

Salivary rheumatoid factor in primary and secondary Sjögren's syndrome.

Factor reumatoide salival en síndrome de Sjögren primario y secundario.

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Receipt: 08/25/2018 **Revised:** 12/18/2018
Acceptance: 03/22/2019 **Online:** 08/05/2019

Cite as:

Triana-Reyes S, Martínez-Sandoval G, Rodríguez-Franco N, Chapa-Arizpe M, Rodríguez-Pulido J, Alcázar-Pizaña A & Riega-Torres J. Salivary rheumatoid factor in primary and secondary Sjögren's syndrome.

J Oral Res 2019; 8(3):196-200.
Doi:10.17126/joralres.2019.030

Abstract: Sjögren's syndrome is a chronic autoimmune disease, characterized by the presence of hyposalivation and xerophthalmia, which in addition to other factors is diagnosed by the presence of rheumatoid factor in blood. The objective of the present study is to evaluate the presence of rheumatoid factor (IgG-IgM) in the saliva of patients with primary and secondary Sjögren's syndrome. Materials and methods: Forty samples from patients with primary and secondary Sjögren's syndrome previously diagnosed by the Arthritis and Rheumatism Specialist Center of the Autonomous University of Nuevo Leon were analyzed. Samples were taken from the saliva using the Carlson-Crittenden device to evaluate the IgG-IgM immunocomplex using the ELISA method. Results: No significant difference was found between the presence of IgM in primary (0.099±0.016) and secondary Sjögren syndrome (0.098±0.017), however, a high presence of IgG was found in the group of patients with secondary Sjögren's syndrome (0.134±0.054). Conclusion: The search for diagnostic tools using salivary biomarkers has come with economic and clinical advantages, however, in the present study no significant changes were found in salivary rheumatoid factor between both groups.

Keywords: Sjogren's syndrome; rheumatoid factor; saliva; immunoglobulins; Enzyme-Linked Immunosorbent Assay

Resumen: El síndrome de Sjögren es una enfermedad autoinmune crónica, caracterizada por la presencia de hiposalivación y xeroftalmia, la cual además de otros factores es diagnosticada por la presencia del factor reumatoide en sangre. El objetivo del presente estudio es evaluar la presencia del factor reumatoide (IgG-IgM) en saliva parotídea de pacientes con síndrome de Sjögren primario y secundario. Materiales y métodos: Se analizaron 40 muestras de pacientes con síndrome de Sjögren primario y secundario previamente diagnosticados por el Centro de Especialistas en Artritis y Reumatismo de la Universidad Autónoma de Nuevo León, a los cuales se les tomó una muestra de saliva parotídea mediante el dispositivo Carlson-Crittenden para evaluar mediante el método ELISA el inmunocomplejo IgG-IgM. Resultados: No se encontró diferencia significativa entre la presencia de IgM en el síndrome de Sjögren primario (0.099±0.016) y secundario (0,098±0,017), sin embargo en cuanto a la presencia de la IgG se encontró elevada en el grupo de pacientes con síndrome de Sjögren secundario (0,134±0,054). Conclusión: La búsqueda de herramientas diagnósticas mediante biomarcadores salivales ha traído consigo ventajas económicas y clínicas, sin embargo en el presente estudio no se encontró un cambio significativo en el factor reumatoide salival entre ambos grupos.

Palabras Clave: Síndrome de Sjögren; factor reumatoide; saliva; Inmunoglobulinas; ensayo de inmunoadsorción enzimática.

INTRODUCTION.

Sjögren's syndrome is a chronic autoimmune disease, of unknown etiology with a slow and progressive course,¹ which occurs in 0.1-3.0% of the population.^{2,3}

This condition can occur in isolation as primary Sjögren's syndrome,⁴ or it can manifest as secondary when presenting together with other autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis,⁵ sclerosis, among others.⁶

Due to a decrease in salivary flow, patients with Sjögren's syndrome have an increase incidence of dental caries, periodontal disease,⁷ mucositis, neoplasms, tooth loss,⁸ as well as *Candida albicans*⁹ infections.

The rheumatoid factor is used for the diagnosis of Sjögren's syndrome.¹⁰ The rheumatoid factor is an antibody of the IgM type produced against the Fc portion of the IgG, which is detected by a blood test that quantifies the levels of IgM against the IgG,¹¹ and when levels above 20 IU/mL IgG-IgM immunocomplexes are formed that activate the complement system and other inflammatory factors that then produce tissue destruction.¹²

Currently, non-invasive diagnostic techniques are being developed that use biomarkers, which are substances found in biological fluids such as saliva,¹³ which is easy to collect fluid, as well as containing elements that reflect the systemic and local health status of patients. However, the status of the rheumatoid factor in saliva is currently poorly described in the literature.¹⁴ The objective of this study is to evaluate the presence of rheumatoid factor (IgG-IgM) in parotid saliva of patients with primary and secondary Sjögren's syndrome.

MATERIALS AND METHODS.

Population and study design

In the present study, we estimated a sample number $n=40$, which was divided into two groups: primary Sjögren's syndrome (SS) and secondary Sjögren's syndrome ($n=20$). The study design was comparative, open, with only one examiner, observational and cross-sectional, and was performed on patients with primary and secondary Sjögren syndrome previously diagnosed by the Arthritis and Rheumatism Specialist Center of the Autonomous University of Nuevo Leon.

Patients with primary and secondary Sjögren's syndrome

(SS), of both genders and within an age range 40 to 80 years were included. Patients who could have another determinant involving hyposalivation, such as head and neck radiation treatment, history of hepatitis C virus infection, human immunodeficiency virus, pre-existing lymphoma, sarcoidosis, patients with graft-versus-host disease, diabetes mellitus, patients taking anticholinergic and parasympathomimetic drugs or with a history of intake within the last four months were excluded. Patients who failed to produce at least 0.5mL of saliva during the salivary sample were also removed from the study.

Saliva collection

Following diagnosis, parotid saliva was samples, obtained from the Stenon duct using a device Carlson-Crittenden. Salivary flow was stimulated with 2% citric acid placed on the back of the tongue at intervals of 30 seconds for a period of 10 minutes and collected in sterile 1.5 ml microtubes.

In order to avoid biases during sampling, an information leaflet was given to the patients with the indication to show up to the appointment without having eaten or drunk, and without having brushed their teeth or taken any medication at least 12 hours prior to the simple taking, and were instructed to remain seated without moving facial muscles while the saliva was being obtained.

Rheumatoid factor assay

The procedure for processing parotid saliva samples correspond to the instructions of the manufacturer of the ELISA kit employed (Rheumatoid factor IgG, IgA, IgM Elisa, Tecan Trading AG, Switzerland), which is a test that assays the antigen-antibody complexes in order to evaluate the presence of a specific compound. Prior to the dilution of the samples, the reagents to be used were prepared as follows: 20 mL of sample buffer concentrate was diluted in 80 mL of distilled water, and 20 mL of wash buffer concentrate was diluted in 980 mL of distilled water.

One thousand microliters of the sample buffer was added to 10 μ L of each saliva sample and placed in new microtubes which were divided according to group.

Using a micropipette, 300 μ L of the wash buffer was placed in each well, incubated for 20 seconds and

removed; this procedure was repeated twice more.

The positive and negative controls as well as the diluted samples to be tested were placed in the corresponding wells of the plate, in duplicate. The plate was incubated for 30 minutes at 20-32°C, then the wells were washed with 300 ul of wash buffer and 10 ul of the conjugate (IgG-IgM) was added to each well followed by an incubation at 20-32°C for 30 minutes. The wells were then washed with 300 ul of wash buffer three times and 10 ul of the TMB substrate was placed in each well and incubated for 30 minutes at 20-32°C. Subsequently, 10ul of the stop solution was aliquoted per well and incubated for 5 minutes to finally read the absorbance of the samples at 450nm.

Ethical considerations

The study was approved by the Bioethics Committee of the Autonomous University of Nuevo León, where each patient was given informed consent prior to taking the clinical history.

Data analysis

An analysis of variance (Anova) was performed with 95% confidence (IBM SPSS Statistics, Version 20, USA and Microsoft Excel 2010).

RESULTS.

During the study, no patients were excluded or eliminated, but it was found the diseases most frequently associated with secondary Sjögren's syndrome were rheumatoid arthritis and systemic lupus erythematosus.

No significant difference was found in the assay of the rheumatoid factor in saliva between the presence of IgM in primary (0.099±0.016) and secondary (0.098±0.017) Sjögren's syndrome; however IgG was found to be high in the group of patients with secondary Sjögren's syndrome (0.134±0.054) compared to those with primary Sjögren's syndrome (0.090±0.012), however the difference was not statistically significant (Table 1).

Table 1. Quantification of rheumatoid factor by ELISA in saliva of patients with primary and secondary Sjögren's syndrome.

	Primary Sjögren		Secondary Sjögren		p-value
	Mean	SD	Mean	SD	
IgM	0.099	0.016	0.098	0.017	0.4584
IgG	0.090	0.012	0.134	0.054	0.011

SD: Standard deviation.

DISCUSSION.

The definition commonly used for the diagnosis of Sjögren's syndrome is that of the American-European Consensus Group of 2002, where six criteria based on ocular and oral symptoms and signs, salivary gland histopathology, glandular dysfunction and presence of the anti-Ro (SSA) and anti-La (SSB) autoantibodies are described, where at least 4 of the 6 criteria must be present in order to qualify for Sjögren's syndrome diagnosis;¹⁵ however a new classification proposed in 2012 by the Sjögren International Collaborative Clinical Alliance, and approved by the American College of Rheumatology, states that the diagnosis of SS can be established with the presence of two or more of the following findings:

Positive antinuclear antibodies, positive minor

salivary gland biopsy with a score of inflammatory focus $\geq 1/4 \text{ mm}^2$, dry keratoconjunctivitis with an ocular staining score \geq and presence of anti-Ro/SSA and/or anti-La/SSB or the presence of positive rheumatoid factor, representing an important modification to the first diagnostic classification.¹⁰

The presence of rheumatoid factor in serum can be found in patients with non-rheumatic diseases, such as bacterial, viral or parasitic infections, among others, however the frequency of occurrence in patients with Sjögren's syndrome ranges from 70 to 90%, even though it can also be found in 5% of healthy subjects under 50 years of age and in between 10 and 25% in those over 70 years of age.¹⁶

Recently, attempts have been made to identify using quantitative and qualitative methods salivary

biomarkers for the diagnosis of Sjögren's syndrome by identifying salivary protein profiles, such as IgA, IgG and albumin.¹⁷

To our knowledge there are no recent studies evaluating the presence of rheumatoid factor in saliva of patients with primary and secondary Sjögren's syndrome, but it has been reported that it is possible to quantify the presence of salivary IgG in these patients.

However by using saliva the presence of rheumatoid factor may be due to gingival inflammation present and not to the syndrome itself, which is why we decided to use parotid saliva in the present study with the aim of avoiding this bias.¹⁸

This is in agreement with a study conducted by German *et al.*,¹⁹ where the relationship of IgG, IgM, IgA and albumin in serum, saliva and tears was evaluated, and no relationship was found regarding the presence of immunoglobulins in serum and in the other assayed fluids. However in the present study, although no statistically significant changes were found, levels of IgG were higher in secondary Sjögren's syndrome.

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CONCLUSION.

The use of new diagnostic tools using salivary biomarkers has resulted in less invasive, simple and less costly methods. Within the limitations of the study it can be concluded that there was no significant difference in the presence of IgM and IgG between primary and secondary Sjögren's syndrome, however IgG was found to be higher in the group of patients with secondary Sjögren's syndrome compared to those with primary syndrome.

Conflict of interests: The authors declare no conflict of interest in relation to published results.

Ethics approval: The study was approved by the Bioethics Committee of the Autonomous University of Nuevo León, Mexico.

Funding: No funding.

Authors' contributions: The manuscript was carried out, written, and approved in collaboration with all authors.

Acknowledgements: We thank CONACYT for scholarship funding, and Dr. Gustavo Israel Martínez González for statistical advice.

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