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Literature Review

Salivary diagnosis of oral cancers by salivary samples: a systematic literature review

Marine Guillon[1], Nathalie Pham Dang[2,3], Joannice Thévenon[1], Laurent Devoize[1,3,*]

[1] CHU Clermont-Ferrand, Service d'Odontologie, 63001 Clermont-Ferrand, France
[2] CHU de Clermont-Ferrand, Service de Chirurgie Maxillo-Faciale, 63001 Clermont-Ferrand, France
[3] Université Clermont Auvergne, Inserm, Neuro-Dol, 63000 Clermont-Ferrand, France

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Abstract – The aim of this article is to carry out a systematic analysis of the literature concerning the diagnosis of oral cancers by salivary samples. Different biomarkers, produced by the tumor itself or by its environment, show significant variations in their concentration at the salivary level, correlated or not at the blood or tissue level. After studying 239 articles, we included 36 in this analysis. This allowed us to extract 46 potential molecules for salivary diagnostics. Only 26 demonstrated a good level of evidence. 8 clusters have also been proposed for better specificity and sensitivity. To date, the protocols of the studies carried out do not allow to affirm that one or more biomarkers are effective for a salivary diagnosis of oral cancers. Part of the results contradict or sometimes lack precision. In addition, the studies included here do not have a good methodological quality and do not always take into account other factors influencing the concentrations (systemic diseases, age, sex, tobacco, alcohol, *etc.*). Additional studies are still necessary, notably with larger groups of patients, representative of the general population and standardization of the protocols for the study and quantification of biomarkers.

Introduction

Oral Squamous Cell Carcinoma (OSCC) is one of the most common cancers with 354,864 new cases diagnosed in 2018, with an incidence of 2.3 and 5.8 cases per 100,000 people in women and men, respectively. It represents 3% of cancers in men, and 2% in women.

Tobacco, alcohol, poor diets, persistent Human Papillomavirus (HPV) infections or the chewing of certain types of nuts (betel nut or areca nut, specific to certain regions of the world) are identified factors risks. Men, aged 50–60, are mainly affected.

The synergistic effect of the various risk factors leads to modifications of the oral mucosa but also along the upper air and digestive tracts. Before malignant transformation, several histological and clinical alterations are identified: leukoplakia, erythro-leukoplakia hyperkeratosis, dysplasia, then carcinoma.

The average overall survival rate at 5 years remains one of the lowest of all cancers (50%) despite the progress made in surgery, radiotherapy or chemotherapy. The chances of survival decrease with the advancement of the stage of the tumor and according to its site.

It has been shown that appropriate and early management of pre-cancerous and cancerous lesions gives better results and a 75–90% chance of survival, as well as an increase in the quality of life with less invasive and extended treatments.

The current gold standard for the diagnosis of oral lesions remains the biopsy. It is nonetheless an invasive, technical, relatively expensive method, which is often traumatic for the patient. Faced with this, diagnostic techniques that can be reliable, precise, inexpensive and non-invasive must be evaluated.

Saliva is about to offer another alternative. It is a complex and dynamic biological fluid, composed of various secretions: salivary glands, oral mucosa sweating, gingival sulcus, compounds derived from blood and other substances from the gastrointestinal and respiratory systems. It is specific to each individual but is also used, thanks to modern technologies, to identify specific markers for certain bacterial, viral or

* Correspondence: laurent.devoize@uca.fr

systemic diseases, drugs, hormones, pollutants, *etc*. It is less invasive to obtain than a blood or tissue sample, easy and quick to collect, transport, store and treat (little training time required, stable when stored at −80 °C). In addition, the saliva is closest to any tumors.

According to an increasing number of studies [1–3], it is an excellent means of first-line diagnosis, already developed in many other fields (hormonal assays, Human Immunodeficiency Virus (HIV),...). Already more than 100 molecules have been identified as potential biomarkers, including DNA, RNA, proteins or metabolites. Various methods have enabled these results, such as the Enzyme-Linked Immunosorbent Assay (ELISA), polymerase chain reaction (RT-PCR), DNA chips or mass spectrometry by liquid chromatography.

An ideal biomarker, alone or in combination, would be an easy-to-dose molecule, specific for the tumor or having a different concentration compared to the healthy population. It must be stable over time and in the sample analyzed, which can differentiate between pre-cancerous and cancerous lesions. It would give a reliable indication as to the diagnosis, the response to treatment and the patient's survival potential, and therefore to his overall prognosis, during and after follow-up.

This is why researching specific biomarkers for oral cancers, applicable to the general population, for their early detection by saliva samples would present a good alternative to standard biopsies. The purpose of this study is therefore to take stock of knowledge and advances in salivary diagnosis of oral cancer (Tab. I).

Table I. Summary of characteristics of included studies.

Characteristics of studies	N studies
Year of publication	
2014	5
2015	13
2016	6
2017	6
2018	4
2019	2
Type of study	
Case control	30
Transverse	5
Cohort study	1
Concentrations	
Salivary	36
Blood	11
Tissue	3
Population	
Healthy	36
Having other types of benign lesions	1
Having a pre-cancerous lesion	16
Having a cancerous lesion	36
Mean of patients by studies ± SD	
Healthy pop	35 ± 21
Having other types of benign lesions pop	20 ± –
Having a pre-cancerous lesion pop	41 ± 39
Having a cancerous lesion pop	43 ± 26

Summary of articles by population: SD = Standard Deviation.

Material and methods

Search strategy, selection of studies and inclusion criteria

We conducted this systematic review of the literature using the Preferred Reporting Items for Systematics Review and Meta-Analyzes (PRISMA) method. An electronic search was carried out via the PubMed, Cochrane Library and Science Direct databases in May 2019, specific biomarkers for oral cancer (OSCC) found by salivary samples.

Our keywords "Medical Subject Heading" (MeSH) have been determined beforehand; we used the terms "Diagnosis AND Biomarker AND Saliva".

Two observers first determined the potential for inclusion of each article, by reading the titles and abstracts. Subsequently, the articles were fully read by an examiner, to include or not include the first selection, as well as their data [4–39].

The exclusion criteria are: literature reviews, editorials, articles not in French or English and dating back more than 5 years, articles dealing exclusively with exosomes, the Human Papilloma Virus (HPV), produced *in vitro* condition, concerning a target population or not including clear salivary measures and/or measures in patients with cancer of the oral cavity and finally articles extended to cancers of the head and neck.

The articles included relate to various types of studies, including a population with oral cancer and a control/control population, or even one or more additional populations with various lesions (pre-cancerous or others such as apthous lesions). Salivary concentrations of biomarkers are provided, whether or not accompanied by blood or tissue concentrations.

These included articles bring together prospective studies of different types: case-control, cross-sectional or cohort study.

The included studies could also present the blood or tissue concentrations of the selected biomarkers. We have reported this data in our study table; they were compared to saliva concentrations in an identical manner, in order to identify possible links between saliva, blood and tumor.

Data processing

The data processing was carried out by a single examiner, using a standard data extraction model. A second reviewer could also be consulted if an article warranted discussion.

The data collected were:
- Characteristics of the population studied (number of patients by group, sex, type of lesion (cancerous, pre-cancerous, others).
- The characteristics of biomarkers (the type and function of the biomarker, the method of analysis, their role in diagnosis).
- The salivary and/or blood and/or tissue concentrations (and sensitivity (Se) and specificity (Sp) if provided).
- Any other conclusions of the study (other associated factors, *etc.*).

Risk of bias assessment

The PEDro score (the Physiotherapy Evidence Database) was calculated for each article included, to assess its level of relevance, compared to its methodological quality [40].

All of these results give an indication of the reliability and level of scientific evidence of our study.

This scale, taken from an evidence-based physiotherapy database, groups 11 items to analyze:
- Item 1: external validity (relating to its "applicability").
- Items 2–9: internal validity (randomization, blind procedure, quality of monitoring and analysis of all patients).
- Items 10 and 11: the amount of statistical information provided.

One point is awarded for an affirmative answer to each of the 11 internal validity criteria, except for item 1 (not counted). The overall score varies between 0 and 10. The cut-off values are: 9–10 = excellent/6–8 = good/4–5 = fair/<4 = poor. This scale does not, however, provide information as to the validity of the article's conclusions.

Definition of groups

We determined four groups, including variable subgroups according to the studies:
- Control population: healthy subjects, without particular pathology, inclusion/exclusion criteria variable according to the studies.
- Pre-cancer population: non-systematic group, lesions identified and separated into sub-groups or not depending on the studies.
- Cancer population: global or separated into subgroups according to the stage of the cancer, histological type of the lesion, presence of metastases or not.
- Other population: only one study is concerned, including benign lesions such as canker sores.

Results

Selection and characteristics of studies

The selection of studies is described in the flow diagram (Fig. 1). Our research selected the studies carried out between 2014 and 2019 (Tab. I). A total of 239 articles were listed, then

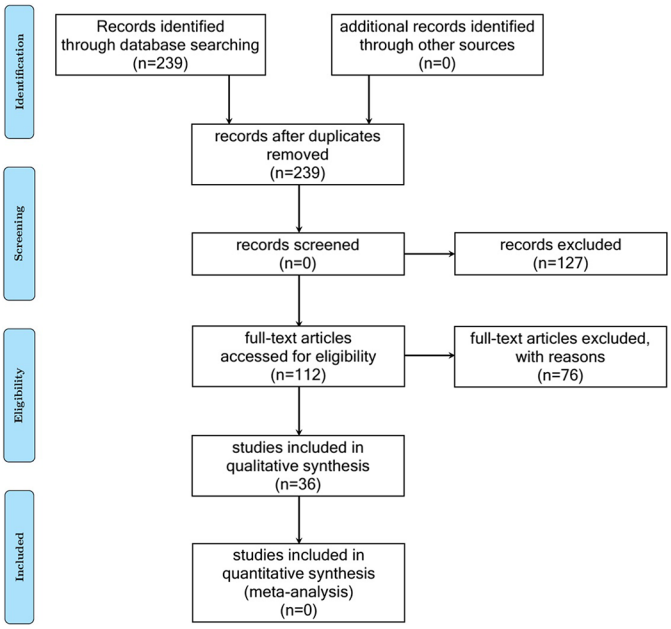

Fig. 1. Flow chart, Preferred Reporting Items for Systematics Review and Meta-Analyzes (PRISMA) method.

36 articles were included in the protocol (30 case-control studies, 5 cross-sectional studies, 1 cohort study). All studies are prospective.

Excluded items could be excluded for one or more criteria

Among the 36 articles included, all provided saliva concentrations, while 11 articles also provided blood concentrations and 3 articles tissue concentrations, obtained from biopsies. All samples were taken before any surgery, chemotherapy or radiotherapy.

The average number of patients per study with oral cancer is 43 ± 26 patients, without cancer is 35 ± 21 healthy patients and 41 ± 39 patients with a pre-cancerous lesion (20 articles concerned). An article also included 20 patients with benign lesions (apthous lesions).

The majority of patients included are men.

Identification of biomarkers

After studying these 36 articles, we were able to identify 46 molecules proposed for salivary diagnosis of oral cancers (Tab. II). These are biomarkers produced by the tumor itself or by its environment, presenting significant variations in their concentrations at the salivary level.

We have classified them according to their nature: tumor markers, metabolic markers (including ions, antioxidants, micronutrients), belonging to the proteome and belonging to the transcriptome. Their role in the cancer process can result in different actions: cell proliferation, limitation of apoptosis, altered cell metabolism or even increased vascularity.

Table II. Summary of published concentrations by biomarkers and by population groups.

Biomarkers	N studies	Δ salivary concentration pop cancerous	Δ salivary concentration pop pré-cancerous	Variation of blood concentration	Variation of tissue concentration
Tumor markers					
CEA (Carcinoembryonic antigen)	1	↑*	–	–	–
Cyfra 21–1 (Cytokeratin fraction 21–1)	3	↑*	↑	↑*	=
Metabolic markers					
Zinc	2	↓*A/B	↓*A/B	↓*A	–
Copper	1	↑*B	↑*B	–	–
Iron	1	↓	↓	–	–
GSH (Gluthatione)	2	↓*A	↓*A	↓*A	–
SOD (Superoxyde dismutase)	2	=	–	↓	–
LDH (Lactate dehydrogenase)	3	↑	↑	–	–
SialicAcid (N-acetyl neuraminic acid)	4	↑*	↑*	↑*	–
8-OHdG (8-hydroxy-2-deoxyguanosine)	1	↑*C	↑*C	–	–
MDA (malondialdehyde)	1	↑*C	↑*C	–	–
Vitamin C (acid ascorbic)	2	↓*C	↓*C	↓*	–
Vitamin E	1	↓*C	↓*C	–	–
Proteome					
LDOC 1	1	=	–	–	–
Glycine	1	↓*	↓	–	–
Proline	1	↓*	↓	–	–
CA19-9	1	=	–	–	–
Amylase	1	↓	↓	–	–
S100A8	1	↑*	–	–	↑*
IL 4	1	↑^{D}	–	–	–
IL 6	2	↑*	↑	=	–
IL 8	2	↑*	↑*	↑*	–
IL 10	1	↑^{D}	–	–	↑
IL 13	1	↑^{D}	–	↑	–
CD 44	1	↑	–	↑	–
CD44v6	1	↑*E	↑	=	–
CD44v10	1	↑*E	↑	–	–
RETN (Resistin)	1	↑	–	–	–
CCNA1	1	↑^{F}	–	–	–
DAPK	1	↑^{F}	–	–	–
DCC	1	↑^{F}	–	–	–
TIMP 3	1	↑^{F}	–	–	–
EGFR (Epidermal growth factor receptor)	1	↑*	–	↓*	–
AKR1B10 (Aldo-keto reductase family 1 member B10)	1	↑*	–	–	–
MMP 9	1	↑*	–	↓	–
SYNE 1	1	↓^{E}	–	–	–
Global proteome	2	↑ou↓	–	–	–
Transcriptome					
miR-139-5p	1	↓*	–	–	–
miARN 145	1	↓*	↓*	–	–
miARN 184	1	↑*	↑*	–	–
miR34a	1	↓*E	↓	=	–
OAZ mARN	1	↑^{G}	↑	–	–
IL8 mARN	1	↑*G/H	↑	–	–
IL1B mARN	1	↑*G/H	↑	–	–
SAT mARN	1	↑*	–	↑	↑
Transgelin mARN	1	↑^{G}	↑	–	–
IL 1-RA	1	↑^{D}	–	–	–

Δ = variation; ↑ = increase of concentration; D = decrease of concentration; without asterisk = $p > 0.05$; with asterisk = $p < 0.05$; = : no significant variation; $^{A\ à\ H}$ = Proposed biomarkers combinations (^{A}: Zinc- GSH/^{B}: Zinc – Copper/ ^{C}: 8-OHdG- MDA- Vitamin C – Vitamin E/^{D}: IL4 – IL10 – IL13 – IL1RA/^{E}: CD44v – SYNE1 – miR34a/^{F}: CCNA1 – DAPK – DCC – TIMP3/^{G}: OAZ mRNA – SAT mRNA – IL8 mRNA – IL1B mRNA/^{H}: SAT – IL8).

Two articles have studied the variations of the proteome in its entirety, without differentiating one or more molecules.

An article, studying micro-RNA 145 and 184 (=miRNA 145 and miRNA 184), included an additional control group of 20 patients with benign lesions (aphtous lesions). It is not shown in the table because the concentrations are the same as for the group of healthy patients.

26 biomarkers demonstrated a good level of evidence for these diagnoses with a p-value <0.05 (with an asterisk in Tab. II), while the others showed insignificant variations in concentration when isolated.

Most of the selected articles study variations in the concentration of a specific molecule. A small number of them study several biomarkers, or even propose to use groups of biomarkers: clusters. Indeed, according to the results, a certain number of biomarkers are significant only when combined in a cluster. This is the case for 11 of them.

Several clusters have therefore been proposed, with better levels of evidence (higher Sensitivity (Se) and Specificity (Sp)) than the molecules alone (Tab. II):
- [A]: Zn — GSH.
- [B]: Zn — Cu.
- [C]: 8-OHdG — MDA — Vitamin C — Vitamin E.
- [D]: IL4 — IL10 — IL13 — IL1RA.
- [E]: CD44v — SYNE1 — miR34a.
- [F]: CCNA1 — DAPK — DCC — TIMP3.
- [G]: OAZ mRNA — SAT mRNA — IL8 mRNA — IL1B mRNA
- [H]: SAT — IL8.

However, despite good Sensitivity (Se) and Specificity (Sp), each cluster is only offered in one of the articles included. Their validity remains questionable to this day, these remain proposals of the authors.

A single study studied the concentrations of miR-139-5p before and after intervention (surgical, radiotherapy, chemotherapy): it demonstrated the return to normal of these rates, 4–6 weeks after treatment for the majority of patients.

An article dealt with the LDOC1 molecule, in order to determine if it could detect cancerous lesions. It was concluded that it was not significant for the detection of cancer, but that it was specific to the sex of the patient. In men, it decreases during oral cancer while in women, it increases.

With an article number >2 and a p-value <0.05, the following molecules present the best levels of evidence compared to the 46 isolated molecules: Cyfra 21-1, Zinc, Gluthatione, Sialic Acid, Vitamin C, IL6 and IL8. Most of them are mentioned in the cluster proposals.

Following their data, the authors proposed, for each biomarker, a role (s) in the management of oral cancer. Several have been mentioned: detection, diagnosis, differentiation of pre-cancerous/cancerous lesions, surveillance and monitoring, evaluation of the response to treatment, prognosis. For example, the following biomarkers have been recognized as significant in the differentiation of pre-cancerous and cancerous lesions: Copper (Cu), LDH, sialic acid, IL8, micro-RNA 184 (=miRNA 184).

The results being too inhomogeneous (units of measurement, protocol analysis, quantification method), we were unable to perform a meta-analysis (Tab. III).

Risk of bias assessment and data interpretation

Concerning the quality of the included studies, several limits have to be taken into account. The number of patients included is generally limited and varies from one study to another. Larger samples of the population are needed to homogenize the results.

The characteristics of the population samples studied are not systematically similar from one group to the other (case/control groups) due to the prevalence of cancer: age, sex, *etc*. Sometimes it is difficult to include patients with the same characteristics on reduced patient samples. In addition, men, aged 50–60, are more affected by oral cancers. The results are therefore not always applicable to the greatest number.

We assessed the methodological quality (see Tab. III), and therefore the credibility, of the studies collected using the PEDro scale: the Physiotherapy Evidence Database.

Here, the inclusion or exclusion criteria as well as the conditions for carrying out the study vary from one article to another and are more or less described. Most studies are not randomized or double blind for the full procedure. For example, only 6 studies have evaluated certain parameters “blind” such as the diagnosis of lesions by two independent assessors. The PEDro (Physiotherapy Evidence Database) scores are affected (Tab. III).

After analyzing the articles, we count:
- 2 articles with a score of 3/10 (poor).
- 11 articles with a score of 4/10 (fair).
- 19 articles with a score of 5/10 (fair).
- 4 articles with a score of 6/10 (good).

The average of the scores being 4.69/10, we conclude that the level of evidence in our study is considered to be fair.

We therefore chose not to limit the values included in our study to those considered significant with $p < 0.05$ associated with a PEDro score >6 (good level), since few articles present a sufficient level of evidence to answer to these criteria (Tab. III). Few data would then have been usable.

Discussion

We therefore included in this systematic review of the literature 36 articles to determine which biomarkers could be used for the diagnosis of oral cancer, by salivary sampling.

Salivary, blood and even tissue concentrations were compared.

Several molecules appear promising, according to a statistical significance ($p < 0.05$) reported by several articles, such as zinc, sialic acid or vitamin C. The data, both in terms of concentrations and protocols analyzes remain very heterogeneous, their comparison is not possible. To date, they cannot therefore be used alone for the detection of oral tumors.

Saliva remains very promising for future diagnoses of oral cancer and deserves more research. It is also called "liquid biopsy" (although this term can also be used for blood samples) and is used for other cancer diagnoses (lungs, gastrointestinal, pancreas, *etc.*).

It meets the criteria mentioned above, that is to say: less invasive to obtain than a blood or tissue sample, easy and quick to collect, transport, store and treat. It is a good substitute for blood tissue by the quality of its components. In addition, the cancer process involves many mechanisms. This therefore offers a wide range of potential biomarkers. They therefore have every interest in being used in a cluster rather than alone.

Saliva would then allow earlier diagnoses of oral cancers, thus offering better chances of survival (75–90%) and an increase in the quality of life of patients.

The level of evidence for this study remains fair, given the PEDro scores of the articles included in particular. If we had limited ourselves to data with $p < 0.05$ and a PEDro score > 6, few articles could have been included.

However, to date, according to our research, there is still no protocol for conducting a randomized, double-blind study for salivary diagnoses of oral cancers. Patients cannot be randomly exposed to cancer. The evaluation of the articles using the CONSORT directives was made, but the results would have been the same or even worse. We therefore made the choice to use the PEDro score in order to adapt to the quality of the selected bibliography.

In view of these low levels of scientific evidence and the very limited number of studies, the functions (detection, prognosis, *etc.*) possibly assigned to biomarkers remain questionable. In addition, according to the previous results, the molecules cannot be used alone for the diagnosis of oral cancers because they do not have sufficient sensitivity and specificity. It is necessary to create clusters of biomarkers, applicable to the general population.

On the other hand, specific populations are sometimes studied. For example, several studies have been carried out in India or Taiwan. These are populations particularly affected by oral cancers, with a higher prevalence than in the rest of the world. They have a different diet with the consumption of betel nut or areca nut in India for example, known to be a risk factor for oral cancer. They also have specific physiological and genetic characteristics such as greater susceptibility to periodontitis for example, which can modify oral physiological parameters.

Another study was conducted on a population with poor oral hygiene [19]. The oral bacterial flora is changed, which can affect their chance of developing oral cancer.

Another factor that can influence the measurements is the heterogeneity of the types of clinical and methodological analyzes of biomarkers. There are many different quantification methods: Quantification by Polymerase Chain Reaction (qRT-PCR), Enzyme-Linked Immunosorbent Assay (ELISA), electrochemiluminescence method (ECLIA), chromatography… Their description is more or less described and precise.

As explained above, studies carried out on larger groups of patients and more representative of the general population are necessary. Standardization of protocols and quantification techniques would be strongly recommended. In addition, some of the studies do not present its tables, its statistical analyzes or even its concentration measurements. More encrypted data would be desirable.

Each study having its own protocol and research objective (differentiation of pre-cancerous/cancerous lesions, tumor detection, prognosis, monitoring, *etc.*), the assignment of one or more roles to biomarkers is not always of one sufficient level of evidence.

It depends on the orientation and therefore on the authors' interpretations of the results. This may still help guide future studies.

It is also known that other parameters influence salivary concentrations such as certain systemic diseases (lupus, human papilloma virus (HPV), *etc.*), periodontitis, oral hygiene, age, sex, *etc*. These parameters are not systematically taken into account in the articles.

After study, it is systematically demonstrated that potential biomarkers show better results (Se and Sp) when they are combined in a cluster. One or more clusters are to be defined so that they are applicable to the general population.

In the discussions of the articles, there are regularly results in contradiction with other previous studies, such as for example the two dealing with superoyxde dismutase (SOD) [9,14]: Its concentration decreases not significantly in one of the studies, while the other shows that it increases in a pathological situation.

Within these two studies, the discussions admit contradiction with other previous studies (not included here).

The conclusion of each article included, just like ours, is much the same: more research is needed, with progress both in terms of protocols and patients included. We are only at the beginning of research in the field of oral cancer diagnoses by salivary sampling.

Conclusion

After studying these 36 articles and 46 molecules, we were unable to identify any biomarker applicable to date for the diagnosis of oral cancer by salivary samples. Studies with better methodologies, sampling and quantification protocols and population samples (number and characteristics) are necessary. In addition, other parameters influencing the concentration of biomarkers such as systemic diseases, tobacco, age and sex of patients, *etc*. must be taken into account during analyzes. Saliva remains a major asset in the diagnosis and early management of oral cancer: it is a non-invasive method, reliable, easy to use, economical and fast. It will be a good tool in addition to the biopsy.

Table III. All PEDro scores per item.

	Duz Mehmand Bugrahan *et al.* (2015)	Zanotti Laura *et al.* (2017)	Zahran F. *et al.* (2015)	Wu Chih-Ching *et al.* (2015)	Varun Chopra *et al.* (2015)	Shetty Shishir Ram *et al.* (2015)	Shetty Shishir Ram *et al.* (2014)	Shah Kavan *et al.* (2018)	Seyedmajidi Seyedali *et al.* (2018)	Rajkumar K. *et al.* (2015)	Rajkumar K. *et al.* (2014)	Rajaram Suganya *et al.* (2017)	Peisker *et al.* (2017)	Patel Shrikant et Metgud Rashmi (2015)	Panneer Selvam et Sadaksharam *et al.* (2015)	Michailidou Evangelia *et al.* (2016)	Malhotra Rewa *et al.* (2016)	Lohavanichbutr Pawadee *et al.* (2018)	Liu Chung-Ji *et al.* (2019)	Ko Hui-Hsin *et al.* (2018)	Kaur, Politis et Jacobs *et al.* (2016)	Kallalli Basavaraj N. *et al.* (2016)	Jou Yu-Jen *et al.* (2014)	Jacob Tharun Varghese *et al.* (2016)	Honarmand Marieh Honarmand *et al.* (2016)	Gonçalves Andréia Souza *et al.* (2015)	Gallo Crescenzio *et al.* (2016)	Dadhich M. *et al.* (2014)	Bu Jingqiu *et al.* (2015)	Bhat Supriya *et al.* (2017)	Aziz Salman *et al.* (2015)	Awasthi Nidhi (2017)	Arduino Paolo G. *et al.* (2015)	Arantes L.M.R.B. *et al.* (2015)	Achalli Sonika *et al.* (2017)	Babiuch Karolina *et al.* (2019)
Q1	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*0*	*1*	*1*	*1*	*1*	*0*	*1*	*1*	*1*	*0*	*1*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*0*	*0*	*1*	*1*	*1*	*0*	*1*	*1*	*1*
Q2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q4	1	1	0	1	0	1	1	0	1	1	1	0	1	0	1	1	1	0	0	0	1	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1
Q5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q7	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Q8	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Q9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Q10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Q11	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
Score	**5**	**5**	**4**	**4**	**5**	**5**	**5**	**4**	**5**	**5**	**6**	**4**	**5**	**4**	**5**	**5**	**6**	**3**	**4**	**4**	**6**	**4**	**5**	**5**	**5**	**6**	**5**	**4**	**4**	**5**	**5**	**5**	**5**	**3**	**4**	**5**

PEDro score of 36 included studies, allowing their methodological evaluation. **Q1** (in italic, because will not be counted) = Eligibility criteriahave been clarified; **Q2** = Subjects were randomly assigned to groups; **Q3** = the allocation respected a secret assignment; **Q4** = the groups were similar at the start of the study with regard to the most important prognostic indicators; **Q5** = all subjects were «blind»; **Q6** = all therapists who administered treatment were «blind»; **Q7** = all reviewers were «blind» for at least one of the essential judgment criteria; **Q8** = the measures, for at least one of the essential judgment criteria, were obtained for more than 85% of the subjects initially divided into the groups; **Q9** = all subjects for whom results were available received treatment or followed the control intervention according to their distribution or; when this was not the case, the data of at least one of the essential endpoints were "intention to treat" analyzed; **Q10** = the results of the intergroup statistical comparisons are indicated for at least one of the essential criteria; **Q11** = for at least one of the essential judgment criteria, the study indicates both of the effects and the estimation of their variability.

Conflicts of interests

The authors have no conflicts of interest to declare.

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