Presence of Hepatitis C Virus RNA in the Salivary Glands of Patients with Sjögren's Syndrome and Hepatitis C Virus Infection

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ABSTRACT. ***Objective.*** To determine whether hepatitis C virus (HCV) RNA could be detected in the salivary glands of patients with both a diagnosis of Sjögren's syndrome (SS) and HCV infection.

Methods. Five patients with primary SS (European criteria) and chronically infected by HCV and 3 controls (one with primary SS without HCV infection, another with HCV infection without sicca syndrome, and a third without SS and HCV infection) were tested for the presence of HCV-RNA (using reverse transcriptase-polymerase chain reaction) in their saliva, serum, and salivary glands.

Results. In the patient group, HCV-RNA was detected in the serum and saliva of all cases and RNA extracted from salivary gland specimens tested positive in 3 cases. In the control group, HCV-RNA was not detected in the serum, saliva, or salivary glands from subjects without HCV infection. Only the control subject with HCV but without sicca syndrome tested positive for the presence of HCV-RNA in the serum, saliva, and salivary gland tissue.

Conclusion. Our results showed that HCV may propagate and reside within salivary gland tissue, leading to HCV associated sialadenitis or Sjögren's-like syndrome in some cases, a phenomenon that does not seem specific. However, a direct role for HCV in the physiopathology of certain cases of primary SS is suggested. (J Rheumatol 2002;29:2382–5)

Key Indexing Terms:
SJÖGREN'S SYNDROME
SALIVARY GLANDS
HEPATITIS C VIRUS
POLYMERASE CHAIN REACTION

Sjögren's syndrome (SS) is an autoimmune disease mainly affecting exocrine glands and presenting with dryness of the mouth and eyes. Lymphocyte infiltrates of lachrymal and salivary glands characterize this syndrome as well as the presence of antinuclear antibodies (ANA) and rheumatoid factors (RF)[1]. In the absence of an associated specific autoimmune disease, patients are classified as having primary SS.

The etiology of primary SS remains unknown, but environmental factors are thought to play a role. Among these factors, viruses have been suspected to be a potential cause of SS and several have been incriminated (retroviruses, Epstein-Barr virus)[2]. For 10 years, attention has been focusing on the relationship between SS and hepatitis C virus (HCV)[3-6]. In 1992, Haddad, *et al* reported the occurrence of characteristic SS histological changes in the salivary glands of patients with chronic HCV infection[7]. Since this description, several authors have reported symptoms of ocular and oral dryness in HCV infected patients[4,6,8]. HCV-RNA can be detected using polymerase chain reaction (PCR) technique and in patients with chronic HCV infection, viral RNA has been detected in different biological fluids including saliva[4]. Further, ANA and RF have been observed in patients with HCV infection[4].

On the other hand, the prevalence of HCV antibodies in different series of primary SS has been evaluated to range from 3 to 75% using PCR, and the detection of HCV viremia in these patients ranges between 0 and 19%[9-11].

HCV-RNA has been found in salivary tissue from patients with chronic HCV infection, but without clinical or histological features suggestive of SS[12]. We investigated the presence of HCV-RNA in the salivary glands from patients with both SS and chronic HCV infection.

MATERIALS AND METHODS

Patients. Patients with diagnosis of both primary SS and HCV infection were included. They fulfilled 4 or more of the 1996 diagnostic criteria for SS proposed by the European Study Group[13] (Patient 4 fulfilled only 3 of the criteria and was considered to have a probable primary SS, according to the 1993 European criteria[14]; for this reason, this patient was included). They were all diagnosed as having primary SS and none had signs or biological evidence of associated autoimmune disease. Schirmer test, rose bengal dye test, and salivary gland biopsy were performed in each patient. The presence of arthritis, parotidomegaly, peripheral neuropathy, cutaneous involvement, and hepatomegaly was noted in each case. Risk factors for HCV infection were also recorded.

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Controls. Controls were required for HCV-RNA detection in salivary glands. They included one patient with primary SS without HCV infection (SS control), another with HCV infection without sicca syndrome (HCV control), and a third without SS or HCV infection (negative control).

Identification of HCV infection. Serum samples from all patients and controls were tested for HCV antibodies by an enzyme immunoassay (EIA) (Innotest HCV Ab IV®, Innogenetics, Gand, Belgium). Positive results were confirmed by a second test (Axym® HCV version 3.0, Abbott, Wiesbaden, Germany). Other serological tests included the detection of hepatitis B surface (HBs) antigen and core (HBc) antigen (DiaSorin, Saluggia, Italy), and human immunodeficiency virus (HIV) antibody (Vironostika®, Organon Teknica, Boxtel, Netherlands, and Genscreen®, Biorad, Marne la Coquette, France).

Identification of HCV RNA. Salivary gland biopsies were digested in a 250 mg proteinase K solution. Total RNA extraction was performed from 200 ml of serum, saliva, and digested salivary gland solution as described by the manufacturer (Roche Diagnostics, Indianapolis, IN, USA). Reverse transcription and amplification were realized using the Amplicor® HCV amplification kit version 2.0 (Roche) with a detection limit of 100 HCV RNA copies/ml.

HCV RNA was quantified in sera by Quantiplex® HCV RNA 2.0 assay (bDNA) (Chiron Corp., Emeryville, CA, USA). HCV genotypes were determined by the Inno-LIPA® HCV II method (Innogenetics).

Immunological assessment. Immunological tests included detection of RF (nephelometry), ANA (indirect immunofluorescence), and precipitating antibodies to the extractable nuclear antigens SSA and SSB (ELISA tests). Serum cryoglobulins were obtained after centrifugation. Blood samples were stored at +37°C and then centrifuged. The supernatant was removed from the serum, and fresh centrifuged serum was then incubated at 4°C for 7 days, and examined for cryoprecipitation. Cryoglobulins were classified according to Brouet, *et al*[15]. Other laboratory investigations included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) concentrations, and HLA-DRB1 and DQB1 typing using PCR followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO)[16].

RESULTS

Between 1996 and 2001, 5 patients with a diagnosis of both primary SS and HCV infection were observed in our department (all Caucasian women, mean age 57.2 ± 11.2 yrs). The demographic and clinical features of these patients are summarized in Table 1. In 3 patients (Patients 1, 3, and 4), HCV infection was diagnosed before SS and only 2 had received interferon therapy before SS diagnosis (Patients 1 and 3). The 5 patients had xerostomia and 4 complained of ocular dryness. All patients had arthritis, and one had cutaneous vasculitis and peripheral polyneuropathy (diagnosed on clinical examination and electromyography). No parotidomegaly was observed. Finally, neither hepatomegaly nor splenomegaly was observed in this series. Four patients were positive for ocular dry tests and salivary gland biopsy showed lymphocytic infiltrates in all cases (grades 1–2 from Chisholm and Mason classification[17] in 2 cases and grades 3–4 in the others). Only 2 patients had elevated ESR (mean ± SD of the series: 21.2 ± 11.3 mm/h), while CRP levels were in the normal range. No patient had elevated transaminase levels. Immunological features included positive ANA in 3 cases and anti-SSA/SSB antibodies in 2 cases. Type III cryoglobulin was present in each patient. RF were found in 2 cases and 3 patients were typed as HLA-DRB*03.

No risk factors for HCV infection were found. All patients were negative for HIV and HBs antigen, while 3 had positive antibodies to HBc antigen. Antibodies to HCV were present in all cases. The determination of HCV genotypes showed that 4 patients had the HCV-1b genotype and one the 2a/2c genotype. Mean serum HCV RNA titer was 13.9 MEq/ml (± 12.7). HCV RNA was detected in serum and saliva from all patients and 3 patients also tested positive for HCV RNA in their salivary glands. These 3 patients had the highest serum HCV RNA titers among the patient group (Table 1).

The control group included one primary SS control (with ocular dryness, positive anti-SSA/SSB antibodies and grade 3 sialadenitis; age 45), one control with HCV infection (HCV antibodies, 90 MEq/ml RNA, 1b genotype) but without sicca symptoms and sialadenitis (age 51), and a negative control (with primary biliary cirrhosis but without HCV infection or sicca syndrome; age 62). HCV RNA was not detected in the serum, saliva, or salivary glands from controls without HCV infection (SS control and negative control). By contrast, the HCV control with HCV antibodies tested positive for the presence of HCV RNA in serum, saliva, and salivary gland tissue.

DISCUSSION

HCV is a small, single stranded RNA virus classified within the flaviviridae family identified in 1989. HCV infection was recognized as common, leading to progressive and insidious liver disease[5,18]. Besides liver involvement, HCV is responsible for a wide range of systemic and/or autoimmune diseases, including mixed cryoglobulinemia[3,18]. Sicca syndrome has emerged as one of the extrahepatic manifestations of HCV infection[4-6,19]. The most convincing argument for this association was the presence of HCV RNA in the saliva from chronically infected HCV patients, a finding also observed in SS patients with positive HCV antibodies[4,5]. HCV infected patients can develop sialadenitis with morphological characteristics similar to those observed in SS patients[20,21]. Another argument is the production of transgenic mice carrying HCV envelope genes that develop a sialadenitis resembling SS[22]. For these different reasons, HCV may rationally be proposed as an etiologic agent for SS.

The mechanisms that may explain such an association include direct destruction of the glandular tissue by the virus, or may be indirect, via the formation of immune complexes or autoimmune phenomena secondary to HCV infection. Another link between HCV and SS is the detection of HCV RNA in salivary gland tissue from HCV infected patients[12,23]. Indeed, HCV RNA has been found in salivary glands obtained at necropsy from chronically infected patients[12]. However, these reports are derived from case reports and have not been confirmed: in another study,

Table 1. Clinical and biological features of 5 patients with Sjögren's syndrome and hepatitis C virus infection.

Patient	1	2	3	4	5
Age/Sex	42 F	58 F	72 F	62 F	52 F
HCV diagnosis	1994	1993	1990	1996	1996
SS diagnosis	2000	1990	1996	1997	1996
Sicca symptoms	Xerostomia	Xerostomia, xerophthalmia	Xerostomia, xerophthalmia	Xerostomia, xerophthalmia	Xerostomia, xerophthalmia
Clinical features	Arthritis	Arthritis	Arthritis, cutaneous vasculitis, polyneuropathy	Arthritis	Arthritis
Ocular dry tests	+	+	–	+	+
Salivary gland biopsy	Sialadenitis grade 3–4	Sialadenitis grade 3–4	Sialadenitis grade 1–2	Sialadenitis grade 1–2	Sialadenitis grade 3–4
ESR (mm/h)	18	16	13	38	ND
ANA	1/160	–	1/640	1/80	–
ENA: SSA/SSB	+	–	+	–	–
RF	+	–	+	–	–
HLA DRB1*	*03/11	*01/12	*03/15	*03/13	*01/13
DQB1*	*02/03	*05/03	*02/06	*04/06	*05/06
HCV genotype	1b	1b	1b	1b	2a/2c
RNA titer, MEq/ml	3.0	2.1	20.5	11.7	32.3
HCV RNA					
Serum	+	+	+	+	+
Saliva	+	+	+	+	+
Salivary glands	–	–	+	+	+

ANA: antinuclear antibodies; RF: rheumatoid factors; HCV: hepatitis C virus; SS: Sjögren's syndrome; ENA: antibodies to extractable nuclear antigens.

Taliani, *et al* failed to find HCV RNA using reverse transcriptase PCR in salivary tissue from 12 unselected patients with active HCV[24]. Moreover, they found that only 15% of their patients had HCV RNA in the saliva, and this weak positivity was explained by a capillary diffusion of HCV RNA. A role for a minimal blood to saliva gradient was also suggested by these results. Another negative result was the lack of HCV antigen detection in salivary glands from 5 HCV positive patients using immunohistochemical analysis[19]. These discrepancies in the detection of HCV RNA in salivary tissue from HCV infected patients could be explained by the variability of HCV serum titers among the patients and the different viral genotypes involved. In addition, in these different studies, the patients had no laboratory and histological proofs of SS. However, a recent study by Arrieta, *et al* reported that HCV RNA and HCV core antigen could be detected in epithelial cells from salivary glands of patients with chronic sialadenitis or SS using *in situ* hybridization and immunochemistry methods, respectively[25].

We detected the presence of HCV RNA in 3 HCV patients with primary SS. Interestingly, we found that the 2 patients (1 and 2) with the lower serum HCV RNA titers showed negative detection in their salivary glands, contrasting with the others, who had the highest viremia. It could be speculated that the HCV patients with high circulating level of HCV had a higher probability of infecting their salivary glands. Alternatively, this could also mean that the HCV RNA in the salivary glands could diffuse from blood during the biopsy. However, the recent study by Arrieta, *et al* using *in situ* hybridization and immunohistochemistry methods suggests that HCV can reside and replicate in epithelial cells from salivary glands of patients with sicca symptoms, and thus the data with PCR HCV RNA detection also provide another argument for the presence of HCV in salivary tissue.

Taken together, our results and those of previous studies suggest that HCV, a sialotropic virus, can reside within the salivary gland cells, and thus may induce a local, direct inflammatory reaction that could alter the salivary tissue. This supports the notion of a direct infection or a direct cytotoxic mechanism, via the replication of the virus within the glandular tissue, that may explain the clinical features of sicca syndrome. Alternatively, a reaction directed against certain very highly conserved antigens could be initiated (in the periphery or locally in the salivary glands), leading to salivary gland histological changes and an immunological reaction responsible for the production of antibodies, some features characteristic of SS[26]. However, the detection of HCV RNA directly in the salivary tissue strongly argues for the first hypothesis. In 2 cases of our series, we did not find HCV RNA in salivary tissue. This could be related to the low level of serum RNA titer in these patients. It could also be speculated that HCV may have transiently infected the

salivary glandular cells and thus virological assessments may have been performed late in the disease evolution. Since patients were harboring different HCV genotypes, it does not seem that a specific genotype has a preferential tropism for the salivary glands. On the other hand, SS has a complex pathophysiology including a genetic component. Thus, an individual genetic predisposition may also play a role in the development of sicca symptoms in HCV infected patients.

Our HCV control tested positive for the presence of HCV RNA in the serum, saliva, and salivary tissue samples. These results agree with some findings of previous studies[12,23]. However, this subject had no sicca symptoms or sialadenitis, and the presence of HCV RNA in the salivary glands suggests that HCV can reside within this tissue, and be excreted in saliva but without damaging the salivary glands. This could also mean that the presence of HCV RNA in salivary glands is not a specific phenomenon without the induction of a sicca syndrome in all cases. Alternatively, it can be hypothesized that this HCV control may develop a sicca syndrome in a few years.

Although the interpretation of our results is limited by the small number of patients and controls, we can conclude that HCV may actively propagate to salivary glands and reside in this tissue, leading to an HCV associated sialadenitis or Sjögren's-like syndrome in some patients. This phenomenon is not specific, since other viruses such as Epstein-Barr virus may also reside in salivary glands. However, a direct mechanism in the association between SS and HCV infection is suggested, giving another argument for the involvement of this virus in the pathophysiology of certain cases of SS. This relationship remains very intriguing but is not fully understood, and deserves further study.

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