Articoli originali

Original articles

High prevalence of parotideal abnormalities among HCV-infected patients

Elevata prevalenza di patologie della parotide nei pazienti con infezione da HCV

Bruno Cacopardo, Marilia Rita Pinzone, Maria Gussio, Giuseppe Nunnari

Department of Clinical and Molecular Biomedicine, Division of Infectious Diseases, University of Catania, Catania, Italy

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae family and is a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma; its natural history is characterized by the progression of 50 to 80% acutely infected individuals to persistent infection as well as by gradual extension of fibrosis throughout the liver: 5-20% of them will develop liver cirrhosis [1-3].

While non-invasive methods for fibrosis detection are actively being pursued, liver biopsy remains the gold standard for grading and staging of chronic hepatitis C (CHC) [4].

Treatment relies on the administration of pegylated (PEG)-interferon and ribavirin both to acutely and chronically infected individuals [5, 6]. Recently, a triple therapy including protease inhibitors, such as telaprevir and boceprevir, has become available for HCV genotype 1-infected patients and new therapeutic options are currently under evaluation [6, 7]. Nevertheless both the virus itself and the related treatment might be associated with different autoimmune manifestations [8, 9]. HCV may localize in several tissues besides the liver, including oral epithelial cells and salivary glands [10, 11]. These tissues may represent a reservoir for HCV and contribute to both viral persistence and reactivation.

Corresponding author
Bruno Cacopardo
E-mail: cacopardobruno@inwind.it

Although it has been demonstrated that HCV can replicate in oral epithelial cells of patients without specific manifestations of oral diseases, a number of reports demonstrated that several salivary diseases, such as Sjogren's syndrome (SS), chronic lymphocytic sialadenitis and parotid non-Hodgkin's lymphoma may occur among HCV-infected patients [11-14].

As for SS, it is an autoimmune disease characterized by lymphocytic infiltration of lacrimal and salivary glands.

Even though its etiology is still unknown, viral infections have been implicated in the development of this disease.

Epidemiological studies have shown that up to 40% of patients with SS may have anti-HCV antibodies [13].

Patients with chronic HCV infection frequently have a mild form of lymphocytic sialadenitis, which is clinically different from the classical SS [13]. Finally, some authors reported the localization of HCV within a parotid non-Hodgkin's lymphoma lesion developed in the course of HCV-related type essential mixed cryoglobulinemia [14].

In this case HCV RNA and HCV-related core proteins were also found in the residual epithelial cells of the salivary gland [15].

Since HCV seems able to localize either in epithelial oral cells and salivary gland acini as well as to produce a wide spectrum of salivary diseases, we aimed at evaluating the prevalence of parotid abnormalities in a cohort of patients affected with CHC attending the Unit of Infectious Diseases of the Garibaldi Nesima Hospital of Catania.

PATIENTS AND METHODS

Between January 2011 and January 2012, 355 consecutive CHC patients attending the Outpatient Clinic of the Unit of Infectious Diseases at the Garibaldi Nesima Hospital of Catania were enrolled. All the enrolled patients were submitted to a specific clinical diagnostic protocol to study the parotid gland with the following schedule:

a) a detailed clinical history was collected, including symptoms or signs such as xerostomia, xerophtalmia, pain in the parotid region, pain when chewing or swallowing, transient or persistent enlargement of the parotid region;
b) a manual clinical exam was conducted on the parotid gland by an expert physician;
c) blood exams including routine liver function tests, anti-HIV antibodies, HBsAg, amylase, complete autoantibody panel including anti-SSA/SSB autoantibodies;
d) Schirmer's test;
e) ultrasound scan and magnetic resonance imaging (MRI) of the facial skull with gadolinium contrast to identify parotideal abnormalities.

Only those patients who exhibited evident or suspect parotid masses or lesions underwent further diagnostic procedures, such as fine needle aspiration, parotid biopsy or even surgical excision.

As a control group, 188 patients were enrolled, all HbsAg positive and anti-HCV negative, affected with biopsy-proven chronic hepatitis B. Controls were submitted to the same diagnostic protocol adopted for the CHC group.

RESULTS

Overall, 45 patients were excluded from the study, 25 because of refusal to sign the informed consent, 17 because of HIV coinfection and 3 due to lack of adherence to the clinical follow up. Thus, 310 patients were enrolled. Mean age was 47.5±14 years; 200 (64.5%) were males. All were anti-HCV positive, had detectable serum HCV RNA and hyper-alanine aminotransferase (ALT) (ALT >2 times the upper limit). Mean ALT value was 86±22 IU/l; mean HCV RNA was 5.56±2.2 $x10^5$ IU/ml. 250 patients (80.6%) had genotype 1b HCV infection, 20 (6.5%) genotype 1a, 12 (3.9%) genotype 2 and 28 (9%) genotype 3 infection. As for risk factors for HCV infection, 110 patients (35.4%) were intravenous drug users, 100 (32.3%) reported previous major surgical interventions, whereas in 100 cases (32.3%) route of infection was unknown. As for liver histology, 300 of 310 patients underwent liver biopsy: 210 (67.8%) had a minimal to mild chronic active hepatitis C, 90 (29%) exhibited a moderate to severe chronic active disease. 10 patients (3.2%) had a

Table 1 - Characteristics of the study population (310 patients).

Patients' characteristics	
Age (years)	47.5±14
Men/Women N (%)	200 (64.5)/110 (35.5)
Alanine aminotransferase (IU/l)	86±22
HCV RNA (IU/ml)	5.56±2.2*105
HCV genotype 1a-1b-2-3, N (%)	20(6.5)-250(80.6)-12(3.9)-28(9)
Risk factors for HCV infection - Intravenous drug use, N (%) - Major surgical interventions, N (%) - Unknown, N (%)	 110 (35.4) 100 (32.3) 100 (32.3)
Liver disease stage - Cirrhosis N (%) - Minimal to mild chronic active disease N (%) - Moderate to severe chronic active disease N (%)	 10 (3.2) 210 (67.8) 90 (29)
Data are expressed as means ± standard deviation, except where otherwise noted.	

Table 2 - Anatomopathological characteristics of the cases submitted to fine needle aspiration (n=11), parotid biopsy (n=10) or surgical excision (n=1).

No. patients	*Sample*	*Description*	*Diagnosis*
4	Biopsy	Epithelial component with epithelial and myoepithelial cells showing divergent growth patterns, including trabecular and papillary architecture; cuboid epithelial cells	Pleomorphic adenoma
2	Biopsy	Noncaseating epithelioid cell granulomas	Sarcoid granuloma
4	Biopsy	Markedly hyperplastic lymphoid infiltrates with loss of acini; ducts infiltrated by lymphoid cells	Chronic lymphocytic sialadenitis
4	FNA	epithelioid cells, multinucleate giant cells, lymphocytes, plasma cells and a variable number of neutrophils	Aspecific granuloma
7	FNA	Lymphoepithelial cell clusters	Sjogren's syndrome
1	Gland excision	Diffuse proliferation of lymphoid cells with loss of ductal and acinar architecture Intense positive staining for LCA and CD20 on the cytoplasmic side of the cell membrane	Non-Hodgkin's lymphoma

FNA: Fine Needle Aspiration; LCA: leukocyte common antigen.

Table 3 - Summary of the parotideal abnormalities found in the study population of CHC patients.

Parotideal abnormalities	*N=53*
Lymphoepithelial cysts	24 (45.3)
Benign tumors	6 (11.3)
- Pleomorphic adenoma	4
- Cystic adenoma	2
- Granulomatous lesions	6 (11.3)
- Sarcoid granulomas	2
- Aspecific granulomas	4
Sjogren's syndrome	12 (22.7)
Chronic lymphocytic sialadenitis	4 (7.5)
Non-Hodgkin's lymphoma	1 (1.9)

Data are expressed as N (%).

clinical diagnosis of liver cirrhosis (Table 1). Comprehensively, 53 patients out of 310 (17%) had abnormalities of the parotid gland: only 5 patients (9.4%) reported symptoms consistent with parotid involvement, such as local pain (3 cases) or stiffness during chewing or swallowing (2 cases); 13 out of 53 (24.5%) had parotid swelling at manual examination; in 30 cases (56.6%) ultrasound scan documented different parotid abnormalities, such as cystic lesions (18 cases, 60%), hypoechogenic masses (10 cases, 33.3%) and diffuse echographic dishomogeneity (2 cases, 6.7%). Finally, MRI documented clear-cut parotideal abnormalities in all cases. Eleven patients (20.7%) required fine needle aspiration and 10 (18.9%) a biopsy to reach the final diagnosis. In one case (1.9%), surgical excision was performed (Table 2).

In detail, 24 patients (45.3%) had lymphoepithelial cysts of the salivary gland; 6 patients (11.3%) had a benign tumor (4 a pleomorphic adenoma and 2 a cystic adenoma); 6 patients (11.3%) had granulomatous lesions, which in two cases were recognized as sarcoid granulomas and in 4 cases were classified as aspecific granulomas; 12 patients (22.7%) were diagnosed as being affected with SS on the basis of persistent xerostomia and xerophtalmia, positive Schirmer's test, positive anti-SSA/SSB antibodies and diffuse dishomogeneity at imaging procedures; 4 patients (7.5%) were diagnosed as having chronic lymphocytic sialadenitis on the basis of clinical symptoms and presence of lymphocytic aggregates in biopsy specimens. Finally, one patient (1.9%) had parotid non-Hodgkin's lymphoma and underwent gland excision (Table 3).

As refers to the HBV-positive group, only 2 out of 188 (1%) exhibited parotideal abnormalities throughout the diagnostic procedures: one patient had a lymphoepithelial cyst of the parotid,

the other one had symptomatic chronic sialolithiasis.

DISCUSSION

In the present study we demonstrated a 17% prevalence of parotideal abnormalities in a cohort of HCV-infected patients. Previous studies have already reported that several parotideal diseases, including SS and chronic lymphocytic sialadenitis, occur more frequently among CHC patients [13, 16, 17].
SS seems to be one of the systemic autoimmune diseases (SAD) more closely associated with HCV. García-Carrasco et al. indicated that HCV infection was present in 14% of patients with SS, which is significantly higher than in the general population (1.2%) [18]. Smyth et al. demonstrated that symptoms consistent with SS were considerably over-represented in CHC subjects [15]. They also indicated that DQB1*02 was significantly associated with viral persistence. Different mechanisms may participate in the pathogenesis of HCV-associated SS [15]. Possible explanations include direct infection and proliferation of HCV in salivary glands, molecular mimicry between HCV and salivary glands and formation of immune complexes containing HCV. HCV replication within salivary gland epithelial cells of patients with SS and HCV infection has been clearly shown. Arase et al. also reported that 16.1% of patients with HCV had chronic sialadenitis [19]. Arrieta et al. indicated that HCV was able to infect and propagate in epithelial cells from salivary glands of patients with chronic sialadenitis [20]. It may be hypothesized that, in people with a particular genetic background, infection of salivary epithelial cells could result in lymphoid proliferation and secondarily in extensive chronic destruction of salivary glands [21, 22].
The hypothesis that HCV is involved in a number of salivary gland diseases is reinforced by the detection of HCV RNA by RT-PCR in saliva and total RNA isolated from salivary glands. Arrieta et al. found positive *in situ* hybridization signals in the biopsies of salivary glands from all anti-HCV-positive patients but in none of the anti-HCV-negative controls [20].
The present study demonstrates that the parotid gland is a relevant target for HCV. The clinical relevance of this observation is related not only to SS and chronic lymphocytic disease but also to a wide spectrum of clinical features ranging from granulomatous lesions to non-Hodgkin's lymphoma. De Vita et al. have already reported a case of parotid non-Hodgkin's lymphoma in a cryoglobulinemic HCV-infected patient [14]. In our report, parotid involvement was more often subtle and asymptomatic, frequently escaping to the manual exam performed by an experienced physician. In conclusion, we believe that targeted diagnostic protocols may help identifying parotid involvement among HCV-infected individuals, as parotideal abnormalities are extremely frequent in this population of subjects.

Conflict of interest: None
Funding sources: None

Keywords: chronic hepatitis C, HBV, HCV, parotid, salivary gland, Sjogren's syndrome.

SUMMARY

Several salivary diseases, such as Sjogren's syndrome (SS), chronic lymphocytic sialadenitis and parotid non-Hodgkin's lymphoma, may occur in the setting of HCV infection. Our aim was to evaluate the prevalence of parotid abnormalities in a cohort of 310 patients with chronic hepatitis C (CHC) attending the Unit of Infectious Diseases of the Garibaldi Nesima Hospital of Catania. Our control group consisted of 188 patients with chronic HBV infection. We found that the prevalence of parotideal diseases was significantly higher among HCV-infected patients in comparison with HBV-infected (17% vs. 1%). Indeed, 53 CHC subjects had parotideal abnormalities: 24 patients (45.3%) had lymphoepithelial cysts of the salivary gland, six patients (11.3%) had a benign tumour, six patients (11.3%) had granulomatous lesions, 12 patients (22.7%) had Sjogren's syndrome and four patients (7.5%) were diagnosed as having chronic lymphocytic sialadenitis. Finally, one patient (1.9%) had parotid non-Hodgkin's lymphoma. In conclusion, parotideal abnormalities are common among HCV-infected individuals and targeted diagnostic protocols may help to identify parotid involvement in this population of subjects.

RIASSUNTO

Nell'ambito dei pazienti con infezione da HCV sono state riportate diverse patologie a carico delle ghiandole salivari, quali la sindrome di Sjogren, la sialoadenite cronica linfocitaria e il linfoma non Hodgkin della parotide. Il nostro studio si è posto l'obiettivo di valutare la prevalenza di patologie parotidee in una coorte costituita da 310 pazienti con infezione cronica da HCV, seguiti presso l'Ambulatorio dell'Unità Operativa di Malattie Infettive dell'Ospedale Garibaldi Nesima di Catania. Il gruppo di controllo era costituito da 188 soggetti con infezione cronica da HBV. La prevalenza di patologie della parotide è risultata significativamente più elevata nei soggetti con infezione da HCV (17% vs. 1%). Infatti, 53 pazienti con epatopatia cronica HCV-correlata presentavano anomalie parotidee: 24 pazienti (45.3%) avevano cisti linfoepiteliali, 6 pazienti (11.3%) una neoplasia benigna della parotide, 6 pazienti (11.3%) lesioni granulomatose, 12 pazienti (22.7%) la sindrome di Sjogren e 4 (7.5%) la sialoadenite cronica linfocitaria. In un caso (1.9%), si è giunti alla diagnosi di linfoma non Hodgkin della parotide, con escissione della ghiandola. In conclusione, le patologie della parotide appaiono molto frequenti nei soggetti con infezione da HCV, per i quali possono essere utili dei protocolli diagnostici mirati, finalizzati alla precoce individuazione di eventuali anomalie a carico delle ghiandole salivari.

REFERENCES

[1] Nature Outlook. Hepatitis C. *Nature* 474, S1-S21, 2011.
[2] Nunnari G., Pinzone M.R., Cacopardo B. Lack of clinical and histological progression of chronic hepatitis C in individuals with true persistently normal ALT: the result of a 17-year follow-up. *J. Viral. Hepat.* 20 (4), e131-137, 2013.
[3] Pinzone M.R., Celesia B.M., Di Rosa M., Cacopardo B., Nunnari G. Microbial translocation in chronic liver diseases. *Int. J. Microbiol*. 2012, 694629, 2012.
[4] Rizzo L., Nunnari G., Berretta M., Cacopardo B. Acoustic Radial Force Impulse as an effective tool for a prompt and reliable diagnosis of hepatocellular carcinoma preliminary data. *Eur. Rev. Med. Pharmacol. Sci.* 16 (11), 1596-1598, 2012.
[5] Nunnari G., Montineri A., Portelli V., Savalli F., Fatuzzo F., Cacopardo B. The use of peginterferon in monotherapy or in combination with ribavirin for the treatment of acute hepatitis C. *Eur. Rev. Med. Pharmacol. Sci.* 16 (8), 1013-1016, 2012.
[6] Casey L.C., Lee W.M. Hepatitis C virus therapy update 2013. *Curr. Opin. Gastroenterol.* 29 (3), 243-249, 2013.
[7] Nunnari G., Schnell M.J. MicroRNA-122: a therapeutic target for hepatitis C virus (HCV) infection. *Front. Biosci. (Schol Ed).* 3, 1032-1037, 2011.
[8] El-Serag H.B., Hampel H., Yeh C., Rabeneck L. Extrahepatic manifestations of hepatitis C among United States male veterans. *Hepatology* 36 (6), 1439-1445, 2002.
[9] Cacopardo B., Pinzone M.R., Nunnari G. Development of sarcoidosis during treatment for chronic hepatitis C with pegylated interferon alfa-2a and ribavirin: case report and literature review. *Infez. Med.* 21 (1), 36-40, 2013.
[10] Liou T.C., Chang T.T., Young K.C., Lin X.Z., Lin C.Y., Wu H.L. Detection of HCV RNA in saliva, urine, seminal fluid and ascites. *J. Med. Virol*. 37, 197-202, 1992.
[11] Arrieta J.J., Rodríguez-Iñigo E., Ortiz-Movilla N., et al. In situ detection of hepatitis C virus RNA in salivary glands. *Am. J. Pathol.* 158 (1), 259-264, 2001.
[12] Toussirot E., Le Huédé G., Mougin C., Balblanc J.C., Bettinger D., Wendling D. Presence of hepatitis C virus RNA in the salivary glands of patients with Sjögren's syndrome and hepatitis C virus infection. *J. Rheumatol.* 29 (11), 2382-2385, 2002.
[13] Scott C.A., Avellini C., Desinan L., et al. Chronic lymphocytic sialadenitis in HCV related chronic liver disease: comparison with Sjogren's syndrome. *Histopathology* 30, 41-48, 1997.
[14] De Vita S., Sansonno D., Dolcetti R., et al. Hepatitis C virus within a malignant lymphoma lesion in the course of type II mixed cryoglobulinemia. *Blood* 86 (5), 1887-1892, 1995.
[15] Smyth C.M., McKiernan S.M., Hagan R., et al. Chronic hepatitis C infection and sicca syndrome: a clear association with HLA DQB1*02. *Eur. J. Gastroenterol. Hepatol.* 19 (6), 493-498, 2007.
[16] Ramos-Casals M., García-Carrasco M., Cervera R., et al. Hepatitis C virus infection mimicking primary Sjögren syndrome. A clinical and immunologic description of 35 cases. *Medicine. (Baltimore)* 80 (1), 1-8, 2001.
[17] Mariette X. Sjögren's syndrome and virus. *Rev. Med. Interne* 15 (9), 601-606, 1994.
[18] García-Carrasco M., Ramos M., Cervera R., et al. Hepatitis C virus infection in 'primary' Sjögren's syndrome: prevalence and clinical significance in a series of 90 patients. *Ann. Rheum. Dis*. 56 (3), 173-175, 1997.
[19] Arase Y., Ikeda K., Tsubota A., et al. Serum levels of gamma-globulin and total bilirubin influence the prevalence of multiple extrahepatic complication in patients with hepatitis C virus infection. *Hepatol. Res.* 25 (1), 14-21, 2003.
[20] Arrieta J.J., Rodriguez-Inigo E., Casqueiro M., et al. Detection of hepatitis C virus replication by in situ hybridization in epithelial cells of anti-hepatitis C virus positive patients with and without oral lichen planus. *Hepatology* 32, 97-103, 2000.
[21] Takamatsu K., Okayasu I., Koyanagi Y., Yamamoto N. Hepatitis C virus propagates in salivary glands. *J. Infect. Dis*. 165, 973-974, 1992.
[22] Grossmann Sde M., Teixeira R., Oliveira G.C., et al. Xerostomia, hyposalivation and sialadenitis in patients with chronic hepatitis C are not associated with the detection of HCV RNA in saliva or salivary glands. *J. Clin. Pathol*. 63 (11), 1002-1007, 2010.