELISA (Table 1 and 2).

4. Discussion

The primary aim of this systematic review was to explore the potential salivary biomarkers of pain, infection, and inflammation. Forty-nine studies were included in two systematic reviews of this work, and thirty-seven different biomarkers were identified (Table 1 and 2).

4.1 Potential salivary biomolecules related to nociception pain

4.1.1Cortisol

Pain responses in humans are determined via various conflicts and peripheral and central nervous systems (Kyle and McNeil, 2014). The autonomic nervous system (ANS) reaction to induce pain is best described via sympathetic nervous system activity rise and a reduction in parasympathetic vagal activity (Koenig et al., 2014). Furthermore, nociception is associated with hypothalamic– pituitary–adrenal (HPA) axis response, as painful stimulation cause an increase of cortisol secretion (Goodin et al., 2012b). In addition to this, cortisol is an essential neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis component, which can also secrete in response to stress as it is involved in stressful cognitive assessment conditions (McEwen, 2008).

Koenig et al. (2017) recruited 30 adolescents aged (15.27± 1.36), which after inducing acute pain with CPT saliva cortisol concentration level, increased. In addition to this, Goodin et al. (2012) used CPT to induce acute pain in 10 healthy participants aged (20.2± 2.7) and reported a high level of saliva cortisol concentration level after inducing pain (Goodin et al., 2012a). Also, higher salivary cortisol level in acute dental pain was reported (Haug and Marthinussen, 2019). They also considered the stress response of cortisol and assessed the stress level by a simple questionnaire, and stated that none of the participants reported acute pain-related stress.

Muller (2011) compared the saliva cortisol level in 64 male volunteers (aged 25.1±3.2) assigned to receive 32 controllable (self-administered) and 32 uncontrollable (experimenter-administered) painful electric skin stimuli. Muller, reported a significant

relationship between salivary cortisol concentration (P<0.01) and in subjective helplessness ratings and salivary cortisol concentrations (P<0.05).

Moreover, the rise in saliva cortisol level is seen in all four studies. However, stress can play an essential role in increased cortisol levels; thus, stress-free way of sample collections is a key to assess pain intensity levels precisely and subjectively (Kirschbaum and Hellhammer, 1989). he typical, non-stressful cortisol secretion in adults has a circadian rhythm, (Figure 6) therefore, the timing of sample collection is an essential factor to assess the pain intensities and reported at 11:00 am.

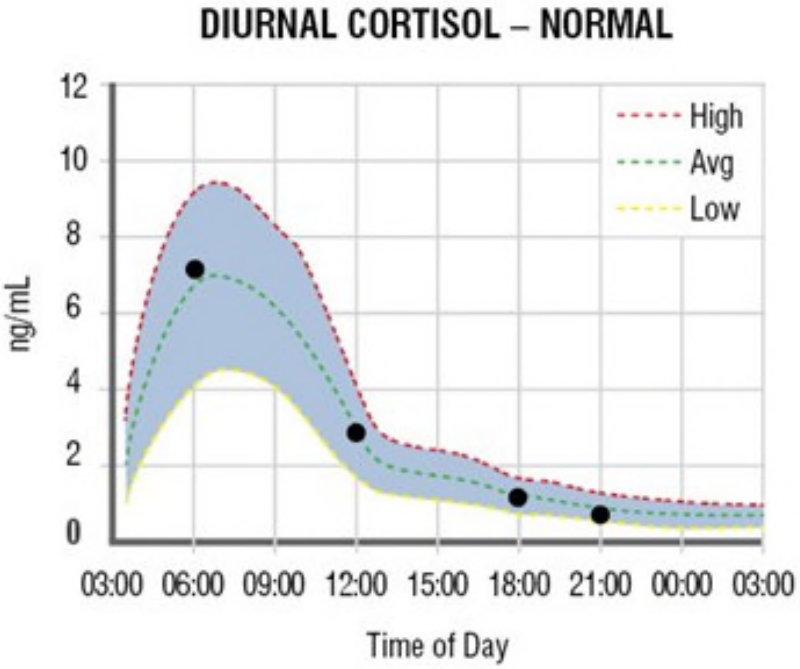

Figure 6: Represents the diurnal cortisol curve in saliva (ZRT Laboratory, 2020).

It can also be seen from the results; the cortisol level concentration differs in age and gender groups. Gender is a significant factor in cortisol secretion in response to inducing pain (Allen et al., 2009) as there are sex differences in stress reactivity due to different HPA reaction patterns (Verma et al., 2011). A study by Dixon et al. (2004) conducted that men have considerably higher cortisol in response to the CPT (Dixon et al., 2004). Similarly, Uhart et al. (2006) found a higher level of cortisol in men than

females in response to stress (Uhart et al., 2006). These studies align with our findings (Koenig et al., 2017, Muller, 2011, Goodin et al., 2012a). Conversely, it is worth to consider the female menstrual cycle of subjects, as female sex hormone has been reported to reduce HPA responsiveness, which can control the stress response (Verma et al., 2011), and this can be the reason that why the cortisol concentrations are higher in men (Verma et al., 2011).

Nevertheless, 2 of 4 studies used CPT to induce acute pain in healthy participants. Hence, different ways of inducing acute pain, such as heat stimulus or chemical stimulus, are expected to test future studies. To summarise, saliva cortisol can be used as a pain intensities biomarker in acute or nociceptive pain, but it should be taken into account that cortisol is a stress-related hormone and to be able to separate stress from pain can be a challenge. Also, age, gender, and the time of sample collection must be considered.

4.1.2 Salivary Alpha-amylase

Saliva is composed of many proteins, and about 50% of the salivary proteins are consist of alpha-amylase (Davis and Granger, 2009). Experimental and clinical evidence suggests that there are connections between nociceptive pain pathways and the sympathetic nervous system (SNS) at all neuraxis levels (Benarroch, 2006, Bantel and Trapp, 2011). sAA secreted via the salivary gland from 6 months of age onwards. sAA has become as a potential surrogate biomarker of autonomic nervous system activity (ANS) and can be also biomarker of sympathetic nervous system (SNS) activity caused by stress and anxiety (Nater et al., 2005, Nater and Rohleder, 2009, Bosch et al., 2011).

Ahmadi-Motamayel et al. (2013) recruited 36 patients with acute dental pain (20 female and 16 male) aged (34.2± 11.4) and reported sAA level was directly correlated with age ($p = 0.001$) and severity of pain ($p = 0.032$). They also concluded that patients' level of pain was proportional to the sAA level; however, it was different for males and

females. In contrast, the male had a higher level of sAA (up to 42.131 unit/mL). This finding is in line with previous studies (Shirasaki et al., 2007, Noto et al., 2005).

Another study measured dispositional positive affect in 73 children with leukaemia cancer aged (16.7± 3.79) and given them emotion regulation strategy conditions randomly (distraction, reappraisal, or reassurance). The children used their own strategy in an experimental method of inducing acute pain (CPT). This study conducted that children in the reassurance group had an increase in the level of sAA after CPT in comparison with children in the distraction ($p = .021$) and reappraisal conditions ($p = .084$) (Jenkins et al., 2018). Also, these findings confirm that reassurance about acute pain may increase distress (Chorney et al., 2013).

Shen et al. (2012) recruited 473 AMI in chest pain patients (291 men and 182 women; 85 AMI aged (63.6± 12.9) and 388 non-AMI aged (60.6± 16.5)), they found that the level of sAA in AMI group (266 ± 127.6 U/mL) was considerably higher than the non-AMI group 130 ± 92.8 U/mL) and ($p < 0.001$). However, more studies need to be done to consider different sample sizes, age, and gender in future research to confirm as no relevant studies were found.

Wittwer et al. (2016) recruited 27 healthy volunteers, 13 women aged (25±3), and 14 men aged (27±4), and the heat pain tolerance was measured on their forearm. They reported that the level of sAA was correlated with the intensity and unpleasantness ratings of acute heat pain stimuli. sAA is suggested to be an indirect physiologic correlate of heat pain perception. However, more research needs to be done on the association of sAA and heat pain method.

Age, gender, and physiological stress can affect the level of sAA during acute pain perception. Ahmadi-Motamayel et al, (2013) reported that the level of sAA was higher in men and older patients than women and younger patients, respectively, during

acute dental pain. Also, sAA is another stress-related biomarker. In addition to this Jenkins et al. (2018) reported that physiological stress could considerably increase the level of sAA in children (Jenkins et al., 2018). Also, Psychosocial Stress Increases sAA activity (Petrakova et al., 2015).

Consequently, Shen et al, (2012) stated AMI chest pain, significant changes between the AMI and non-AMI group in gender distribution, and males reported a higher level of sAA. Also, pain and acute stresses in AMI patients to increase the sympathetic nervous system activation, which causes the salivary gland adrenoceptor activation and results in increased sAA activity (Shen et al., 2012).

To summarise, saliva sAA can be used as a pain intensities biomarker in acute pain, but it should be taken into account that as sAA is related to stress response and to be able to separate stress from pain can be a challenge. Moreover, as age, gender, stress confounds the activity of sAA levels, these factors needed to be considered in future research.

4.1.3 Substance P

SP, a primary neurotransmitter (a molecule with 11 amino acid residues, which belongs to tachykinin neuropeptide family) is also involved in the acute pain perception and sensation (Regoli et al., 1994). SP is involved in several biological processes: vasodilation, contraction of smooth muscle, and immune responses. SP is also released at spinal dorsal horn via A and C fibers and performs as an excitatory neurotransmitter in response to nociceptive stimuli (Romero-Reyes and Uyanik, 2014).

Few studies concern SP and pain. Increasing the level of saliva SP in acute pain intensity is seen in one study. Ahmad M et al. (2014) recruited 30 (7 male and 23 female) patients with self-reported dental pain and 35 healthy volunteers in the control group (25 male and 10 female) and they reported, salivary SP level in males without

dental pain (498.5± 54.6 pg/ml) were considerably higher in comparison with females without dental pain (335.5 ± 73.6 pg/ml, P < 0.001) however the level of salivary SP were higher in females with dental pain (891.4 ± 43.7 pg/ml) vs males (823.2 ± 50.7 pg/ml). Few studies reported an elevated salivary SP chronic migraine and burning mouth syndrome (Jang et al., 2011, Borelli et al., 2010). As a result, it is difficult to conclude whether salivary SP can be a biomarker of acute pain, and more research is needed. Also, more studies are needed to focus on age and gender-related changes at the SP level.

Moreover, the methods of sample collection may affect the SP concentration level. Additionally, a study compares different saliva collection methods in healthy individuals and reported stimulated sublingual saliva (SSS) has the highest saliva SP concentration (Jasim et al., 2018). Hence, saliva collection methods need to take into account in future research.

Overall, one of the hypotheses of this review was that the biomolecules in saliva that exhibit a change in the presence of acute pain. From the results, it is concluded that, the concentration of salivary pain-related biomolecules changed with acute pain.

4.2 Potential biomolecules related to infection and inflammation

During inflammation response, the human body recruited pro-inflammatory cytokines, chemokines, and other inflammatory mediators. When the immune system is activated, it stimulates the secretion of phagocytosis and macrophages of pro-inflammatory molecules such as a variety of cytokines including IL-6, IL-1β, and TNF-α (Figure 7) (Zhang and An, 2007).

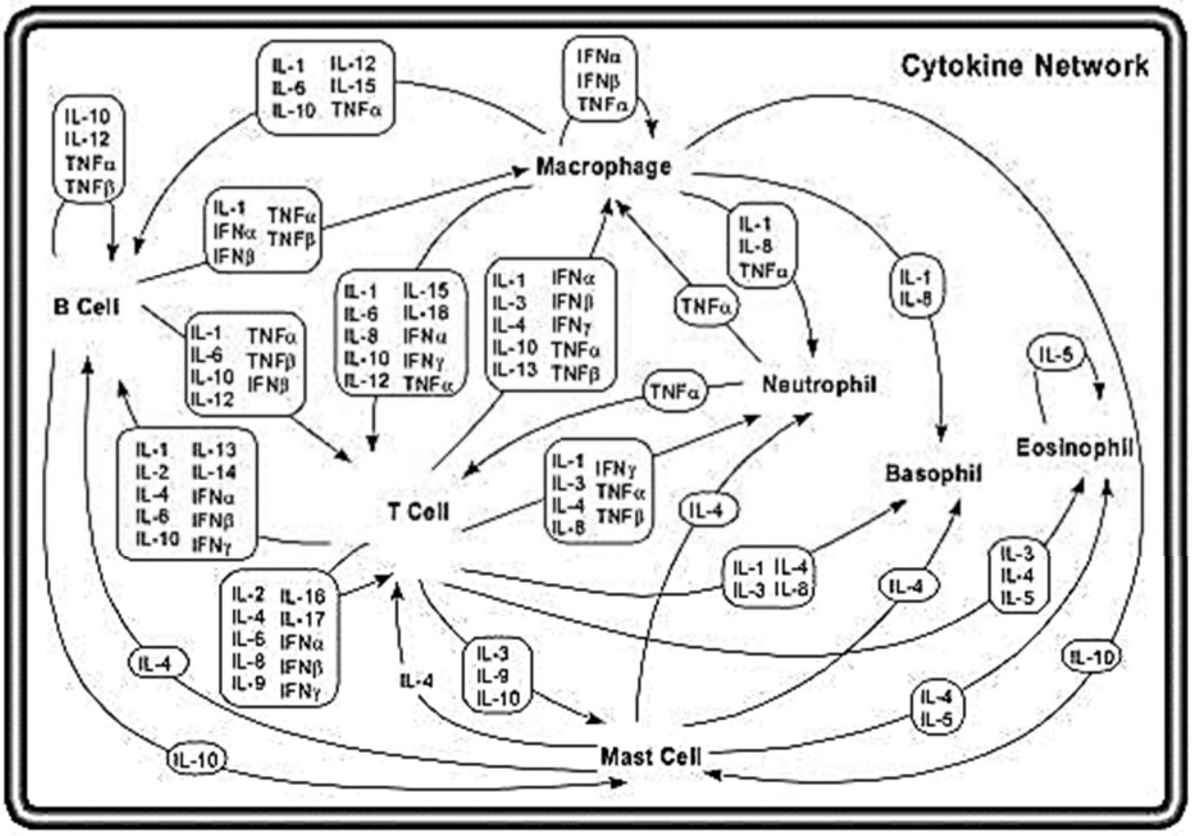

Figure 7 : *Represents the cytokines network (Zhang and An, 2007).*

In the past decades, researchers investigated the importance of saliva as a diagnostic tool in early prediction, prevention, and treatment of local gland disease such as inflammatory and autoimmune disease For instance, in dental caries, saliva has an essential role in enamel caries prevention and development. The majority of potential biomarkers of inflammation summarised in table 2 are for oral diseases such as periodontitis and gingivitis and OLP disease. The most common types of periodontal disease are periodontitis and gingivitis (Taylor, 2014). Periodontitis is the disruption of natural homeostatic processes by different bacterial species present in subgingival dental plaque (Darveau, 2010) while gingivitis is the inflammation of the gingiva (gums) (Pihlstrom et al., 2005). As periodontal disease is time-consuming and expensive to treat; hence, salivary biomarkers can play an important role in the prevention, early detection, and management of the disease (Slots, 2013).

Moreover, periodontitis can be classified based on the three-stage of progression (inflammation, degradation of connective tissue, and bone turnover), which are associated with different salivary biomarkers in terms of the different stages of periodontal disease. As an example, IL-1, IL-6, and TNF-α levels increased at the beginning of the inflammatory stage. In addition to this, when the disease becomes more advanced, TNF, and IL-1 levels are significantly increased (Korte and Kinney, 2016). In this review, the infection and inflammation biomarkers are further divided into several classes: protein/enzyme and cytokines/chemokines (Table 2).

4.2. Cytokines/chemokines biomolecules

The immunological system, such as cytokines, plays a vital role in the pathogenesis and development of inflammation and infectious disease (Zhang and An, 2007). Cytokine is "small secreted proteins released by cells that have a particular effect on the interactions and communications between cells" (Zhang and An, 2007). By looking at table 2, this review studied IL-1, IL-1 α, IL-1β, IL-6, IL-4, IL-17, IL-17A, IL-23, IL-10, IL-18, IL-8, CSF-1, TNF-α, sTNFR-2, MIP-1a, MCP-1 and IFN-γ as potential biomarkers of periodontal diseases.

4.2.1 Interleukin 1β

IL-1β is one of the pro-inflammatory cytokine and is produced via macrophages. It plays an important role in the inflammatory cascade and promotes TNF-α and IL-6 release (Dinarello, 2009). An increase in the level of salivary IL-1β was seen in 5 studies in periodontitis (Miller et al., 2006, Ebersole et al., 2013, Rangbulla et al., 2017, Mirrielees et al., 2010, Hassan et al., 2018). These findings are in line with previous studies (Giannobile et al., 2009, Taba et al., 2005).

Moreover, in terms of variability, IL-1β showed no detectable age-related trend in inflammation (Cavallone et al., 2003, Jylha et al., 2007, Panickar and Jewell, 2015, Navarro et al., 2016). Also, saliva IL-1β concertation is not associated with smoking (Ebersole et al., 2013). However, it has been reported that IL-1β linked with obesity (Stienstra et al., 2011, Speaker and Fleshner, 2012).

To summarise, salivary IL-1β is a robust biomarker for periodontal disease.

4.2.2 Interleukin 6

Similarly, IL-6 is both a pro-inflammatory and anti-inflammatory cytokine which is involved in inflammatory diseases (Hirano et al., 1988). The trend of increase in the concentration of IL-6 was seen in OLP in 3 studies (Zhang et al., 2008, Juretic et al., 2013, Rhodus et al., 2005), which is in line with previous studies (Kaur and Jacobs, 2015, Brailo et al., 2012, Gu et al., 2004).

Kaur and Jacobs (2015) recruited 54 OLP patients and 50 healthy age-matched volunteers, and checked the smoking and alcohol consumption and found no significant difference. Similarly, Rhodus et al (2005) concluded that smoking has no effect on the level of IL-6. Hence, this finding shows that smoking cannot considerably increase the level of IL-6 in OLP disease. However, these studies also concluded that the level of IL-6 is significantly higher in advance stages of OLP disease compared to early stages (Kaur and Jacobs, 2015, Gu et al., 2004, Zhang et al., 2008).

The majority of studies in this review reported that salivary IL-6 has no association with periodontitis (Ramseier et al., 2009, Gursoy et al., 2009,Teles et al., 2009). In contrast, one study reported increased levels of IL-6 in periodontitis (Ebersole et al., 2013) (Table 2) which is consistent with previous studies (Costa et al., 2010, Prakasam and Srinivasan, 2014).

To summarise, IL-6 can be a potential biomarker of OLP disease and periodontitis however more researches need to be done. The limitation of these studies can be sample size, the method of detection and in some studies, OLP disease patients and healthy controls were not age and sex-matched, should consider these in future research.

4.2.3 Tumor necrosis factor alpha

TNF-α was reported higher in concentration in oral OLP disease in 7 studies (Pezelj-Ribaric et al., 2004, Zhang et al., 2008, Juretic et al., 2013, Rhodus et al., 2005, Ghallab et al., 2010, Liu et al., 2014, Frodge et al., 2008). However, 4 studies reported no significant differences in TNF-α levels in periodontitis (Mirrielees et al., 2010, Aurer et al., 2005, Gursoy et al., 2009, Teles et al., 2009).

Another key biomarker in periodontal disease is TNF-α. TNF-α is a pro-inflammatory cytokine, which is produced by macrophages (Mika et al., 2013). Additionally, it is playing an important role in the inflammatory cascade and promotes IL-1β and IL-6 release (Dinarello, 2009). TNF-α has been reported high in concentration in OLP disease (a chronic inflammatory disease of the mucus membrane in the oral cavity) in 6 studies of this review (Pezelj-Ribaric et al., 2004, Zhang et al., 2008, Juretic et al., 2013, Rhodus et al., 2005, Ghallab et al., 2010, Frodge et al., 2008). These findings are in line with previous studies (Malarkodi and Sathasivasubramanian, 2015, Kaur and Jacobs, 2015).

Moreover, several parameters can change the concentration of saliva TNF-α in patients such as smoking (Singh et al., 2014, Petrescu et al., 2010) which increases the level of TNF-α and consuming alcohol (Nagler et al., 2002) which inhibits the level of TNF-α. Also, the concentration of TNF-α is varied in different clinical types of OLP disease (elevated in the erosive and atrophic form), hence, the level of TNF-α is correlated with the severity of OLP disease (Nagler et al., 2002).

On the other hand, the level of TNF-α had no significant change in periodontitis in 4 studies (Mirrielees et al., 2010, Aurer et al., 2005, Gursoy et al., 2009, Teles et al., 2009). These findings are consistent with previous studies. Salivary TNF-α level has shown no association with periodontitis (Rathnayake et al., 2013, Ebersole et al., 2013). Hence, it cannot be the potential biomarker of periodontitis.

To summarise, TNF-α has the potential to be a biomarker in OLP disease. However, as the concentration of TNF-α is varied in different types of OLP disease; stress, alcohol consumption and smoking must be taken into consideration in future research as they can interpret the results. In addition, the limitation of these studies can be the timing of saliva collection, different methods of detection, variation in selecting the healthy control groups, the severity of OLP across the studies.

Overall, IL-1β, IL-6, TNF-α are important biomolecules that play a critical role in oral inflammation disease. However, more studies need to be done on other biomolecules; IL-1, IL-1, IL-4, IL-17, IL-17A, IL-23, IL-10, IL-18, IL-8, CSF-1, sTNFR-2, MIP-1a, MCP-1, and IFN-γ as potential biomarkers of periodontal diseases.

4.3 Enzyme/protein biomolecules

Other potential enzymes/protein-based biomarkers were studied in this review including ECM-1, CPR, MMP-8, MMP-9, MMP-7, salivary IgA, IgA, IgM, IgG, HbsAg, HbsAb, HbcAb, ProCT, Calprotectin, ANXA1, Mucin 4, Salivary SP-D and β-glucuronidase in infection and inflammation (Table 2).

4.3.1 C- reactive protein

CRP is an acute inflammatory protein that rises at infection or inflammation (Sproston and Ashworth, 2018). CRP is secreted via the liver in response to IL-6 (Marnell et al., 2005) and which also helps the secretion of the pro-inflammatory cytokines in response to infection (TNF-α, IL-1β) (TNF-α, IL-1β) (Du Clos, 2000).

Omran et al. (2018) recruited 70 neonates (35 with sepsis and 35 healthy controls) and reported higher salivary CRP between septic neonates and controls (12.0±4.6ng/L vs. 2.8±1.2ng/L) respectively and concluded salivary CRP revealed 94.3% sensitivity and 80% specificity in diagnosing of neonatal sepsis (bacterial bloodstream infection in babies). Iyengar et al. (2014) were the first to report the use of salivary CRP in neonate's detection and concluded that salivary CPR is a useful biomarker for detecting neonates. However, future studies need to consider a larger sample and control group (Iyengar et al., 2014).

Moreover, one of the studies recruited 104 males (32 had TB, and 72 had other ORD) aged (38.8± 11.9) and reported salivary CPR was increased in TB patients vs ORD ($p<0.0001$) with a sensitivity of 75% and specificity of 85% (Jacobs et al., 2016b). However, the positive correlation between CRP salivary biomarker and TB have been previously shown in other studies as well (Rohini et al., 2016).

Furthermore, CPR is also a potential biomarker of periodontal disease (Christodoulides et al., 2005, Noack et al., 2001, Jayaprakash et al., 2014, Shojaee et al., 2013, Metgud and Bajaj, 2016, Christodoulides et al., 2007). These findings are in line with previous studies (Pitiphat et al., 2008, Wohlfeil et al., 2012, Haba et al., 2011).

One study concluded that factors such as age and smoking could affect the CRP level, and reported CRP level was considerably higher in the older and smoker patients than in the healthy group (Pitiphat et al., 2008). Another study compared the salivary CPR level of smokers and non-smokers and found that smokers had higher salivary CPR concentration levels (Azar and Richard, 2011). A study exposed 15 healthy students to the Trier Social Stress Test (TSST) and reported that there were no significant changes in the level of salivary CPR (Campisi et al., 2012).

To summarise, salivary CPR can be a potential biomarker in inflammation and infection disease; however, age and smoking need to be considered in future samples analysis.

4.3.2 Matrix metalloproteinase 8

Following the cytokines secretions in response to inflammation and infection, neutrophils release various enzymes. For instance, matrix metalloproteinase (MMP), and other inflammatory mediators. MMPs are one of the main proteases involved in periodontitis (Sorsa et al., 2011). Currently, MMP-8 is considered to be the potential biomarker for periodontitis in saliva (Rathnayake et al., 2015). Moreover, in this review, 12 studies reported that the salivary level of MMP-8 concentration in saliva has a

positive correlation with the stage of periodontitis, gingivitis disease (Miller et al.,2006, Johnson et al., 2016, Gursoy et al., 2010, Ebersole et al., 2013, Ebersole et al., 2015, Kushlinskii et al., 2011, Gupta et al., 2015, Rangbulla et al., 2017, Martinez et al., 2017, Ramseier et al., 2009, Mirrielees et al., 2010, Christodoulides et al., 2007). These findings are consistent with previous studies (Sorsa et al., 2016, Noack et al., 2017). Thus, MMP-8 can be used as a potential biomarker in oral cavity inflammation.

However, Scannapieco et al. (2007) reported no significant differences in MMP-8 level in periodontitis disease; therefore, few factors such as a method of detection (ELISA, IFMA, and Luminex), use of antimicrobial agents may explain the variation in results. Besides, Johnson et al. (2016) concluded that age and gender, smoking status did not considerably affect the salivary concentration level of MMP-8.

To summarise, MMP-8 is one of the critical biomolecules in periodontitis; however, more studies need to be done to confirm whether age and gender can vary the concentration of salivary MMP-8 level.

4.3.3 Immunoglobulins (IgA, IgM, IgG)

Immunoglobulins (IgA, IgG, and IgM) are significant anti-inflammatory biomolecules in infectious diseases. From the results, IgA was detected in HIV infection (Mandal et al., 2016), which is in line with current literature (Dang et al., 2019).

Moreover, Oba et al. (2000) reported that IgA, IgM was detected in hepatovirus infection patients with a sensitivity of 80.8% and specificity of 100%. This finding is consistent with previous studies (Parry, 1993, Piacentini et al., 1993).

Salivary immunoglobulins are acceptable alternative specimens for diagnosing infectious diseases.

To summarise, CPR, MMP-8, IgA, IgM, and IgG are essential biomolecules that play a critical role in infection and inflammation diseases. However, more studies need to be done on other biomolecules; ECM-1, MMP-9, MMP-7, salivary IgA, IgA, IgM, IgG,

HbsAg, HbsAb, HbcAb, ProCT, Calprotectin, ANXA1, Mucin 4, Salivary SP-D and β-glucuronidase as potential biomarkers of infection and inflammation.

Overall, the second hypotheses of this review were that biomolecules in saliva exhibit a change in the presence of infection and inflammation. From the results, it is concluded that the concentration of salivary biomolecules changed with infection and inflammation disease.

5. Limitations

One of the main limitations of this systematic review is that the studies included in this review are screened and selected by a single author; therefore, it may risk bias. Also, this review's results were not quantified (meta-analysis) as the results of each study were varied.

6. Conclusion

This systematic review supports potential salivary biomarkers for diagnosing and monitoring acute pain, infection, and inflammation disease. The findings of this study suggest that salivary cortisol and sAA can be potential biomarkers of acute pain. Salivary IL-1β is a robust biomarker for periodontal disease. IL-6 can be a potential biomarker of OLP and periodontitis. TNF-α has the potential to be a biomarker in OLP disease. Salivary CPR can be a potential biomarker in inflammation and infection disease. MMP-8 is one of the critical biomolecules in periodontitis.

Therefore, the use of salivary biomarkers in pain, infection, and inflammation poses a number of advantages over measuring biomarkers in blood. For instance, patients are free of stress from venepuncture, costs reductions as there is no need of specialised physicians for sample collection, the sample does not clot and and less likely to transmit disease from blood and is safer.

Moreover, several studies concluded that factors such as age, gender, physiological stress, smoking, and consuming alcohol could affect the biomarkers' level during acute pain intensity, infection, and inflammation diseases. To conclude, further research is required to ascertain salivary biomarkers' use as a method to quantify acute pain intensity, infection, and inflammation disease.

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