Review Article

Salivary biomarkers in pain assessment: an integrative review

Biomarcadores salivares na avaliação da dor: revisão integrativa

Biomarcadores salivales en la evaluación del dolor: revisión integradora

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Descriptores
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Abstract

Objective: To identify the main salivary biomarkers described and the techniques used for saliva sample collection in studies related to pain assessment in patients undergoing painful procedures or experiencing painful diseases

Methods: An integrative literature review was conducted via bibliographic searches in the Virtual Health Library (VHL), MEDLINE/PubMed, CINAHL, and EMBASE databases for the period from 2009 to 2019; data were collected in October and November 2019. The DeCs health descriptors and the Medical Subject Headings (MeSH) were used to answer the guiding question: "Which salivary biomarkers are used in pain assessment and how are they employed?" A descriptive analysis of the articles was performed; data were collected and recorded in a spreadsheet developed for the present study.

Results: Of the 126 published articles identified, 22 articles were included for analysis. The articles were mainly regarding adults undergoing painful procedures or patients experiencing painful diseases. The main salivary biomarkers evaluated were alpha-amylase and cortisol, and the main saliva collection techniques were Salivette® and passive collection.

Conclusion: The studies indicated that objective pain measurement is a challenge. The main salivary biomarkers evaluated were cortisol and alpha-amylase, and the main technique employed for saliva sample collection was Salivette®. The dosage of salivary molecules is emerging for use as a complement in pain assessment in patients of different ages undergoing painful procedures or experiencing painful diseases.

Resumo

Objetivo: Identificar os principais biomarcadores salivares descritos, assim como as técnicas empregadas para coleta das amostras de saliva, em estudos relacionados à avaliação da dor em pacientes submetidos a procedimentos dolorosos ou portadores de patologias dolorosas.

Métodos: Revisão integrativa da literatura, realizada pelas buscas bibliográficas nas bases Biblioteca Virtual em Saúde (BVS), MEDLINE/PubMed, CINAHL e EMBASE, com recorte temporal de 2009 a 2019 e período de coleta de dados entre outubro e novembro de 2019. Foram utilizados Descritores em Saúde (DeCs)e Medical SubjectHeadings (MeSH), para responder à pergunta norteadora: Quais são e como são utilizados os biomarcadores salivares na avaliação da dor? Foi realizada uma análise descritiva dos artigos, sendo os dados extraídos e registrados em uma planilha desenvolvida para o presente estudo.

Resultados: Das 126 publicações identificadas, 22 artigos foram incluídos para a análise. Constatou-se que os artigos são, majoritariamente, desenvolvidos com adultos durante realização de procedimentos dolorosos ou portadores de patologias dolorosa. Os principais biomarcadores salivares avaliados foram a alfa-amilase e o cortisol, e as principais técnicas para coleta de saliva foram o Salivette® e a coleta passiva.

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Conflicts of interest: Nothing to declare.

Conclusão: Os estudos indicam que a mensuração objetiva da dor é um desafio. Os principais biomarcadores salivares descritos são o cortisol e a alfa-amilase, sendo o Salivette®a principal técnica utilizada para coleta das amostras de saliva. A dosagem das moléculas salivares é incipiente e empregada de forma complementar na avaliação da dor em pacientes de diferentes faixas estárias, submetidos à procedimentos dolorosos ou portadores patologias dolorosas.

Resumen

Objetivo: Identificar los principales biomarcadores salivales descriptos, así como las técnicas utilizadas para la recolección de las muestras de saliva en estudios relacionados con la evaluación del dolor en pacientes sometidos a procedimientos dolorosos o con patologías dolorosas.

Métodos: Revisión integrativa de la literatura, realizada por medio de búsquedas bibliográficas en las bases Biblioteca Virtual em Saúde (BVS), MEDLINE/PubMed, CINAHL y EMBASE, con un recorte temporal del 2009 al 2019 con un período de recolección de datos de octubre a noviembre de 2019. Se utilizaron Descriptores en Salud (DeCs) y Medical SubjectHeadings (MeSH), para responder a la pregunta orientadora: ¿Cuáles son los biomarcadores salivales en la evaluación del dolor y cómo se utilizan? Se realizó un análisis descriptivo de los artículos y los datos extraídos y registrados en una planilla desarrollada para el presente estudio.

Resultados: De las 126 publicaciones identificadas, se incluyeron 22 artículos para análisis. Se constató que los artículos están, mayoritariamente, desarrollados con adultos durante la realización de procedimientos dolorosos o con patologías dolorosas. Los principales biomarcadores salivales evaluados fueron alfa-amilasa y cortisol, y las principales técnicas para la recolección de saliva fueron Salivette® y la recolección pasiva.

Conclusión: Los estudios indican que la medición objetiva del dolor es un desafío. Los principales biomarcadores salivales que se describen son el cortisol y la alfa-amilasa y Salivette® la principal técnica utilizada para la recolección de muestras de saliva. La dosificación de las moléculas salivales es incipiente y utilizada de forma complementaria en la evaluación del dolor en pacientes de distintos grupos de edad, sometidos a procedimientos dolorosos o con patologías dolorosas.

Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or an experience similar to that associated with tissue damage.[1] The manifestation of pain shows considerable variation between individuals and produces several behavioral and physiological responses that can be employed as clinical assessment tools.[2] Owing to the complexity associated with pain assessment, numerous tools have been developed and validated in different age groups and clinical conditions to perform this assessment. Some of these instruments include behavioral, physiological, and contextual parameters that are used as indicators. These indicators are considered to increase the specificity of these instruments.[3]

Factors such as the type or cause of pain as well as patient age and clinical condition must be considered in pain assessment; in addition, the proficiency of the health professional who applies and interprets various assessment instruments should be considered.[4] Therefore, the complexity of pain assessment and the need to use specific and accurate methods are acknowledged, particularly in nonverbal populations, wherein pain is often underestimated and undertreated.[3]

Despite advances in the general understanding of pain pathophysiology as well as in pain assessment, numerous aspects related to pain assessment and its consequent management in clinical practice are emerging. Therefore, additional parameters, such as dosages of salivary molecules, have been investigated as complementary physiological measures for pain assessment.[5,6] In addition to the association of salivary dosages with pain scores that were measured using assessment scales[7-10], these dosages reflect the plasma and urinary levels of different molecules.[11] Although the use of salivary molecules in pain assessment is considered clinically essential and promising, currently, evidence to support any such molecules as isolated "objective" measures of pain is lacking.[12]

Nevertheless, the development and dosage of new biomarkers is a constantly evolving field, and salivary molecules have gained special attention—particularly for conducting clinical research—because obtaining saliva samples is a simple, noninvasive procedure that is devoid of stress and discomfort to the patient; these are the fundamental attributes of pain assessment methods.[13]

In clinical practice, pain assessment and management remain inadequate and inconsistent.[14] Elucidating the multidimensional experience of pain and its management is challenging, because repeated and untreated pain can result in deleterious consequences in the short, medium, and long term.[15,16] Considering the above, the role of the nursing team—

involving pain assessment and reassessment in terms of multiple aspects, such as pain intensity, quality, location, and duration, as well as the use of pain prevention and relief strategies including pharmacological and non-pharmacological measures—is essential. The increase and appropriation of knowledge regarding this topic by the nursing team as well as the adoption of innovative techniques and use of biomarkers can contribute to improving care.

Considering the complexity of pain assessment, the inclusion of complementary parameters, such as the dosage of salivary biomarkers, may aid in future studies aimed at improving pain assessment and management in different scenarios. Therefore, it is essential to identify of the main salivary molecules evaluated in cases of painful diseases or during potentially painful procedures.

Accordingly, the present study aimed to identify the main salivary biomarkers described in studies related to pain assessment in patients undergoing painful procedures or experiencing painful diseases and to identify the techniques used for saliva sample collection in these studies.

Methods

The present study was performed as an integrative literature review, a research method used in evidence-based practice that facilitates the incorporation of this evidence into clinical practice. This method gathers and summarizes the scientific knowledge of available research on a defined theme, thereby contributing toward deepening the knowledge on the investigated topic.[17] For study development, the following steps were followed: selection of the guiding question; establishment of selection criteria; literature search; definition of the information to be extracted from selected studies; evaluation of included studies; interpretation of the results; and presentation of the review.[17]

The guiding question for this review was "Which salivary biomarkers are used in pain assessment and how are they employed?" Accordingly, bibliographic searches in the Virtual Health Library (VHL) as well as the MEDLINE/PubMed, CINAHL, and EMBASE electronic databases were performed. The combinations of descriptors and keywords were adapted to the acronym PICO[18] as follows:

- P (Patient): adults, children, and newborns
- I (Intervention): dosage of salivary biomarkers in pain assessment
- C (Comparison): standard or routine care (where applicable)
- O (Outcome): pain assessment

For the search in the VHL, health descriptors standardized in the DeCs project of the Latin American and Caribbean Center for Information in the Health Sciences (BIREME) were used: ((Patients) AND ("biomarkers") AND ("Saliva") AND ("Pain Assessment")). For the search in the CINAHL and PubMed databases, descriptors from the Medical Subject Headings (MeSH) were used: (("Biomarkers") AND ("Pain") AND ("Saliva")).

The review was conducted in October and November 2019; inclusion criteria for articles were as follows: articles available electronically, with full text in English, Spanish, or Portuguese, published between 2009 and 2019 and articles that addressed the use of salivary biomarkers simultaneously with the application of an instrument for pain assessment in adults, children, or newborns. Exclusion criteria were as follows: short communications (such as Comments, Letters to the editor, or Editorials), studies that did not present content related to the topic, research on animal models, and duplicate articles.

Titles and abstracts were independently analyzed by two authors (VR and PF). Disagreements were discussed among the authors and resolved by a third researcher (MB), if necessary. Thereafter, the articles considered relevant for the present research were obtained and descriptively analyzed thoroughly. The level of evidence (LE) was identified based on study design and was determined as follows: Level I, systematic review or meta-analysis; Level II, randomized controlled clinical trial; Level III, nonrandomized controlled clinical trial; Level IV, well designed cohort or case-control studies; Level V, systematic review of qualitative and descriptive studies; Level VI, descriptive or qualitative studies; and Level VII, opinion of authorities or expert's re-

port.[19] This hierarchy classifies Levels I and II as strong, III to V as moderate, and VI and VII as weak.[19]

The information from each publication was collected and recorded in a Microsoft Excel spreadsheet (Microsoft Office Enterprise 2007) that was developed for the present study and previously tested; the data collected included the following: author, year, country, number of participants, age group (adults, children, or newborns), study design, objectives, saliva sample collection techniques used, salivary biomarker(s) evaluated, pain assessment scale, correlation with pain scales or blood and urine samples, main results, and conclusions.

Because this was a literature review, the submission and ethical review of the study was not required.

Results

In total, 126 articles were identified, including 12 duplicates. Of the 114 remaining articles, 67 were excluded after reading the titles and abstracts because these articles did not fulfil the inclusion criteria. From the 47 selected studies, 25 were excluded after reading the full text: 1 article addressed only saliva collection techniques; 1 case report, 2 experimental studies, and 5 articles were related to the treatment of different diseases; and 13 articles mainly addressed diagnostic aspects. Finally, 22 articles were included in the review (Figure 1).

All articles were published in English, and during the period analyzed, no review was published. Regarding the articles, 4 (18.2%) were published in 2018; 3 (13.6%) each in 2016, 2013, and 2011; 2 (9.1 %) each in 2014, 2012, and 2010; and 1 (4.5%) each in 2019, 2017, and 2015.

Regarding the countries where the studies included in the review were conducted, 7 (31.8%) were conducted in the United States, 3 (13.6%) in Brazil, 2 (9.1%) in Turkey, 2 (9.1%) in The Netherlands, 1 (4.5%) in South Korea, 1 (4.5%) in Japan, 1 (4.5%) in India, 1 (4.5%) in Austria, 1 (4.5%)) in Spain, 1 (4.5%) in Australia, 1 (4.5%) in Italy, and 1 (4.5%) in Switzerland (Table 1).

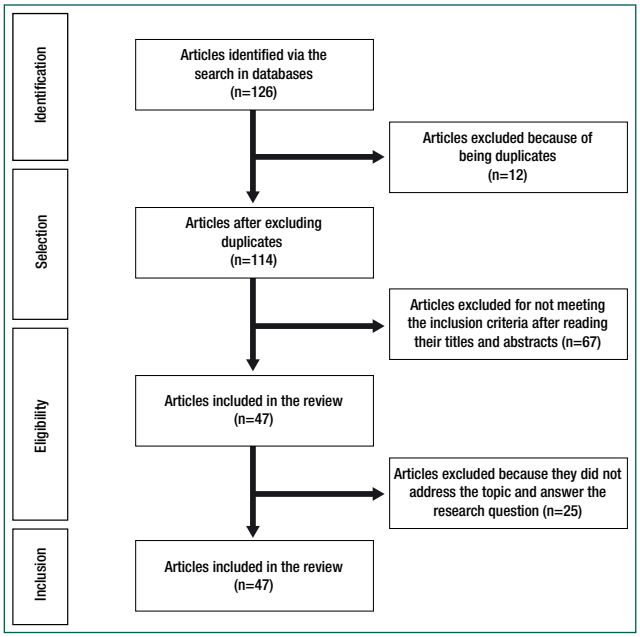

Figure 1. Flowchart representing the eligibility and inclusion of articles

Regarding the level of evidence, 5 (22.7%) articles were categorized as Level II,[20–32] 2 (9.1%) as Level III,[20,22] 7 (31.8%) as level IV[9,26,30–37], and 8 (36.4%) as level VI[7,8,10,24,27,28,33–36] (Tables 1 and 2). It was observed that 5 (22.7%) articles presented strong LEs (levels I-II), 9 (40.9%) presented moderate LEs (levels III-V), and 8 (36.4%) presented weak LEs (levels VI-VII) (Table 1).

Table 1. Distribution of studies according to classification of the level of evidence

Level of evidence		n(%)	Classification	n(%)
Level I	Systematic review or meta-analysis	0 (0)	Strong	5 (22.7)
Level II	Randomized controlled clinical trial	5 (22.7)		
Level III	Nonrandomized controlled clinical trial	2 (9.1)	Moderate	9 (40.9)
Level IV	Cohort or case-control studies	7 (31.8)		
Level V	Well-designed systematic review of qualitative and descriptive studies	0 (0)		
Level VI	Descriptive or qualitative studies	8 (36.4)	Weak	8 (36.4)
Level VII	Opinion of authorities or report of experts	0 (0)		

Regarding the investigated population, 14 (63.6%) studies included adults, followed by 5 (22.7%) studies involving children and 3 (13.6%) with newborns. Regarding pain etiology, 9 (40.9%) studies investigated orofacial pain and temporomandibular disorders, musculoskeletal pain, abdominal pain, cancer-related pain, rheumatoid arthritis, epilepsy, and migraine and 13 (59.1%) publications

assessed procedure-related pain. Among numerous pain measurement tools, the visual analog scale (VAS) was applied in 27.3% of the studies (Table 2). Regarding saliva sample collection, 13 (59.1%) studies used Salivette® and 7 (31.8%) used passive collection. In 2 (9.1%) studies, the collection methodologies employed included saliva aspiration with a Levine probe and syringes and the use of an oral swab (Figure 2A) (Table 2).

Regarding salivary biomarkers, 7 (31.8%) studies assessed alpha-amylase, 4 (18.2%) measured cortisol, and 3 studies (13.6%) assessed both cortisol and alpha-amylase. In addition, 4 (18.2%) studies analyzed other molecules; 1 (4.5%) evaluated opiorphin; 1 (4.5%) quantified cytokines, chemokines, hormones, and neuropeptides; 1 (4.5%) evaluated 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and total antioxidant status (TAS); and 1 (4.5%) analyzed interleukin 1b (IL-1b), tumor necrosis factor (TNF), and matrix metalloproteinase-8 (MMP-8). Further, 1 (4.5%) study evaluated cortisol, alpha-amylase, C-reactive protein, IL-1b and interleukin 6 (IL-6); 1 (4.5%) analyzed cortisol, alpha-amylase, secretory immunoglobulin A (sIgA), testosterone, and TNF receptor (sTNFRII); 1 (4.5%) quantified cortisol and TNF; and 1 (4.5%) analyzed alpha-amylase and salivary chromogranin (sCgA) (Figure 2B; Table 2). Finally, 2 (9.1%) studies included blood sample measurements in addition to saliva sample measurements (Table 2).

Furthermore, regarding salivary biomarkers, 15 (68.2%) studies identified variations in the level of the salivary molecule evaluated and 4 (18.2%) studies found a positive correlation between the levels of salivary molecules and pain scores (Chart 1) .

Discussion

In total, 22 articles were included in the present review; overall, there was wide variability in terms of the research design followed, population studied, pain etiology investigated, pain assessment scores or tools used, biomarkers evaluated, and saliva collection methods employed.

Pain assessment is a multifactorial, complex, and challenging process[38]; despite numerous pain assessment tools involving self-reporting or observation methods, evaluating and defining biomarkers as potential objective measures for pain assessment and management are required. Therefore, salivary diagnosis is gaining attention because salivary glands are integrated into the neuroendocrine system and contain a wide variety of molecules that play important roles in pain pathophysiology.[39,40] Numerous plasma constituents enter saliva by passive diffusion, active transport, or extracellular ultrafiltration.[41] Consequently, most substances found in blood are also present in saliva; therefore, saliva is considered to be functionally equivalent to serum, reflecting the physiological state of the body.[13,25,42]

The studies evaluated indicated that saliva sample collection is easy and can be performed in a non-invasive and safe manner, rendering it advantageous compared with blood collection. Therefore, there are compelling reasons to investigate the potential of saliva as a diagnostic and prognostic approach in

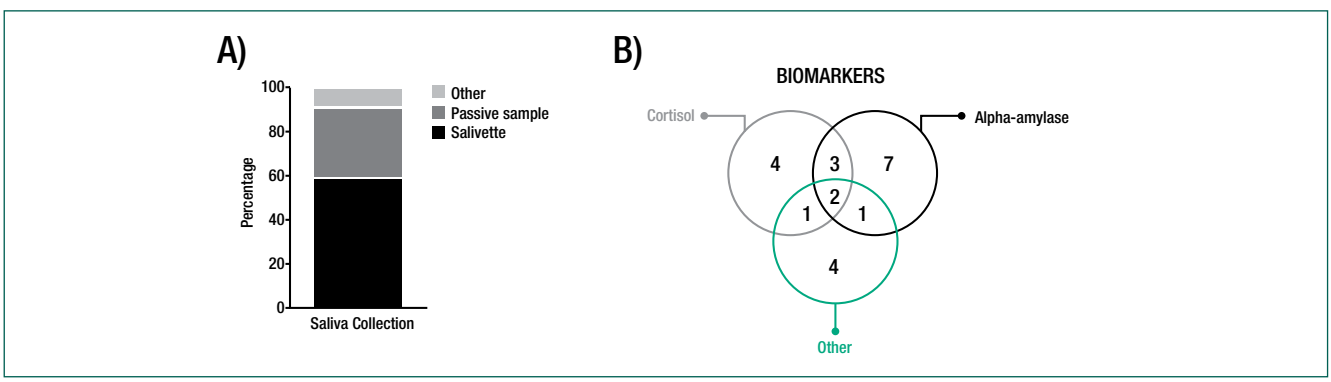

Figure 2. Description of the analyzed scientific production. (A) Saliva sample collection techniques; (B) Salivary biomarkers evaluated

Chart 1. Summary of data collection

Author/Year/ Country	Number of participants/ Age group/Study design/ Level of evidence	Objective	Salivary biomarkers/Pain assessment scale	Results/ Conclusions
Ozdogan et al., 2019 Turkey[10]	N = 39 Adults ≥ 18 years Cross-sectional Level of evidence VI	To determine the concentration of salivary opiorphin in dental pain.	Opiorphin Visual Analog Scale (VAS)	Salivary opiorphin levels increase in dental pain. A strong correlation was observed between the level of pain reported by the individual and salivary opiorphin levels. It was also observed that the extent of inflammation affects opiorphin levels.
Shaw et al., 2018 India[20]	N = 25 Newborns 28 weeks to 34 weeks and 6 days Uncontrolled clinical trial Level of evidence III	To assess cortisol levels before and after a session of motor physical therapy in newborns.	Cortisol Premature Infant Pain Profile (PIPP)	No difference in salivary cortisol was found after motor physical therapy. The PIPP score increased after motor physical therapy, but pharmacological intervention was not needed. Motor physical therapy was well tolerated and did not result in stress for the newborn.
Silva Andrade et al., 2018 Brazil[9]	N = 20 Adults Mean age 17 years Case-control, prospective Level of evidence IV	To assess the levels of stress-related salivary biomarkers in patients in orthodontic treatment with fixed brackets, comparing these patients with individuals with normal mastication.	Cortisol and alpha-amylase (sAA) Visual Analog Scale (VAS)	It was observed that orthodontic patients showed a significant increase in emotional stress, detected by alpha-amylase activity after the arch wire placement, when the patients reported the highest pain scores. Baseline salivary cortisol was not affected by the treatment and the use of isolated endocrine measures is not adequate to predict temporary pain in patients under orthodontic treatment. No stress-related biomarkers were correlated to the pain reports.
Jenkins et al., 2018 USA[21]	N = 73 Children 6 to 8 years Randomized clinical trial Level of evidence II	To assess strategies of emotional regulation and positive affection in children with cancer exposed to a painful experimental procedure (cold pressor task).	Alpha-amylase (sAA) Numeric classification scale	Specific strategies of emotional regulation, such as distraction and reassessment, can mitigate the response to stress and pain in pediatric patients with cancer and modulate salivary sAA levels. The study points out that reports of behavioral pain do not always correspond to the physiological response.
Yennurajalingam et al., 2018 USA[22]	N = 33 Adults Uncontrolled clinical trial Level of evidence III	To determine the viability and effectiveness of stimulation by cranial electrotherapy in depression, anxiety, sleep disorders, and pain scores in patients with late-stage cancer.	Cortisol, alpha-amylase (sAA), C-reactive protein (CRP), interleukin-1β (IL-1β), and interleukin-6 (IL-6) Brief Pain Inventory (BPI)	No significant changes were found in salivary levels of cortisol, alpha-amylase, CRP, IL-1β, and IL-6 after four weeks of stimulation by cranial electrotherapy. The use of stimulation by cranial electrotherapy is associated with improvements in depression, anxiety scores, and pain severity.
Kollmann et al., 2017 Austria[23]	N = 35 Pregnant women ≥ 18 years, with an indication for elective cesarean section, and ≥ 37 weeks of pregnancy Randomized clinical trial Level of evidence II	To investigate the impact of early skin-to-skin contact after a cesarean section on the adaptation of the newborn, on maternal pain, and on the response to stress.	Cortisol and alpha-amylase (sAA) Numeric rating scales (NRS)	Salivary levels of cortisol and alpha-amylase, well-being (reports of intraoperative nausea and vomiting), and maternal pain showed no difference between the groups with early and late skin-to-skin contact.
Sobas et al., 2016 Spain[24]	N = 34 Adults 30 to 40 years Observational Level of evidence VI	To assess the variability of potential biomarkers for pain assessment.	Cortisol, alpha-amylase (sAA), immunoglobulin A (sIgA), testosterone, and tumor necrosis factor alpha receptor II (sTNFαRII) Numeric rating scales (NRS)	It was observed that sIgA and sTNFαRII presented acceptable reproducibility levels in healthy individuals and can be used as potential salivary biomarkers to assess pain.
Wittwer et al., 2016 Switzerland[25]	N = 27 Adults Randomized clinical trial Level of evidence II	To investigate the effects of acute pain caused by heat on salivary alpha-amylase activity.	Alpha-amylase (sAA) Multidimensional Mood State Questionnaire (*Mehrdimensionale Befindlichkeitsfragebogen*, MDBF)	A positive correlation was observed between alpha-amylase levels and the intensity of pain in response to painful stimuli by heat. It is suggested that alpha-amylase is a physiological indicator of painful perception resulting from heat.
Kim et al., 2016 South Korea[26]	N = 137 Adults: 94 with rheumatoid arthritis, 43 healthy Case-control Level of evidence IV	To assess psychological stress and the activation of the stress system in patients with rheumatoid arthritis.	Cortisol and alpha-amylase (sAA) Visual Analog Scale (VAS)	Cortisol levels were higher in patients with rheumatoid arthritis. No difference was found in alpha-amylase levels. The results suggest that depression is more prevalent in patients with rheumatoid arthritis in comparison with the control group. This may be related to the subjective symptoms of pain, because a positive correlation was observed between scores in the Beck Depression Inventory (BDI) and in the VAS for pain.
Symons et al., 2015 USA[8]	N = 10 Nonverbal children with cerebral palsy (mean age 9.2 years) Observational, prospective Level of evidence VI	To assess the viability of magnetic resonance and immunoassays to identify and compare relevant salivary biomarkers in pediatric patients with cerebral palsy, with and without pain.	Cytokines, chemokines, hormones, and neuropeptides. Dalhousie Pain Interview (DPI)	It was observed that the levels of most salivary metabolites, neuropeptides, cytokines, and hormones were higher in children with cerebral palsy with pain (based on the parents' previous report) versus without pain.
Generaal et al., 2014 The Netherlands[27]	N = 1125 Adults Cross-sectional Level of evidence VI	To assess whether hypothalamic-pituitary-adrenal axis dysfunction is associated to the presence and severity of chronic musculoskeletal pain.	Cortisol Chronic pain grade (CPG)	Low blood cortisol levels were observed in chronic musculoskeletal pain. The authors concluded that when chronic pain occurs together with a depressive or anxiety disorder, cortisol levels are masked.
Brown et al., 2014 Australia[7]	N = 77 Children, 4 to 13 years Longitudinal, prospective Level of evidence VI	To establish whether salivary cortisol and alpha-amylase are sensitive to the detection of stress during treatment procedures for burn wounds.	Cortisol and alpha-amylase (sAA) Revised Faces Pain Scale (FPS-R); Visual Analog Scale-Anxiety (VAS-A); Face, Legs, Arms, Cry, Consolability (FLACC) scale; Child Trauma Screening Questionnaire (CTSQ)	Cortisol and alpha-amylase respond to stress from the procedures for treating burn wounds. Alpha-amylase levels were associated to pain and to high CTSQ scores. The authors indicate that cortisol and alpha-amylase can be used to assess stress and pain during the placement of dressings in the treatment of burns.

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Continuation.

Author/Year/ Country	Number of participants/ Age group/Study design/ Level of evidence	Objective	Salivary biomarkers/Pain assessment scale	Results/ Conclusions
Cabral et al., 2013 Brazil[28]	N = 55 Newborns, 30 weeks to 39 weeks and 5 days Observational, prospective Level of evidence VI	To assess the response of newborns to stress during hospitalization in a neonatal intensive care unit	Cortisol Neonatal Facial Coding System (NFCS)	It is believed that the salivary concentration of cortisol is an indicator of neonatal stress, due to the adrenal response to stress during the first days of hospitalization. During the analysis of facial activity to assess acute pain, none of the children presented signs of pain, but this does not mean that the children were not under stress.
Shibata et al., 2013 Japan[29]	N = 47 Newborns, 36 weeks and 7 days to 41 weeks and 3 days	To determine whether salivary biomarkers can be objective indicators of pain in newborns.	Salivary chromogranin (sCgA) and salivary alpha-amylase (sAA) Neonatal Infant Pain Scale (NIPS)	Significantly increased NIPS scores are reported after a painful procedure. Despite the changes in salivary biomarkers (sCgA or sAA) before and after a heel puncture, a large inter- and intra-subject variability was observed. The authors concluded that these biological indicators are not adequate to assess pain in newborns.
Ferrara et al., 2013 Italy[30]	N = 23 Children, 12 with epilepsy and 11 controls (4 to 15 years) Case-control Level of evidence IV	To assess the perception of pain in epileptic children during an invasive procedure (a puncture to collect venous blood) by determining salivary alpha-amylase activity and comparing it to the activity in healthy children.	Alpha-amylase (sAA) Wong–Baker Faces Pain Rating Scale; Pediatric Pain Profile (PPP)	It was observed that children with epilepsy present higher levels of alpha-amylase and greater sensitivity to pain when compared to the control group. A correlation was observed between sAA activity and PPP. The authors indicated that alpha-amylase activity may represent a new, objective, noninvasive biomarker to assess the perception of pain in children with epilepsy.
Robles et al., 2012 USA[31]	N = 76 Adults 18 to 40 years Prospective cohort Level of evidence IV	To evaluate the clinical use of salivary alpha-amylase in assessing responses to the stress related to elective third molar tooth extraction.	Alpha-amylase (sAA) Pain Catastrophizing Scale (PCS)	It was observed that sAA levels were lower during the surgery and postoperative follow-up when compared to the levels measured before surgery. Although sAA did not present the increase expected by the authors, a relationship was observed between the response to pain and increased sAA levels. It was also observed that PCS scores were not significantly correlated to pain.
Goodin et al., 2012 USA[32]	N = 24 Adults 18 to 45 years Randomized clinical trial Level of evidence II	To assess whether hypnosis directly influences the hypothalamic-pituitary-adrenal axis and the proinflammatory reactivity to experimental acute pain (cold pressor task)	Cortisol and tumor necrosis factor alpha (TNFα) Pain Intensity (PI) and Pain Unpleasantness (PU) numeric scales	Hypnosis was associated to a reduction in the pain scores when compared to the control group. However, it was not associated to significant changes in cortisol and TNFα levels.
Campos et al., 2011 Brazil[33]	N = 20 Adolescents and young adults, with a mean age of 18.5 years Cross-sectional Level of evidence VI	To assess the correlation between salivary alpha-amylase levels and the intensity of the pain reported by the patients during an orthodontic treatment of bracket bonding and arch wire insertion.	Alpha-amylase (sAA) Visual Analog Scale (VAS)	No correlation was found between sAA concentrations and pain intensity in patients undergoing orthodontic treatment. However, the patients did present a significant, progressive increase of sAA levels during the evaluation period of 21 days, divided into three phases: pretreatment (days 1 to 7), bonding (days 8 to 14), and initial arch wire insertion (days 15 to 21).
Kiefte-de Jong et al., 2011 The Netherlands[34]	N = 483 Children 14 to 24 months Prospective cohort Level of evidence IV	To assess whether the cortisol circadian rhythm and reactivity to stress are associated to functional constipation and abdominal pain in infancy.	Cortisol Abdominal Pain Index	No difference was found in the cortisol circadian rhythm between children with and without constipation and abdominal pain.
Rodríguez de Sotillo et al., 2011 USA[35]	N = 30 Adults Case-control Level of evidence IV	To determine whether oxidative stress biomarkers measured in the saliva and serum in patients with temporomandibular disorders (TMD) may be associated to increased pain when compared to a healthy control group.	8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and total antioxidant status (TAS) Pain Intensity (PI) score	Salivary levels of 8-OHdG, MDA, and TAS were observed to be both changed and correlated to the respective serum levels in patients with TMD when compared to the control patients. Additionally, these salivary biomarkers were diagnostic predictors of pain severity. A significant association was observed between pain and salivary oxidative biomarkers in patients with TMD.
Mirrielees et al., 2010 USA[36]	N = 105 Adults ≥ 18 years Controlled cross-sectional Level of evidence VI	To test the hypothesis that rheumatoid arthritis influences salivary biomarker levels in periodontal disease.	Interleukin-1β (IL-1β), tumor necrosis factor alpha (TNFα), and metalloproteinase 8 (MMP8) Visual Analog Scale (VAS)	Salivary IL-1β and TNFα levels were found to be significantly higher in patients with rheumatoid arthritis who were not treated with anti-TNFα antibodies when compared to patients treated with anti-TNFα and to healthy controls. It was concluded that salivary levels of IL-1β, MMP8, and TNFα are clearly influenced by the periodontal environment and by systemic inflammatory conditions such as rheumatoid arthritis.
Buğdaycı et al., 2010 Turkey[37]	N = 110 Adults 50 patients with migraine 60 controls Case-control Level of evidence IV	To investigate salivary alpha-amylase levels as a noninvasive tool to assess sympathetic nervous system activity in patients with migraine during attack, post-attack, and headache interval periods.	Alpha-amylase (sAA) Visual Analog Scale (VAS)	Changes in sAA levels were observed in different periods of migraine. No significant differences in sAA levels were found between the interval periods and the control group. It was observed that VAS scores were not correlated to sAA values obtained during migraine attack periods.

pain-related research[24,40] and in diagnosis of various diseases[43,44] in different age groups.[39]

Another point that ought to be highlighted refers to saliva sample collection, considering that the method may vary according to age group. In adult patients, the collection technique is well established, with a predominant use of Salivette®. Salivette® comprises a piece of synthetic cotton packaged in a plastic tube. In this method, individuals are instructed to place the piece of cotton under the tongue or chew it to stimulate salivary flow for 2–3 min. Thereafter, the individual removes the piece of cot-

ton from the mouth and returns it to the Salivette® tube. However, there is no consensus in the literature regarding the most appropriate method for saliva sample saliva sample collection in the pediatric population, particularly in the neonatal population.

There was differences observed in the results from the studies included herein regarding correlations and associations between dosages of salivary molecules and pain assessment tools. For instance, a correlation was observed between reported pain and salivary molecules in studies conducted with children and adults in which alpha-amylase and opiorphin levels were evaluated.[7,10,25,30]

Moreover, a significant association between pain and oxidative biomarkers was identified.[35] Conversely, in the other studies, no correlations were noted between the evaluated salivary molecules and pain scores.[9]

In the included studies, there was a notable use of cortisol and alpha-amylase for pain assessment. Cortisol is the most widely used hormone in pain-related research.[45] Alpha-amylase is one of the most important enzymes present in saliva.[46] It increases under stressful conditions and induces the production of catecholamines, reflecting sympathetic activity.[47] This renders alpha-amylase a potential objective biomarker and its measurement a noninvasive method for pain assessment.[30] The results of the articles included in this review confirmed that there was an increase in the levels of most salivary metabolites, neuropeptides, cytokines, and hormones in response to potentially painful stimuli.

Since 2013, alpha-amylase has been studied for pain assessment in children with epilepsy and is being used as an objective, noninvasive biomarker.[30] In 2018, a study evaluated alpha-amylase level as a biomarker for pain assessment in patients with pediatric cancer—a population exposed to numerous painful procedures.[21] In addition, it is suggested that alpha-amylase is a physiological indicator of the subjective perception of pain from heat.[25] Moreover, alpha-amylase can be used for pain assessment during the placement of dressings.[7]

With regard to the selected articles that evaluated salivary cortisol, an increase in cortisol levels in neonates can be observed as a result of the psychological and physical response to different stimuli.[28] The linear correlation of salivary cortisol levels with its plasma and urinary levels[11] was used for pain assessment in patients with rheumatoid arthritis in a study that observed an increase in cortisol correlated with joint pain intensity.[26] Remarkably, although there was a linear relationship between saliva, blood, and urine levels, the quantification of potential salivary biomarkers may be different.

Conversely, in some studies no correlation was observed among cortisol and alpha-amylase levels, pain intensity, and pain assessment scores in response to a painful procedure or disease.[9] A study conducted in 2013 with newborns showed that painful stimuli did not promote significant changes in the salivary levels of chromogranin and alpha-amylase in newborns. In addition, a large inter- and intrasubject variability was identified in the salivary levels of these biological indicators, which can be considered a hindrance to the use of these salivary molecules for neonatal pain assessment.[29]

In this more vulnerable population, in which exposure to painful procedures is inevitable and pain assessment depends almost exclusively on the observation of behavioral, physiological, and contextual aspects, the establishment of standardized saliva sample collection techniques and the dosage of salivary biomarkers could provide a better understanding of pain and facilitate decision making regarding therapy.[8] It must be emphasized that salivary molecules have the potential to integrate the multidimensional pain assessment in newborns as well as to be investigated for the prognosis and diagnosis of different pain-related pathophysiological processes in different age groups.[13.43]

The introduction of such noninvasive type of method for pain assessment and management can contribute to clinical practice, particularly for the nursing team.[48,49] The development and establishment of consistent and practical techniques and devices for bedside analysis is necessary; in addition, the development of protocols to standardize the assessment of different salivary molecules whose levels vary throughout the day and in response to different situations is important.

Regarding LEs of the included studies, descriptive or qualitative studies (8 articles) classified as Level VI (weak) were predominant, followed by well-designed cohort or case-control studies (7 articles) classified as Level IV (moderate); randomized controlled clinical trial studies (5 articles) classified as Level II (strong) were scarce.[19] These findings emphasize the need for additional studies, particularly well-designed randomized controlled clinical trials with representative samples that can better elucidate the relationship between biomarkers and pain assessment in patients undergoing painful procedures or experiencing painful diseases. Additional studies may encourage new reviews of the scientific literature, including systematic reviews.

Regarding the limitations in the design and development of the present study, it must be highlighted that the variability of study designs restricted the investigation and comparison of the identified results. Similarly, the variability of painful situations and pain assessment tools prevented a further in-depth comparative analysis of the results included in this review.

There was no consensus in the literature regarding the use of salivary biomarkers for pain assessment and the feasibility of this bedside research tool. Nevertheless, it was evident that the present study contributed to the understanding of pain assessment by identifying the main salivary molecules evaluated and the techniques used for saliva sample collection. Therefore, future research to investigate the association between pain and salivary biomarkers may contribute toward further elucidating the characteristics of painful conditions to support decision making in the clinical management of pain.

Conclusion

The studies described in this review indicated that the objective measurement of pain remains a challenge and that the dosage of salivary molecules is emerging for use as a complementary tool in pain assessment in individuals of different age groups undergoing painful procedures or experiencing painful diseases. The main salivary biomarkers evaluated were cortisol and alpha-amylase; Salivette® was the chief technique used for saliva sample collection.

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Rocha VA, Freitas P, Silva IA, and Bueno M collaborated with the project design, data analysis, and interpretation; article writing; a relevant critical review of the content; and approval of the final version to be published.

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