

Diagnosis by Raman Spectroscopy of Pre-Malignant and Malignant Oral Lesions: A Systematic Review

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Abstract

Raman spectroscopy is a technique that uses a monochromatic source of light-generating light of the same and different incident energy, thus allowing to obtain the frequency of atoms and molecules vibration to detect chemical connection or geometry, which allows discovering the chemical composition. This technique has great relevance for the early diagnosis of premalignant and malignant lesions due to its practicality and lack of invasiveness. The aim of this study was to evaluate the success of Raman spectroscopy on early diagnosis of oral lesions with a predisposition to malignancy and malignant lesions. A literature review was carried out in PubMed, SciELO, Google Scholar and Lilacs databases, using keywords "precancerous lesions", "Raman Spectral Analysis" and "oral neoplasia". Thirty-two articles were found and 12 were selected, based on the exclusion criteria (review articles and articles that were not studying oral lesions). The high success rates of Raman spectroscopic technique was observed in the rapid identification of malignant or premalignant cells other forms of oral dysplasias in oral lesions due to the practicality of the laser apparatus and lack of invasiveness. It is concluded that the diagnosis by Raman spectroscopy is effective due to its ease-of-use and a great possibility to provide early diagnosis affording successful treatment of oral precancer and cancer.

Key Words: Precancerous lesions, Raman spectral analysis, Oral neoplasms, Fast diagnosis, Efficacy

Introduction

Premalignant Oral Lesions (POLs), like as leukoplakia, erythroplasia, and oral submucosal fibrosis, often precede oral cancer [1]. Despite oral accessibility, most lesions are diagnosed late in the disease, with most patients suffering from a T3 or T4 tumor at the time of diagnosis [2].

Although few lesions develop into malignancy, their diagnosis is based on the histopathological evaluation, which is invasive and takes longer to show results [3]. A non-invasive, real-time and point-of-treatment method could overcome these problems and facilitate regular screening. Bio-optical methods could overcome these limitations by relying on a common principle: illuminating the tissue with light, the optical spectrum derived from the tissue contains information about the molecular/chemical composition of the tissue and/or its surface character. The information thus obtained allows a characterization of the tissue. Several bio-optical methods-recently presented in the literature show the tendency for an *in situ* identification of pathological alterations in clinical practice [2]. Raman spectroscopy, a bio-optical method, provides an objective, highly accurate and sensitive acquisition of the molecular composition of the tissue through the specific interaction of photons with cellular molecules, generating light of the same incident energy allowing to obtain information of the frequency that the atoms of that lesion vibration [4].

It does not require preparation of the biological samples, which makes it a non-invasive and robust tool for the *in situ* analysis of the biological tissue. In addition, the high spatial and spectral resolution-up to the recognition of a single molecule-can provide an exact location of the lesion and its edges [2]. Raman spectroscopy was evaluated for its ability to discriminate between normal, dysplastic and neoplastic in the head and neck region [3]. This way, an objective classification in the tumor tissue or in the physiological oral tissue can be

derived from the acquired spectra, without the necessity of a subjective clinical interpretation of the results [2].

Raman spectroscopy appears as a noninvasive screening method that can rapidly provide a diagnosis of malignant oral lesions [5]. There is a need in the in-depth study of this subject, due to the increase in the number of cases of oral carcinomas diagnosed in high stages, attenuated mainly by the infeasibility of the histopathological diagnostic methods that are considered invasive, that generates difficulties for the patients. With little knowledge of dentistry professionals about Raman spectroscopy and its possibilities for the clinical diagnosis of several lesions, it would be interesting to have an explanatory review that included the Raman spectroscopy technique, its operation, limitations and recent applications in Stomatology.

Aim

The aim of this study was to review the literature on the evaluation of the success of the use of this technique, Raman Spectroscopy, in the early clinical diagnosis of oral lesions with a predisposition to malignancy and malignant lesions.

Methods

This study was performed following PRISMA protocols (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The research was carried out in the PubMed, SciELO, Google Scholar and LILACS databases with complete articles published in the English language. In the manual search was included the reference list of articles selected on the subject.

Inclusion and exclusion criteria

Inclusion criteria were articles that evaluated laboratory or clinical efficacy of Raman spectroscopy in longitudinal studies. Articles that evaluated the use of this technique in exclusively oral lesions. And studies that provided all

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information about the research, such as the date of onset, the efficacy, and inefficacy of this technique, the reason for the ineffectiveness, and in which types of lesions this was ineffective. Exclusion criteria were studies that did not evaluate the use of Raman spectroscopy in malignant or premalignant oral lesions, duplicate studies, found in different databases, articles of critical or systematic reviews.

Search

All terms used to search for articles were checked records in the Descriptors in Health Sciences (DECs). The terms "precancerous lesions", "Raman spectral analysis", "oral neoplasms", "rapid diagnosis", "efficacy", "rapid diagnosis and precancerous lesions", "Raman spectral analysis and efficacy", "Oral neoplasms and Raman spectral analysis", "Raman spectral analysis and oral neoplasms and precancerous lesions", "Raman spectral analysis and rapid diagnosis and efficacy". The filters used were articles published in the English language.

Selection

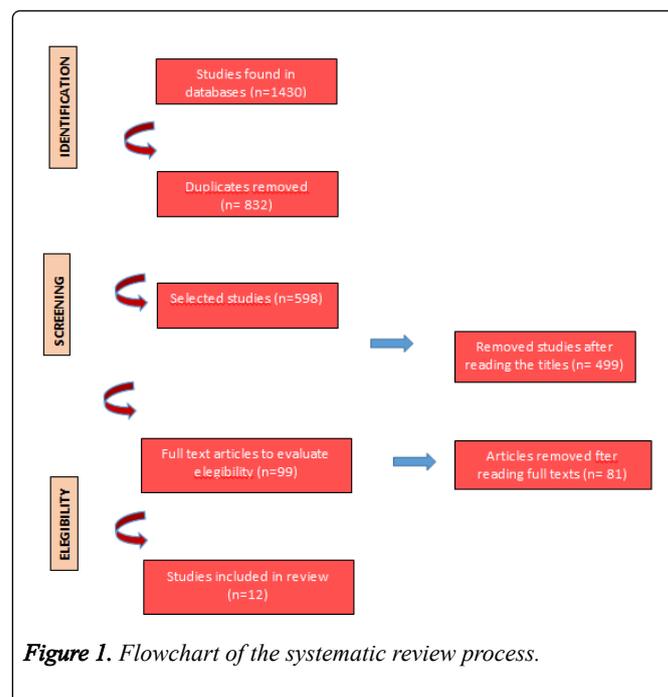
The articles identified in all databases were selected for automatic deletion of duplicates. The studies based on the titles and their abstracts were evaluated, and those that were considered of interest and reviewed twice by the authors of this work were read through until a consensus was reached. After the selection, the list of references of the previously chosen articles was evaluated and another 3 studies with potential for inclusion were selected. The summary of the paper selection is presented in *Figure 1*.

Table 1. Details of selected articles.

Authors	Types of study	Main findings	Efficacy diagnosis	in
Sahu A et al. [1]	Clinical trials	Evaluation of the malignancy of oral lesions in tobacco users, healthy or who already had pre-malignant lesions.	Yes	
Singh SP et al. [5]	<i>In vivo</i>	Evaluation of malignancy of lesions in healthy, cancerous, elderly and smokers patients.	Yes	
Singh SP et al. [5]	<i>In vivo</i>	Evaluation of lesions with the possibility for malignancy in patients who already have cancer in other healthy regions with smoking habits	Yes	
Mian SA et al. [3]	Laboratory	Evaluation of the technique for the diagnosis and differentiation between normal, dysplastic or cancerous lesions.	Yes	
De Carvalho. [6]	Clinical trials	Evaluation of the technique in 14 patients who had hyperplastic lesions, compared with normal tissues	Yes	
Kpnifer et al. [7]	<i>Ex-vivo</i>	Evaluation of the discrimination of the technique, through the excitation of cells	Yes	
Tan Y et al. [8]	<i>Ex- vivo</i>	Evaluation of the technique in squamous cell cancer, visualizing common malignant components.	No	

Results

Most of the studies reviewed included the evaluation of lesions in patients who already had potential for malignancy, using as examples the frequent use of substances such as tobacco and alcohol, and sexually transmitted diseases, comparing them with healthy people and people with injuries, but who did not make use of these potential substances for malignancy.



Data analysis

The selected studies were organized in a table, presenting their authors, years of publications and main information about the Raman spectroscopy tests, demonstrating efficacies, inefficiencies and reasons for inefficiencies (*Table 1*).

In the evaluated patients who had habits that could induce the malignancy the Raman spectroscopy technique was successful in the accurate and fast diagnosis, being able to be useful for the treatment processes, realizing that with the precise and faster diagnosis, there could be an intervention for treatment as early as possible, and there is a greater chance of rehabilitation of the patient with the lesion [1].

The same precision and rapidity of results were observed in patients who were not carriers of these habits and in patients who did not have lesions with malignancy potential, thus

guaranteeing the accuracy of the results in the differentiation of benign, premalignant and malignant lesions [3].

Only one of the studies that were evaluated did not classify Raman spectroscopy as satisfactory, reported in its laboratory study, where it evaluated the technique in squamous cell carcinogens, however, the cells were torn during the use of the technique [8].

Therefore, it is possible to notice that the observed technique has, in general, good results in the fast and accurate diagnosis of oral lesions, considering all the factors that can leave them closer to or distant from the malignancy, demonstrating their capacity to be used in the day-to-day clinical and provide a greater possibility of effective treatment to the patient.

Table 1 presents a summary relationship of the evaluated articles together with their methodologies and main findings.

Discussion

The present results demonstrate the successful use of Raman spectroscopy as a diagnostic technique for oral lesions, highlighting: a higher velocity, with no need for incision for a histopathological evaluation, thus also being able to affirm that this would not be an aggressive technique and its precision as it can identify the molecular and chemical composition of the tissue, differentiating malignant, premalignant and non-malignant lesions. It was also observed the accuracy of the results in patients using substances such as tobacco [9] that were analyzed with aspects of progression and malignancy at different molecular level of patients who would not be users.

This method uses a laser with a variable wavelength which, when the tissue is exposed, contains information on the proteins, vitamins and chemical and physical components of a cell in its cell cycle state, based on fluorescence, allowing an evaluation of the components that can inform the diagnosis of those lesions, based on their molecular aspect.

However, one of the main barriers that the Raman spectroscopic technique has faced for its daily clinical use would be the removal of this fluorescence as a base in the tissues without compromising the spectral characteristics that respond to the biomolecular profile of the tissue, since that fluorescence remains in the tissue [2]. Studies have already been carried out to remove the detection of this fluorescence without compromising the result of the spectroscopy, changing it by other chemical reactions that do not have a permanent effect.

Some studies also point out that the spectral results can be reduced to an algorithm, where each of them can represent a diagnostic order, so that a database can be established for each result obtained in the evaluation of malignant, premalignant lesions or non-malignant, being those of oral cavity or not, further facilitating the diagnosis, standardizing a sequence for each spectroscopic response [9]. Further research should be done to extend the database to even more accurate classification and to validate classification performance through independent data sets.

Another favorable point to this technique would be that oral lesions have the characteristic of being easily torn apart, which may interfere with the diagnosis when there is a need for excision for histopathological evaluation. With the use of Raman spectroscopy, there would be no need to remove the lesion, being able to obtain more fully the composition of that lesion and complete diagnosis, without any interference or loss of material [2].

Studies not included for the results of this paper indicate limitations in the Raman spectroscopic technique due to the lack of spatial information needed to examine more complete biological tissues, expensive equipment and complex algorithms [10]. However, there are already studies that foresee the fusion of the applied spectroscopy in the Raman technique with the use of the image, thus eliminating any risk of bias; the complex algorithms, once discovered, will be stored in a database, so that it is not necessary to unravel it in all situations and with the expansion of knowledge about this diagnostic technique, it will become more popular and there is a tendency to have a lower cost.

It is important to note that Raman technology has been implemented not only in the dental area. In recent years, a colonoscopy endoscope has been developed that performs Raman spectroscopy to detect molecules in the bowel related to inflammatory bowel diseases or to pre-surgically determine the margins of cancerous tumors in patients in real-time. In addition to the identification of the molecules, Raman spectroscopy provides information on the extent of tissue inflammation.

This optical technology will be presented as a way of rapid diagnosis of cancer of the intestinal tract, conducting the study of the biochemical and structural information of the sample without requiring the surgical removal, making it minimally invasive [11], without doing anything prior preparation of the tissue and allowing a start of earlier treatment, and may also use these practices for the formulation of a database for a future formulation of optical biopsy and diagnostic of malignant tissue changes [12].

These approaches allow us to observe the success of the Raman spectroscopic technique in a variety of tissues, however, with the same purpose: a minimally invasive and real-time diagnostic method, allowing the chances of early treatment, good prognosis [13].

Conclusion

As shown in this article, the comparative techniques of Raman spectroscopy and other methods show, for the most part, a superiority of this technique in the diagnosis of malignant and premalignant oral lesions, presenting as a non-invasive, precise and fast result technique not only for the diagnosis, but also for a differentiation of the subtypes of dysplasias and cancers, showing the speed at which the cell cycle is, which is very necessary due to the high incidence of cases of oral lesions already diagnosed at an advanced stage of malignancy. However, it is necessary that further studies on Raman spectroscopy as a method of diagnosis of oral lesions be performed, not only in patients with predisposition to malignancy and without predisposition, but also in patients presenting with other oral pathologies that may to evolve to a

malignant lesion, using more *in vivo* tests for the diffusion of this method in the clinical routine, demonstrating a great advance in the histopathological diagnosis.

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