ORAL AND DENTAL ASPECTS
OF HEPATITIS C VIRUS INFECTION

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My sincerest thanks go to my family and friends for the support I received, and finally, I wish to express my love to Ilaria and the big family of 83 Victoria Park Road for the unforgettable time we spent together during these three years.
Oral and dental aspects of the hepatitis C virus (HCV) infection were investigated, with particular attention to the possible associated oral conditions, such as oral lichen planus (LP) and Sjögren's syndrome (SS), and the risk of nosocomial transmission within dental health care. The prevalence of oral LP in a group of Italian patients with HCV-associated liver disease was investigated as well as the seroprevalence of anti-HCV among Italian patients with oral LP; both the studies confirmed the association between oral LP and HCV infection, at least in countries at high prevalence. HCV 1b was the most common genotype found in a group of patients with oral LP and HCV infection, although no significant link between a particular genotype and HCV-associated oral LP could be demonstrated. A small proportion of the same group was also found to be co-infected by hepatitis G virus (HGV), a novel virus whose pathological potential is still to be elucidated. Anti-epithelial antibodies were found in a significant proportion of another group of patients with HCV-associated oral LP, although confirming the high frequency of autoantibodies in HCV-positive subjects, the exact role of these antibodies remains unclear. The presence of HCV genome in oral LP tissues has been demonstrated for the first time and the expression of HCV antigens investigated by immunohistochemistry. In addition the histological and serological features of some of HCV-associated LP were compared with those of the idiopathic form, showing some differences in the tissue expression and serological levels of some adhesion molecules and immunoglobulins. The first case of malignant change in patient with HCV-associated LP was also presented. A study of a group of English patients with SS showed no association with HCV infection, possibly due to the very low prevalence of HCV in the UK.
A low prevalence of HCV infection was found among a group of dental health care workers, thus indicating that an occupational acquisition of HCV is unlikely in UK dental health care. Finally the knowledge of HCV infection among European dental students was surveyed and revealed the need for improved education about aspects of HCV infection relevant to dentists.
# TABLE OF CONTENTS

## CHAPTER 1. INTRODUCTION

1.1. Hepatitis C virus virology
1.2. Epidemiology of hepatitis C virus infection
1.3. Hepatitis C virus transmission
1.4. Diagnosis of hepatitis C virus infection
1.5. Natural history of hepatitis C virus-related liver disease
1.6. Extrahepatic manifestations and associated conditions of hepatitis C virus infection
1.7. Therapy of hepatitis C virus infection
1.8. Considerations for dental treatment of an HCV-infected patient

## CHAPTER 2. HEPATITIS C VIRUS INFECTION AND ORAL DISEASES

2.1. Hepatitis C virus infection and oral lichen planus
2.1.1. Review of literature
2.1.2. Epidemiological studies of the association between oral lichen planus and Hepatitis C virus
2.1.3. Hepatitis G virus co-infection among patients with hepatitis C virus-associated oral lichen planus
2.1.4. Antibodies to epithelial components in lichen planus associated with hepatitis C Virus infection
2.1.5. Detection of the hepatitis C virus genome and antigens in the oral mucosa of patients with hepatitis C virus-associated oral lichen planus
2.1.6. Idiopathic and hepatitis C virus-associated oral lichen planus: a comparative study of immunohistochemical and serological features
2.1.7. Development of squamous cell carcinoma in hepatitis C virus-associated oral lichen planus
2.2. Hepatitis C virus infection and Sjögren’s syndrome, a British study
CHAPTER 3. HEPATITIS C VIRUS INFECTION
AND THE DENTAL HEALTH CARE WORKER --- 146

3.1. Hepatitis C virus infection as an occupational hazard for the health
care workers ----------------------------------------------------- 147
3.1.1. Review of literature ------------------------------------------ 147
3.1.2. Prevalence of HCV infection in health care workers of a UK dental
hospital ------------------------------------------------------------- 163
3.2 Knowledge of hepatitis C virus (HCV) infection among dental students
from three European countries. -------------------------------- 169

CONCLUSIONS AND FURTHER STUDIES -------------- 182

REFERENCES ---------------------------------------- 184
LIST OF TABLES

Table 1.1: Classification systems of the Hepatitis C Virus genotypes ......16
Table 1.2: Hepatitis C virus infection among volunteer blood donors world­wide ..............................................................22
Table 1.3: Extrahepatic manifestations of HCV infection......................35
Table 1.4: Recommendations for the dental management of an HCV­infected patient with impaired liver function ..........................40
Table 2.1: Studies on the possible association between lichen planus and chronic liver disease .........................................................46
Table 2.2: Prevalence of hepatitis C virus infection in patients affected by lichen planus...............................................................52
Table 2.3: Frequency of distribution of the HCV genotypes 1a, 1b and 2a in 33 Italian patients with oral lichen planus .........................64
Table 2.4: Characteristics of patients with HCV genotypes 1b and 2a .....65
Table 2.5: Clinical details of the patients with HCV-associated oral lichen planus (OLP), co-infected with HGV .................................77
Table 2.6: Characteristics of HCV-infected lichen planus patients with and without HGV co-infection .............................................78
Table 2.7: Frequency of HGV co-infection in patients with chronic hepatitis C.................................................................................82
Table 2.8: Immunofluorescence staining patterns of sera of patients with HCV-related and non-related oral lichen planus, patients with HCV infection and healthy controls..............................88
Table 2.9: Immunohistochemical staining observed in samples of oral and liver tissues ..................................................................100
Table 2.10: Monoclonal antibodies used in immunohistochemical studies .........................................................................................123
Table 2.11: Immunohistochemical staining patterns observed in buccal mucosal tissues ..............................................................124
Table 2.12: Circulating adhesion molecules and IgG levels ..................125
Table 2.13: Studies of malignant change in groups of oral lichen planus patients ...........................................................................133
Table 2.14: Association of HCV infection with sialadenitis or Sjögren's syndrome .............................................................................143
Table 3.1: Hepatitis C virus: definition of occupational exposure according to UK Public Health Laboratory Service Hepatitis Subcommittee .................................................................150
Table 3.2: Recommendations of the Centers for Disease Control for the follow-up of health care workers, after percutaneous or permucosal exposure to potentially HCV-infected blood .......151

Table 3.3: Hepatitis C virus seroconversion rates in health care workers occupationally exposed to blood..............................................156

Table 3.4: Prevalence of HCV infection among health care workers......157

Table 3.5: Seroprevalence of HCV in groups of dental health care workers .................................................................160

Table 3.6: Hepatitis C virus incidence among health care workers .......161

Table 3.7: Occupational status, gender and age of 167 UK dental health care staff ........................................................................164

Table 3.8: Speciality of 167 UK dental health care staff ....................165

Table 3.9: Frequency of HCV seropositivity in 167 UK dental health care staff ........................................................................167

Table 3.10: Demographics of 343 dental European undergraduate students and 63 postgraduates students ..............................176

Table 3.11: Knowledge of dental undergraduates and postgraduates of routes of transmission of HCV ...........................................177

Table 3.12: Respondents' knowledge of natural history, diagnosis and treatment of HCV infection .............................................178

Table 3.13: Respondents’ knowledge of HCV transmission during dental health care.................................................................179

Table 3.14: Respondents’ average score of perceived risk of HCV transmission during clinical activity ......................................180

Table 3.15: Respondents' knowledge of potential oral manifestations of HCV infection ...............................................................181
LIST OF FIGURES

Figure 1.1: Hepatitis C virus genome and encoded proteins ......................17
Figure 1.2: Putative Hepatitis C virus replication .........................................18
Figure 2.1: Oral lichen planus lesion of the buccal mucosa in a HCV-positive patient .................................................................53
Figure 2.2 H&E stained section of HCV-associated oral lichen planus biopsy specimen ........................................................................54
Figure 2.3 Detection of HCV-RNA in sera from patients affected by oral lichen planus. The sera marked with numbers 1, 2, 3, 12, 17, 19 and 22 were HCV-RNA positive ........................................66
Figure 2.4 HCV-genotyping of 3 HCV-RNA-positive patients affected by oral lichen planus. Patients 6 and 7 were infected by the 2a subtype while patient 8 by the 1b subtype ......................67
Figure 2.5 Detection of HGV-RNA in sera from HCV-positive patients affected by oral lichen planus. Representative results of 14 cases. Sera tested in lane 4 and 12 (arrows) were HGV-RNA positive. ......................................................................................79
Figure 2.6 Immunostaining profile of serum from one patient of the HCV+ve/LP+ve group against monkey oesophagus, showing reactivity against epithelial nuclei (pattern I; arrowheads) and against surface-associated epithelial antigen in the basal layer (pattern II; arrows) (original magnification x 400) ...............89
Figure 2.7: Punctate cytoplasmic staining (pattern III) produced by serum from a different patient in HCV+ve/LP+ve group (original magnification x 400). Note the apparent absence of anti-nuclear reactivity in many cells (arrowheads) .................90
Figure 2.8: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Positive cell of the inflammatory infiltrate. Antibody used: anti-c22-0 (mAb 1) (original magnification x 400) .................................................................102
Figure 2.9: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Intense staining of the keratinized layers. Antibody used: anti-c22-0 (mAb 1) (original magnification x 80) .................................................................103
Figure 2.10: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Intense nuclear staining of numerous keratinocytes. Antibody used: anti-c100-3 (mAb 2) (original magnification x 160) .................................................................104
Figure 2.11: Expression of putative HCV-associated antigens in oral lichen planus lesional tissue (paraffin-embedded sample). Positive cells of the inflammatory infiltrate. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400). .............................. 105

Figure 2.12: Expression of putative HCV-associated antigens in oral lichen planus lesional tissue (paraffin-embedded sample). (a) staining of suprabasal keratinocytes. (original magnification x 160), (b) cytoplasmic staining of suprabasal keratinocytes, characterised by a granular appearance. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400). ................................................. 106

Figure 2.13: Expression of putative antigens in HCV-positive liver tissue (paraffin-embedded sample). (a) group of positive hepatocytes (original magnification x 160), (b) cytoplasmic staining of hepatocytes, characterised by a coarse granular appearance. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400). ......................................................................................... 107

Figure 2.14: Graphs showing the distribution of (a) sLFA-3, (b) sICAM-1 and (c) serum IgG levels within each patient group. ..................... 126

Figure 2.14.1: Detection of LFA-3 antigen in (a) idiopathic and (b) HCV-associated oral lichen planus lesional tissue. In both sections LFA-3 is expressed primarily within the inflammatory infiltrate, with lower levels in adjacent regions of the epithelium and connective tissue. ........................................................................ 126 b

Figure 2.15: Severe oral lichen planus affecting the palatal mucosa....... 136

Figure 2.16: Squamous cell carcinoma with associated lichen planus of the dorsum of the tongue..................................................... 137

Figure 3.1: Risk of seroconversion after occupational exposure with different blood-borne viruses. .................................................. 162
CHAPTER 1.

INTRODUCTION
1.1. **Hepatitis C virus virology**

Hepatitis C virus (HCV) is a positive stranded RNA virus, identified for the first time in 1989 by Choo and colleagues at the Chiron Corporation (Emeryville, California) by means of a new molecular biological technique. From a concentrate chimpanzee plasma with an high infectivity titre ($10^6$ infectious doses/ml) they extracted the total DNA and RNA which were both used as a template for the synthesis of complementary DNA (cDNA). The cDNA was inserted in a cloning vector and expressed in *Escherichia coli*. Expressing proteins were immunoscreened with serum from a patient diagnosed with non-A, non-B viral hepatitis and only one clone (5-1-1) out of millions reacted (Choo et al. 1989). This clone represented the basis for the identification of a larger clone (c100-3) which expressed protein was used in the serological tests of first generation. By using overlapping clones, the entire sequence of the HCV genome was subsequently obtained.

Hepatitis C virus is now recognised as the main agent of the previously termed parenteral non-A, non-B viral hepatitis and one of the major causes of chronic hepatic disease world-wide. On the basis of the similarities between its nucleotide and amino acid sequence and that of the flavi- and pestiviruses, HCV has been classified within the Flaviviridae family as a separate genus (Heinz 1992). The HCV plus-stranded RNA genome comprehends approximately 9500 nucleotides and includes two untranslated regions at the 5’ and 3’ ends and an open reading frame encoding a viral polyprotein precursor of 3000 amino acids, cleaved by host and viral proteases into three structural proteins and six non-structural proteins. Structural proteins, encoded in the N-terminal region, comprehend the core protein (C) believed to be the viral capsid, and envelope proteins (E1 and E2); the nonstructural proteins, encoded in the C-terminal region, include six proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) with a number of
enzymatic activities some of which are still not fully elucidated (van Doorn 1994; Brechot 1996) (Figure 1.1). The morphology of the HCV viral particle has as yet not been described in detail, however studies based on membrane filtration (Yuasa et al. 1991) and electron microscopy (Kaito et al. 1994; Li et al. 1995) suggest that the virion might be formed by a 30-50nm icosahedral nucleocapsid, surrounded by an envelope characterised by surface projections.

The precise details of HCV replication are still unclear. Replication may involve the synthesis of a negative strand intermediate which acts as a template for the synthesis of a new positive RNA genome (Yoshikura et al. 1996) (Figure 1.2). Thus the detection of the negative strand should indicate HCV replication; indeed this has been detected in the liver (Sherker et al. 1993), plasma (Sherker et al. 1993), peripheral blood mononuclear cells (Qian et al. 1992) and sperm of HCV-infected patients (Liu et al. 1994), as well as in primary and metastatic hepatocellular carcinoma tissue (Bocher et al. 1994; Kobayashi et al. 1994), and interestingly, in malignant lymphoma tissue of the parotid gland (De-Vita et al. 1995).

Due to the frequent errors in the course of replication (approximately $10^{-3}$ substitutions per site per year) (Brechot 1996) and lack of repair mechanisms, HCV has an extremely variable genome and is present as quasispecies in a single infected individual (Oshima et al. 1991). This genetic variability occurs in all domains but is more frequent in a hypervariable region (HVR1), located at the N-terminus of E2 sequence, where a high rate of non-synonymous mutations (leading to amino acid changes and antigenically distinct variants) has been observed.

Following the complete sequencing of a number of different isolates, it was evident that different HCV strains may show significant differences in the genome's nucleotide sequences (Choo et al. 1991). Nucleotide sequencing of many isolates collected world-wide has permitted the development of
different classification systems (Table 1.1) (Miyakawa et al. 1995). In 1994, on the basis of a collaborative study comparing the NS5b region sequences of a world-wide panel of HCV variants, a classification was developed dividing the known viruses into main “types”, differing by 31-34% of the whole genome and numbered in order of discovery with Arabic numerals; each type is divided into “subtypes” differing up to 25%, which are identified by a lower-case letter, again in order of discovery (Simmonds et al. 1994). Apart from the expensive and time-consuming sequencing of the whole genome, typing of the different viral strains can be based on the analysis of the reverse transcriptase-polymerase chain reaction (RT-PCR) products by means of different techniques such as restriction analysis (Nakao et al. 1991), hybridization with specific probes (Stuyver et al. 1993) or use of type-specific primers during the amplification step (Okamoto et al. 1992). Genotyping can also be undertaken by serological assays (Machida et al. 1992; Simmonds et al. 1993) which, although being easier, cheaper and allowing testing non-viraemic individuals, cannot discriminate between subtypes.

The distribution of the different genotypes in the HCV-positive population is not the same world-wide; genotype 1 is probably the most common variant in Europe, USA and Japan, although its prevalence may vary considerably; genotype 2 is less common in Europe than in Japan and China, genotype 3 is the most frequent in Thailand, Singapore and parts of India, genotype 4 in Egypt, Middle East and Central Africa while genotypes 5 and 6 are present in South Africa and South East Asia (McOmish et al. 1994; Anonymous 1995; Smith and Pontisso 1996). Also the distribution pattern changes according with the geographical area: in some regions particularly of Africa and Asia, only few types but many different subtypes are present, suggesting a presence of HCV for long periods and a common route of transmission; a different pattern is seen in Western Europe and North America, where there are more types in a limited number of subtypes, this pattern is consistent with
a recent introduction of the virus through different routes; in addition in Egypt, a high prevalence of infection is associated with a dominant genotype (4a), suggesting a recent epidemic (Smith and Pontisso 1996).
<table>
<thead>
<tr>
<th>System</th>
<th>Chayama</th>
<th>Simmonds</th>
<th>Okamoto</th>
<th>Enomoto</th>
<th>Tsukiyama</th>
<th>New system</th>
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<tbody>
<tr>
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<td>1a</td>
<td>I</td>
<td>PT</td>
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<tr>
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<td>*</td>
<td>6a</td>
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* not classified
Figure 1.1: Hepatitis C virus genome and encoded proteins
Figure 1.2: Putative Hepatitis C virus replication
1.2. Epidemiology of hepatitis C virus infection

Hepatitis C virus infection is a world-wide health problem. The prevalence of anti-HCV antibodies among individuals at low risk for blood-borne diseases, such as volunteer blood donors without known risk factors (see below), varies considerably between geographical areas. In Europe there is a low frequency of HCV infection in Northern countries (UK 0.04% (McLindon et al. 1995) and Norway 0.1% (Nordoy et al. 1994)), while in countries of Mediterranean area and Eastern Europe the frequency can be ten times greater. A similarly high seroprevalence has been found among 11 million Japanese blood donors (Yamaguchi et al. 1994). The HCV seropositivity rate among US blood donors varies from 0.1% and 0.7% (Sharara et al. 1996). The highest known frequencies of HCV infection has been found in developing countries, particularly in Africa (Saleh et al. 1994; el-Nanawy et al. 1995) and Middle East (Mansell and Locarnini 1995). (Table 1.2) (Abe and Inchauspe 1991; Allain et al. 1991; Amirudin et al. 1991; Arguillas et al. 1991; Chen et al. 1991; Chiaramonte et al. 1991; Dawson et al. 1991; Laskus et al. 1991; Martinez et al. 1991; Par et al. 1991; Park et al. 1991; Richards et al. 1991; Slama et al. 1991; Tsai et al. 1991; Van-der-Poel et al. 1991; Aguelles and Janot 1992; Archer et al. 1992; Darwish et al. 1992; Esteban et al. 1992; Galban et al. 1992; Garson et al. 1992; Giulivi et al. 1992; Hejjas et al. 1992; Hugo et al. 1992; Hyams et al. 1992; Hyland et al. 1992; Kashiwagi 1992; Knodler et al. 1992; Leite et al. 1992; Lin et al. 1992; Malaguti et al. 1992; Novakova et al. 1992; Zhang et al. 1992; Zufferey et al. 1992; Duraisamy et al. 1993; Goncales et al. 1993; Goncales et al. 1993; Khan et al. 1993; Lai et al. 1993; Lytsar' et al. 1993; Ndumbe and Skalsky 1993; Sychev and Mikhailov 1993; Tang 1993; Timan et al. 1993; Tretiskaia et al. 1993; Vanderborght et al. 1993; Zhang et al. 1993; Abdelaal et al. 1994; Bernvil et al. 1994; Ciuffreda et al. 1994; Crawford et al. 1994; Hayashi et al. 1994; Hejjas et al. 1994; Honda et al. 1994; Islas et al. 1994; Klofera et al. 1994;
Liang et al. 1994; Lindholm 1994; Luengrojanakul et al. 1994; MacLennan et al. 1994; Merino-Conde et al. 1994; Myl'nikov et al. 1994; Nordoy et al. 1994; Patino et al. 1994; Schwarz et al. 1994; Song et al. 1994; Suarez et al. 1994; Vasconcelos et al. 1994; Wang et al. 1994; Yamaguchi et al. 1994; Agbodjan et al. 1995; Anderson et al. 1995; Araj et al. 1995; Ayed et al. 1995; Bar et al. 1995; Bassily et al. 1995; Caspari et al. 1995; Choudhury et al. 1995; Develoux et al. 1995; Ehrmann et al. 1995; Garcia-Bengoechea et al. 1995; Ilako et al. 1995; Irshad et al. 1995; Jin et al. 1995; King et al. 1995; Love et al. 1995; McLindon et al. 1995; Mutimer et al. 1995; Ng et al. 1995; Quinti et al. 1995; Stern et al. 1995; Sulaiman et al. 1995; Tsega et al. 1995; Van Hoof et al. 1995; Wang 1995; Wu et al. 1995; Barna et al. 1996; Benjelloun et al. 1996; Darmadi et al. 1996; Guerrero et al. 1996; Hennig et al. 1996; Jaiswal et al. 1996; Lvov et al. 1996; Munoz-Gomez et al. 1996; Murphy et al. 1996; Naman et al. 1996; Oni and Harrison 1996; Salmeron et al. 1996; Soetjipto et al. 1996; Tang et al. 1996; Mison et al. 1997). It must be stressed that the results of most of these surveys are not comparable since a variety of tests with different predictive values have been used and confirmatory tests of the positive cases have not been always undertaken. The frequency of HCV-seropositivity in blood donors may not reflect the real proportion of HCV-infected individuals, as volunteer blood donors are a population that is negatively selected on the basis of risk factors for blood borne infections, thus the prevalence in the general population would be expected to be higher than that of blood donors; indeed few detailed studies of representative samples of the general population in France (Dubois et al. 1997), Italy (Bellentani et al. 1994) and the US (Alter 1995), reported seropositivity rates higher (1.15%, 3.2% and 1.4% respectively) than those of exclusive blood donors.

Little information is available on the incidence of HCV infection among low risk groups. Recently a retrospective study estimated that in the US, during the last decade, 150,000 subjects have been infected per year (Alter 1995),
and prospective studies on large cohorts of blood donors from Italy, Japan and UK, reported incidences of 10, 1.78 and 2.8 per 10,000 person-years respectively (Atrah et al. 1995; Sasaki et al. 1996; Prati et al. 1997).
Table 1.2: Hepatitis C virus infection among volunteer blood donors world-wide

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence (%)</th>
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<tr>
<td>Algeria</td>
<td>0.18</td>
<td>Ayed et al. 1995.</td>
</tr>
<tr>
<td>Bangladesh</td>
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<td>Belgium</td>
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<td>Benin</td>
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<td>0.15-0.3</td>
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<tr>
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<tr>
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<td>Araj et al. 1995; Naman et al. 1996.</td>
</tr>
<tr>
<td>Malaysia</td>
<td>1.49-1.9</td>
<td>Duraisamy et al. 1993; Ng et al. 1995.</td>
</tr>
<tr>
<td>Mauritius</td>
<td>2.1</td>
<td>Schwartz et al. 1994.</td>
</tr>
<tr>
<td>Mexico</td>
<td>0.7-1.47</td>
<td>Guerrero et al. 1996; Islas et al. 1994; Merino-Conde et al. 1994.</td>
</tr>
<tr>
<td>Morocco</td>
<td>1.1</td>
<td>Benjelloun et al. 1996.</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0.1</td>
<td>Van der Poel et al. 1991.</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2.0-3.0</td>
<td>Oni et al. 1996.</td>
</tr>
<tr>
<td>Northern Eurasia</td>
<td>0.7-10.7</td>
<td>Lvov et al. 1996.</td>
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<tr>
<td>Norway</td>
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<td>Philippines</td>
<td>2.2</td>
<td>Arguillas et al. 1991.</td>
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<td>Poland</td>
<td>2.0</td>
<td>Laskus et al. 1991.</td>
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<tr>
<td>Rodrigues Islands</td>
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<td>Singapore</td>
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<td>Spain</td>
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<td>Esteban et al. 1992; Garcia-Bengoechea et al. 1995; Martinez et al. 1991;</td>
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<tr>
<td>Surinam</td>
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<td>Van der Poel et al. 1991.</td>
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<tr>
<td>Sweden</td>
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<td>Lindholm et al. 1994.</td>
</tr>
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<td>Switzerland</td>
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<td>Zuffrey et al. 1992.</td>
</tr>
<tr>
<td>Taiwan</td>
<td>0.8-1.6</td>
<td>Chen et al. 1991; Luengrojanakul et al. 1994; Tsai et al. 1991.</td>
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<tr>
<td>Togo</td>
<td>3.3</td>
<td>Agbodjan et al. 1995.</td>
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<td>Tunisia</td>
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<td>Slama et al. 1991.</td>
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<td>UK</td>
<td>0.18-0.04</td>
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<td></td>
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<td>al. 1995; Mutimer et al. 1995.</td>
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<td>Ukraine</td>
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<td>Tretskaya et al. 1993.</td>
</tr>
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<td>Anderson et al. 1995; Dawson et al. 1991; Hyams et al. 1992; Liang et al. 19</td>
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<td></td>
<td></td>
<td>94; Murphy et al. 1996; Richards et al. 1991.</td>
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<tr>
<td>Vietnam</td>
<td>0.8-20.6</td>
<td>Song et al. 1994.</td>
</tr>
</tbody>
</table>
1.3. **Hepatitis C virus transmission**

The principal route of transmission of HCV is parenteral, through contaminated blood. Almost all subjects receiving a single unit of HCV-RNA-positive blood ultimately become HCV-infected (Aach et al. 1991), hence any recipient of blood or blood products, particularly before 1990, may be considered at risk of HCV infection. It is now evident that HCV has been the cause of up to 90% of the so called “post-transfusion non-A, non-B hepatitis” (Tremolada et al. 1991) and estimates from UK, France, and Australia indicate that 6,000 100,00 and 200,000 individuals respectively have acquired infection following blood transfusion (Zinn et al. 1995). Since the advent of screening of all blood donations for HCV, the risk of HCV acquisition following transfusion has fallen: at present the chance of becoming HCV-infected with a blood donation that passed all the screening tests is 1 in 103,000 (Schreiber et al. 1996). Besides the use of blood and blood products, other iatrogenic routes of transmission include organ (Pereira et al. 1991), tissue (Conrad et al. 1995) and bone marrow transplantation (Shuhart et al. 1994) from infected donors, haemodialysis (Alter 1995), plasmapheresis (Tao et al. 1991), endoscopy (Andrieu et al. 1995) and colonoscopy (Bronowicki et al. 1997). Transmission of HCV from health care workers (HCW) to patients is rare, although recently an HCV-positive cardiac surgeon transmitted HCV to five patients during open heart surgery (Esteban et al. 1996).

As for most blood-borne infections, injecting drug users are at high risk of HCV acquisition. Several studies, from different geographical areas, report similar prevalence rates of about 70% among injecting drug users and in the US they account for half of all newly infected individuals (Marwick 1997). Recently the use of non-injecting drugs, such as intranasal cocaine, has been significantly associated with HCV-infection (Conry et al. 1996).
Different HCV genotypes may be associated with the different routes of transmission: in Europe genotype 3a is prevalent in young people with an history of injecting drug use or tattooing (Shev et al. 1995), while genotype 1b is the most common subtype in elderly persons and those who have received blood transfusion or with no known risk factors of HCV acquisition (Pawlotsky et al. 1995).

A contaminated hollow needle may be a source of HCV infection even in instances of accidental injury. There are several reports of HCW who contracted HCV infection following needlestick injury (Vaglia et al. 1990; Seeff 1991; Tsude et al. 1992) and recently simultaneous occupational transmission of HCV and Human Immunodeficiency Virus HIV, characterised by a very late seroconversion to both the viruses (after more than 9 months), followed by a rapid and fatal progression of the liver disease, has been documented (Ridzon et al. 1997).

Although animal model studies have suggested that HCV transmission via non-percutaneous routes is unlikely (Suzuki et al. 1993), HCV transmission can occur via sexual and vertical routes, and within households. The viral genome has been detected in semen (Fiore et al. 1995) and vaginal discharges (Tang et al. 1996) of infected patients and cases of sexual transmission have been documented (Healey et al. 1995). However the sexual route has a low efficiency of transmission (Meisel et al. 1995) that increases with length of relationship (Kao et al. 1996), chronic liver disease (Scotto et al. 1996) and co-infection with HIV (Eyster et al. 1991; Soto et al. 1994) or other viruses (herpes simplex virus-2) (Shev et al. 1995). Homosexual men seem not to be at higher risk than heterosexual individuals (Corona et al. 1991). Vertical transmission (mother-to-infant) occurs infrequently (0-12%) (Fischler et al. 1996) and although transmission through two generations has been documented (Inoue et al. 1992), retrospective studies suggest that vertical transmission alone is unable to maintain HCV
infection in the human population (Mansell and Locarnini 1995). Co-infection with HIV increases the risk of vertical transmission (Zanetti et al. 1995), perhaps due to the higher serum levels of HCV-RNA, an independent risk factor for the mother-to-infant transmission (Ohto et al. 1994). The precise mode of HCV vertical transmission remains unclear (Kurauchi et al. 1993): it may occur in-utero, perinatally or even via a post-delivery route, although breastfeeding is probably not an at-risk activity (Lin et al. 1995). Household transmission in the absence of sexual contact, as in the case of siblings of multitransfused or hemodialysis patients, is uncommon (Brackmann et al. 1993; Hou et al. 1995). Nevertheless up to 40% of HCV-infected patients have none of the known risk factors for the infection; thus there must be either unidentified or other parenteral routes of transmission -as demonstrated by the spread of HCV within a haematology ward, despite high levels of infection control (Allander et al. 1995).
1.4. Diagnosis of hepatitis C virus infection

A number of different enzyme-linked immunosorbent assays (ELISA) have been developed to detect circulating IgG antibodies to multiple viral epitopes and confirmatory recombinant immunoblot assays (RIBAs) can be utilised to exclude false positives (de Medina and Schiff 1995), which frequently occur, especially during the screening of low risk groups (McFarlane et al. 1990). The first-generation HCV ELISA was based on the c100-3 epitope from the nonstructural NS4 region; second-generation tests include, along with the c100-3 epitope, the putative core c22-3 and c33c from the NS3 region; the c33c and c100-3 epitopes were then substituted by a larger cloned fragment combining both of them into a c200 protein. The third generation anti-HCV ELISA includes also an antigen from the NS5 region (Figure 1.1). The latest generation serological tests can detect HCV infection 6 to 8 weeks after exposure, compared with 11-12 weeks of the first generation assays (Rubin et al. 1994), but they cannot distinguish between past or present infection, do not indicate the current phase of the disease (acute or chronic) nor predict the disease progression. Anti-HCV antibodies are present in saliva of infected patients and an assay developed to detect IgG of the oral fluids has the same sensitivity and specificity of commercially available serological tests (McIntyre et al. 1996).

At present it is not possible to detect viral antigens in blood or related fluids, thus the "gold standard" in the diagnosis of HCV infection is the detection of the viral genome by mean of RT-PCR (Okamoto et al. 1990). This test is commonly employed to distinguish HCV-seropositive patients with a resolved infection from viraemic patients, particularly among individuals with normal alanine aminotransferase (ALT) levels. In fact normal ALT values cannot exclude liver injury, as a high proportion of patients affected by HCV-associated (histologically diagnosed) chronic hepatitis may have persistently normal aminotransferase levels (Yuki et al. 1994). HCV-RNA is detectable in
the serum of the majority of seropositive patients with abnormal hepatic serological tests, although false negative results are possible, due to very low viraemia or HCV-RNA limited to compartments different from serum (Schmidt et al. 1995).

Recently quantitative HCV-RNA assays have been developed and commercialised (Jacob et al. 1997), viraemia levels being found to be a predictive factor of response to therapy.
1.5. **Natural history of hepatitis C virus-related liver disease**

Acute hepatitis C occurs, in a minority of infected patients, 6 to 12 weeks after acquisition of the virus, while HCV RNA may become detectable in serum one week after acquisition (Farci et al. 1991). The clinical manifestations of this phase, if present, are no different from those of other viral hepatitides: mild and non-specific symptoms such as malaise, anorexia and nausea, possibly accompanied by more specific signs like jaundice and steatorrhoea and elevation of ALT serum levels. Hence the majority of infected patients have no signs of acute hepatic disease. Acute fulminant hepatitis, although possible, is rare (Wright et al. 1991; Yanagi et al. 1991).

Infection with HCV tends to cause chronic hepatic disease: in absence of treatment, up to 78% of patients show elevation of serum ALT levels for more than 6 months; but when serum HCV RNA is used as marker of persistent infection, up to 100% of the patients have been found chronically infected (Colombo 1996). With the exception of the late stages of the disease, chronic hepatitis C is usually an asymptomatic disease, often with normal or minimally elevated ALT; however histological abnormalities are present in the majority of, even apparently healthy, patients (McLindon et al. 1995; Prieto et al. 1995); for this reason liver biopsy is essential in the diagnosis and clinical management of chronically HCV-infected patients (Dusheiko et al. 1996).

Hepatic cirrhosis is a common consequence of chronic hepatitis C; in two large studies from Spain and US, cirrhosis was diagnosed in 30% and 50% of patients respectively (Tong et al. 1995; Colombo 1996); nevertheless, none of 83 women who developed chronic hepatitis following administration of HCV-contaminated Rho (D)-immunoglobulin, showed signs of progressive hepatitis or cirrhosis 15 years after the acute attack (Muller 1996). The reason for such large variations is unclear; patterns of progression may be influenced by patient characteristics, such as gender, age or immunological status, viral factors (viral load, genotypes or quasispecies) or even route of
acquisition. However, on the basis of a large cross-sectional study, chronic hepatitis C has been recently defined as a "progressive fibrotic disease" with a progression towards cirrhosis linear according to time (Poynard et al. 1997). In the same study age at contagion, duration of infection, alcohol consumption and male gender were all factors associated with progression of fibrosis. Different HCV genotypes may also affect the severity of HCV liver disease and although data can be conflicting, an analysis of a group of liver transplant patients infected with different strains of HCV showed good evidence for a more pathogenic course of infection with subtype 1b (Feray et al. 1995). The average time from HCV acquisition to diagnosis of cirrhosis varies between 20 and 30 years (Tong et al. 1995; Colombo 1996; Poynard et al. 1997), although a much more rapid progression of the disease is possible among immunocompromised patients, such as renal graft recipients (Chan et al. 1995) and individuals co-infected with HIV (Eyster et al. 1993).

Hepatocellular carcinoma (HCC) is strongly associated with HCV infection; it is a common finding in follow-up studies of patients with HCV infection of less than 30 years duration (Kiyosawa et al. 1990; Tong et al. 1995). Up to 76% of HCC patients may be HCV-infected (Simonetti et al. 1989) and chronic HCV infection has been suggested as the cause of the recent rise in the incidence of HCC in Japan (Okuda 1996). It has been calculated that the annual cumulative risk of developing HCC is approximately 1% in HCV-positive patients without cirrhosis and 3-10% in those with cirrhosis (Benvengù and Alberti 1996). Carriage of hepatitis B surface antigen (Kaklamani et al. 1991; Chiba et al. 1996), alcohol intake (Miyakawa et al. 1996; Tsutsumi et al. 1996) and possibly HCV genotype 1b (Zein et al. 1996; Bruno et al. 1997) have been indicated as possible co-factors in the development of HCV-associated HCC, but the underlying mechanism of the association of HCV with HCC is still under investigation. Most of the patients with HCC have a previous history of cirrhosis, itself a preneoplastic condition.
(Johnson 1996). Besides HCV does not integrate in the hepatocyte genome and no transforming activity has been demonstrated for any HCV protein, thus an indirect role for HCV in the development of HCC has been proposed, although HCC in inactive or mild chronic hepatitis, not preceded by cirrhosis, has been reported (De Mitri et al. 1995).

In a well-designed multicentre prospective study, the mortality from all causes in a group of patients with post-transfusion non A, non B hepatitis was similar to that of transfused patients who did not developed hepatitis, although in the non A, non B group there was a small but statistically significant increase in the number of deaths related to liver disease (Seeff et al. 1992). The apparent good prognosis of HCV-related liver disease shown in this study may be due to the follow-up period (18 years) shorter than the aforementioned average times for the development of cirrhosis and HCC, in addition although longer prospective studies are not available, the results of other surveys suggest a rather poorer long term prognosis (Tremolada et al. 1992; Tong et al. 1995).
1.6. Extrahepatic manifestations and associated conditions of hepatitis C virus infection

Hepatitis C virus infection can give rise to a broad spectrum of non-hepatic manifestations affecting different tissues and organs (Table 1.3) (Koff and Dienstag 1995; Hadziyannis 1997). The presence of extrahepatic manifestations does not seem to relate to HCV serotype (Pawlotsky et al. 1995).

The best documented association is with essential mixed cryoglobulinaemia, a multisystemic disorder characterised by deposition of immune complexes in blood vessels resulting in a range of manifestations that goes from mild vasculitis with purpura and arthralgia to severe vasculitis with peripheral neuropathy and glomerulonephritis. The triad of purpura, weakness and arthralgia is a common presentation of cryoglobulinaemia. A direct aetiological role for HCV in the pathogenesis of essential mixed cryoglobulinaemia has been suggested on the basis of epidemiological data, detection of HCV-associated antigens in skin biopsy specimens of HCV-positive patients with essential mixed cryoglobulin, and by the frequent clinical resolution of the disease with interferon alfa (INF-α) treatment (Gumber and Chopra 1995), in addition, in instances of HCV relapse after INF-α treatment discontinuation, cryoglobulinaemia also relapses. Hepatitis C-associated mixed cryoglobulinaemia may precede the development of non-Hodgkin’s B-cell lymphoma (Ferri et al. 1994) and prevalence of HCV infection is significantly higher among patients with non-Hodgkin’s lymphoma than control groups (Pioltelli et al. 1996); thus it appears that HCV infection may be involved in the aetiopathogenesis of a particular subset of non-Hodgkin’s lymphoma originating from mixed cryoglobulinaemia.

Membranoproliferative glomerulonephritis may be found in association with chronic hepatitis C and is usually part of the lesions associated with HCV-related mixed cryoglobulinaemia (Johnson et al. 1993).
HCV infection may give rise to porphyria cutanea tarda, a condition due to reduced hepatic uroporphyrinogen decarboxylase activity and clinically characterised by increased skin fragility and cutaneous lesions including erythema, blistering, scarring and pseudoscleroderma, occurring on exposure to sunlight. (Cribier et al. 1995). Other dermatological conditions possibly associated with HCV infection include lichen planus, polyarteritis nodosa, erythema nodosum and urticaria (Pawlotsky et al. 1995).

Thyroid disease, sometimes, but not always with an autoimmune basis, may affect more than 10% of patients with chronic HCV infection (Hadziyannis 1997). A high prevalence of diabetes mellitus has been reported in HCV liver disease (Allison et al. 1994; Grimbert et al. 1996) and a raised frequency of HCV infection has been noted among one group of diabetic patients (Simo et al. 1996); however INF-α therapy may be an aggravating factor in both these HCV-related endocrinopathies.

Autoantibodies against specific and non-specific antigens are a common finding in HCV-positive patients (Clifford et al. 1995) and chronic hepatitis showing features of both autoimmune and HCV-related hepatitis has been described (Bellary et al. 1995).

HCV infection has been reported in patients affected by Behcet's syndrome (Munke et al. 1995) and INF may be an effective treatment of such patients (Hamuryudan et al. 1994); however only one of 224 Turkish patients with Behcet's syndrome was HCV-seropositive (Oguz et al. 1995).

Erythema multiforme triggered by HCV affecting one patient has been described, although oral involvement was not mentioned (Antinori et al. 1991).

Apart from these extrahepatic manifestations there are a number of conditions aetiologically unrelated to HCV infection that, owing to similar routes of transmission or other epidemiological factors, are frequently found in HCV-positive patients. They include co-infection with other hepatitis viruses
(B, D and G) and/or human immunodeficiency virus, injecting drug, haemophilia, etc. and their presence may influence the management of the patients and the progression of the HCV-associated liver disease.
Table 1.3: Extrahepatic manifestations of HCV infection*

<table>
<thead>
<tr>
<th>Category</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological and lymphoid</td>
<td>Mixed cryoglobulinaemia, Aplastic anemia, Idiopathic thrombocytopenia, Non-Hodgkin’s B-cell lymphoma</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Thyroid dysfunctions (hypo- and hyperthyroidism), Diabetes mellitus</td>
</tr>
<tr>
<td>Dermatological</td>
<td>Porphyria cutanea tarda, Lichen planus, Polyarteritis nodosa, Erythema nodosum, Erythema multiforme, Malacoplakia, Behcet’s syndrome, Urticaria</td>
</tr>
<tr>
<td>Salivary gland and ocular</td>
<td>Sialadenitis, Mooren corneal ulcer, Uveitis</td>
</tr>
<tr>
<td>Kidney, neuromuscular and joints</td>
<td>Glomerulonephritis, Muscle weakness, Latent muscular abnormalities, Peripheral neuropathy, Arthritis/arthralgias, Rheumatoid arthritis</td>
</tr>
<tr>
<td>Autoimmune and miscellaneous</td>
<td>Pulmonary fibrosis, Pulmonary vasculitis, Hypertrophic cardiomyopathy, CRST syndrome (systemic sclerosis), Antiphospholipid syndrome, Granulomas, Autoimmune hepatitis type 1 and 2, Circulating autoantibodies</td>
</tr>
</tbody>
</table>

*modified from Hadziyannis 1997
1.7. Therapy of hepatitis C virus infection

Spontaneous resolution of chronic HCV infection is rare and currently the treatment of this condition is INF-α. INF-α is a cytokine produced by B lymphocytes and monocytes following viral infection or antigenic stimulus (Wiranowska and Stewart 1981), its antiviral effects include activation of cellular ribonucleases and inhibition of viral penetration and transcription (Davis and Hoofnagle 1986; Harper 1994).

The standard INF-α regimen in the treatment of chronic hepatitis C is 3 million IU administered subcutaneously three times weekly for six months. Most of the patients have a decrease in serum ALT levels and 40 to 50% have a normalisation of ALT levels (Hoofnagle and M. 1997). Suspension of INF-α therapy is recommended in cases of lack of response after three months, as a late response has been suggested to be unlikely (Sanchez-Tapias and Rodes 1995). An alternative to ALT levels in monitoring INF-α therapy is HCV RNA viral load, that in most patients with a sustained response becomes undetectable within four weeks of initiating therapy (Orito et al. 1995). Unfortunately, about 50% responding patients have a relapse of ALT levels, thus only 25% of patients treated with INF-α have long-term improvement. Thus three response pattern can be described: a) non-response, b) transient response, characterised by an improvement of serological parameters that is lost either during (breakthrough) or after the end of treatment (relapse), c) durable response in which the normalisation of serum ALT and clearance of HCV RNA occurs early in course of treatment and remain unchanged until the end of the follow-up period (Ryff 1997).

Since INF-α is an expensive drug that may induce side effects affecting patient quality of life, many studies have been searching for a way to increase the long-term response-rate modifying the regimen adopted (dose and time) or trying to identify the characteristics that make a patient a better candidate for a long-term response to the therapy. Such “predictors of response”
include viraemia, viral genotype, quasispecies heterogeneity, age and sex, liver histology, hepatic iron content, ALT level, duration and source of infection. A Consensus Panel of the National Institute of Health (NIH) has recently recommended that the length of INF-α treatment should be increased to 12 months, as longer regimens more frequently achieve a sustained response and has indicated as factors associated with a favourable response to INF-α treatment infection with HCV genotypes 2 or 3, serum viral levels below 1 million copies per ml, and absence of cirrhosis (Marwick 1997).

A recent meta-analysis of numerous clinical trials found that INF-α is an effective treatment for the acute phase of the infection, achieving frequent HCV RNA clearance and ALT normalisation (Camma et al. 1996). In addition a recent review suggested that INF-α treatment, especially in high dose or long duration, may benefit HCV-related cirrhosis, reducing the risk of decompensation (Idilman et al. 1997), and of HCV-related HCC (Nishiguchi et al. 1995; Mazzella et al. 1996).

Interferon α therapy causes a transient influenza-like syndrome a few hours after initial administration, generally well controlled with paracetamol and self-limiting. A number of other side-effects may occur in the course of treatment (Dusheiko et al. 1996; Hoofnagle and M. 1997), these include gastrointestinal upsets (nausea, vomiting diarrhoea), weight loss, neuropsychiatric manifestations (irritability, anxiety and depression), hair loss, bone marrow suppression (thrombocytopenia, leucopenia), immune disorders (autoimmune antibodies, thyroid dysfunction) and diabetes mellitus. Some life-threatening side-effects such as myocardiopathies or cerebral vascular haemorrhages have been reported (Bailly et al. 1997) as well as deaths due to severe bacterial infection and suicide. Most of INF-α side-effects are dose-dependent and require reduction or cessation of therapy.

Owing to the numerous side-effects, high cost and variable efficacy of INF-α, several alternative therapies have been tested. To date the only drug that has
shown promising results is ribavirin, an antiviral agent that, combined to INF-α, increases the percentage of sustained responses in patients with chronic hepatitis C disease (Schalm et al. 1996; Reichard et al. 1998).
1.8. Considerations for dental treatment of an HCV-infected patient

HCV infection may seriously affect hepatic function; in particular, patients with long-standing liver disease may have bleeding disorders and a defective metabolism of many drugs; thus HCV-positive patients may need special management when requiring dental treatment (Wisnom and Kelly 1993); Table 1.4 summarises the principal features that may influence the dental management of an HCV-positive patient with impaired liver function.
Table 1.4: Recommendations for the dental management of an HCV-infected patient with impaired liver function

1 With invasive procedures, careful preoperative assessment of the coagulation status of the patient, including:
   - prothrombin time (PT)
   - partial thromboplastin time (PTT)
   - platelet count

2 Great caution in the prescription of medication, in particular:
   - CNS depressant (barbiturates, opioids)
   - hepatotoxic drugs (tetracyclines, erythromycin estolate, monoamine oxidase inhibitors, phenylbutazone)
   - medications that may aggravate the haemorrhagic tendency (aspirin)

3 Consider the influence of possible HCV-associated medical conditions - mainly blood-borne infections (HIV, HBV, HGV).

4 Provide effective oral hygiene prophylaxis in cases of xerostomia, in order to reduce need for operative dentistry
CHAPTER 2.

HEPATITIS C VIRUS INFECTION AND ORAL DISEASES
2.1. Hepatitis C virus infection and oral lichen planus

2.1.1. Review of literature

Introduction

Lichen Planus (LP) is a common mucocutaneous inflammatory disorder of uncertain aetiology (Scully and el-Kom 1985). Because of its relatively high frequency (1-2% in the general population), chronic nature and possible malignant transformation, the management of LP has a major role in day-to-day practice the oral physician has to face. Lichen planus can arise in association with a number of systemic diseases. In some instances these associations probably reflect reactions to drug therapy or represent coincidental concurrence because of the relatively high prevalence of LP, and affected patients being in middle to late life and thus liable to systemic diseases.

Associations between LP and immunologically-mediated diseases, infections and malignancies have long been suggested, but often the evidence is equivocal.

For example while sulphonylurea-like agents can cause lichenoid eruptions (Thompson and Skaehill 1994) there is no definitive evidence of a significant increased frequency of diabetes mellitus in patients with LP and vice versa. Associations with a wide range of autoimmune disorders have been reported but most reports have included only small groups of patients and thus few helpful conclusions can be drawn of their relationship with the pathogenesis of LP. Occasionally LP, often bullous in presentation, can arise in patients affected by malignancies.

Lichen planus is unlikely to have a strong autoimmune basis; there is no association with HLA-DR4 haplotypes and a lack of frequent circulating autoantibodies in high titre in affected individuals (Porter et al. 1997). The lesions of LP have a predominantly CD8+ T lymphocyte infiltrate, probably mediated by local up-regulation of adhesion molecules such as intercellular
adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3). In addition there is an accumulation of antigen presenting cells such as Langerhans cells that may mediate local lymphocyte activation. Lichen planus thus probably represents a cell-mediated response to an antigenic trigger (Porter et al. 1997); nevertheless the identity of this trigger remains unknown.

Lichen planus has been observed in patients with syphilis (Lochner and Pomeranz 1974), chronic bladder infection (Shelley and Shelley 1984), intestinal amoebiasis (Wahba-Yahav 1989), herpes simplex virus type 2 (HSV-2) (Kurkuoglu and Oz 1995) and HIV (Ficarra et al. 1993), but to date there is no evidence of LP having a specific infectious aetiology.
Lichen planus and liver disease

In the last 15 years an increasingly strong association between LP and chronic hepatic disease has been suggested (Table 2.1) (Rebora et al. 1982; Korkij et al. 1984; Mobacken et al. 1984; Powell et al. 1984; Rebora and Rongioletti 1984; Katz and Pisanti 1985; Monk 1985; Scully et al. 1985; Ayala et al. 1986; Cottoni et al. 1988; del Olmo et al. 1989; GISED 1991; Gandolfo et al. 1992; el-Kabir et al. 1993; Bagan et al. 1994). Since the first report of erosive LP in a small group of patients with chronic active hepatitis (Rebora et al. 1978), as many as 64% of some groups of patients with LP, in Spain and Italy, have been reported to have chronic hepatic disease (Ayala et al. 1986; Cottoni et al. 1988; Bagan et al. 1994). In addition, a large case-control study carried out in 27 Italian hospitals between 577 patients with LP and 1031 controls, indicated that the risk of developing LP was increased among patients with a history of liver disease requiring hospital admission or specialist consultation, those who had liver biopsy and those with history of viral hepatitis; in particular the authors suggested that hepatotropic viruses different from the hepatitis B virus, namely the non-A non-B hepatitis virus, might play a significant role in the association between LP and liver disease (GISED 1991).

An association between LP and hepatitis B virus (HBV) infection has been suggested as hepatitis B surface antigen (HBsAg)-positive patients may have double the risk of developing LP compared with HBsAg-negative patients (GISED 1991). In addition there are reports of anti-HBV antibodies in LP patients (Divano et al. 1992; Rebora 1994), of lichenoid eruption following administration of different HBV vaccines (Ciaccio and Rebora 1990; Trevisan and Stinco 1993; Aubin et al. 1994), and of an association of LP with hepatocellular carcinoma - an HBV/HCV linked malignancy (Virgili et al. 1992). Nevertheless the majority of patients with both LP and chronic hepatic disease are not HBV-infected.
A chronic liver disease sometimes described in association with LP is primary biliary cirrhosis (PBC) but, although some cases of oral lichenoid lesions have occurred in PBC in the absence of penicillamine treatment (Graham-Brown et al. 1982; Powell et al. 1982; Oleaga et al. 1995), the association of LP and PBC is mostly due to the administration of this agent (Powell and Rogers 1981; Powell et al. 1982).
Table 2.1: Studies on the possible association between lichen planus and chronic liver disease

<table>
<thead>
<tr>
<th>Reference</th>
<th>Lichen planus study group (n° cutaneous/oral)</th>
<th>Abnormal liver function (%)</th>
<th>Chronic hepatitis (%)</th>
<th>Hepatic cirrhosis (%)</th>
<th>Liver disease overall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rebora et al. 1982</td>
<td>37 mucocutaneous LP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rebora and Rongioletti, 1984</td>
<td>44 mucocutaneous LP</td>
<td>40.9</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powell et al. 1984</td>
<td>3897 LP&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Mobacken et al. 1984</td>
<td>54 oral LP (70.7% erosive)</td>
<td>11.1</td>
<td>3.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Korkij et al. 1984</td>
<td>73 LP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.0</td>
<td>4.0</td>
<td>4.0</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Katz et al. 1985</td>
<td>15 oral LP (100% erosive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monk et al. 1985</td>
<td>55 LP&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scully et al. 1985</td>
<td>113 oral LP (22.1% erosive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ayala et al. 1986</td>
<td>21 oral LP (100% erosive)</td>
<td></td>
<td>9.5</td>
<td>52.3</td>
<td>61.9</td>
</tr>
<tr>
<td>Cottoni et al. 1988</td>
<td>62 LP&lt;sup&gt;c&lt;/sup&gt; (17.7% erosive)</td>
<td></td>
<td>9.6</td>
<td>14.5</td>
<td>25.8</td>
</tr>
<tr>
<td>Del Olmo et al. 1989</td>
<td>65 oral LP</td>
<td></td>
<td>7.6</td>
<td>10.7</td>
<td>33.8</td>
</tr>
<tr>
<td>GISED, 1991</td>
<td>577 LP (3.1% erosive)</td>
<td></td>
<td>18.3</td>
<td></td>
<td>21.4</td>
</tr>
<tr>
<td>Gandolfo et al. 1992</td>
<td>96 oral LP</td>
<td></td>
<td>5.2</td>
<td>7.2</td>
<td>24.0</td>
</tr>
<tr>
<td>El Kabir et al. 1993</td>
<td>180 oral LP (33.3% erosive)</td>
<td></td>
<td>25.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bagan et al. 1994</td>
<td>187 oral LP (42.7% erosive)</td>
<td></td>
<td></td>
<td></td>
<td>21.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> precise characteristics of LP not stated  
<sup>b</sup> significant difference with the control groups  
<sup>c</sup> 62.9% mucosal involvement  
<sup>d</sup> no significant difference with the control groups
Lichen planus and hepatitis C virus infection

Recently an association between hepatitis C virus infection and LP has been suggested that perhaps explains some aspects of the association of LP with chronic hepatic disease.

As previously mentioned, HCV infection can give rise to a wide variety of extrahepatic immunologically-mediated abnormalities including the generation of a number of tissue specific and non-specific autoantibodies (Clifford et al. 1995), cryoglobulinaemia (Gumber and Chopra 1995), autoimmune thyroiditis (Tran et al., 1993), lymphocytic sialadenitis resembling Sjögren's syndrome (Haddad et al. 1992), diabetes (Allison et al. 1994) and possibly non-Hodgkin's lymphoma (Ferri et al. 1994).

The first case of HCV-associated LP was reported by Mokni and colleagues in 1991 (Mokni et al. 1991), the patient was a drug addict with a HCV-related chronic active hepatitis, who develop cutaneous LP few years after HCV acquisition (the oral status was not mentioned). Following this case, many other groups reported cases of possible association between LP and HCV infection; HCV-associated hepatic disease may precede LP onset or may be diagnosed together with it; in the majority of cases mucosal involvement is present together with cutaneous lesions or even on its own (Amichai et al. 1994; Benchikhi et al. 1994; Cecchi et al. 1994; Jubert et al. 1994) and there can be coincident resolution of cutaneous and oral LP with INF-α therapy of chronic HCV-associated hepatic disease (Doutre et al. 1992). Recently a case of coincident oral LP and lymphocytic sialadenitis, resembling Sjögren's syndrome, has been reported (Tanei et al. 1997).

The few studies investigating the frequency of LP among HVC-positive subjects showed that five to 8% of patients with HCV-related chronic hepatic disease may have LP (Pawlotsky et al. 1994; Sata et al. 1996). More data are available on the HCV seroprevalence in groups of patients with LP (Table 2.2). (Divano et al. 1992; Rebora et al. 1992; Bagan et al. 1994; Cribier et al.
Gandolfo et al. 1994; Santander et al. 1994; Bellman et al. 1995; Favia et al. 1995; Nagao et al. 1995; Tanei et al. 1995; Carrozzo et al. 1996; Mignogna et al. 1996; Sanchez-Perez et al. 1996; Dupin et al. 1997; Imhof et al. 1997; Ingafou et al. 1997) The frequency of anti-HCV antibodies in European groups of unselected LP patients varies in different studies from about 4% (Rebora et al. 1992; Cribier et al. 1994) to 34% (Mignogna et al. 1996) but none of 55 English patients with oral LP were HCV seropositive (Ingafou et al. 1997). Twenty three percent of 40 American LP patients were HCV-seropositive by second-generation enzyme linked immunosorbent assay (ELISA) (Bellman et al. 1995), and two studies from Japan reported a prevalence of HCV infection of 37.8% (Tanei et al. 1995) and 62% (Nagao et al. 1995). Notably, in almost all of these investigations the percentage of subjects with HCV infection in the LP study groups was much higher than that of the general population. Indeed in a recent controlled study the prevalence of HCV antibodies in a group of Italian oral LP patients was significantly higher than control subjects such that the authors recommended that patients affected by LP should be systematically screened for the presence of HCV infection (Carrozzo et al. 1996). In the same study a significant association was found between HCV infection and the erosive form of oral LP, confirming the finding of a Spanish survey (Sanchez-Perez et al. 1996) and of a previous study that found a tendency in patients with erosive oral LP to have chronic hepatic disease (Bagan-Sebastian et al. 1992).

It thus appears that there may be geographical differences in the association between HCV infection and LP. Antibodies to the GOR epitope (GRRGQKAKSNPNRPL) (Mishiro et al. 1990) are found almost exclusively in a sub-group of autoimmune hepatitis type 2 patients who are also anti-HCV positive and who represent the majority of autoimmune type 2 hepatitis patients in Italy (80%) but not in the UK (10%) (Michel et al. 1992). While 2 of 36 Italian patients with LP without chronic hepatic disease were HCV
seropositive, none were anti-GOR positive, whereas 11 of 20 patients with LP and chronic hepatic disease were HCV positive and 8 were also anti-GOR positive (Divano et al. 1994). This, considered with the aforementioned data, suggests that the association of LP and chronic hepatic disease may reflect infection with a particular form of HCV infection, although even the great variability of prevalence of HCV infection within countries may have a role. Nevertheless data of French, Japanese and Italian patients, do not suggest any association between a specific HCV genotype and the development of LP (Pawlotsky et al. 1995; Nagao et al. 1996); recently, a German study found that 83% of the patients with HCV-associated LP were infected by subtype 1b; however the authors stated that such a high proportion was more likely to be related to age than that it represents an actual association of the subtype HCV-1b and LP (Imhof et al. 1997). In addition it has been shown that there are no significant differences in serum levels of HCV RNA in infected patients with or without oral LP. Similarly co-infection with hepatitis GBV-C virus, a newly described hepatitis virus sometimes also designated hepatitis G virus (HGV), seems unlikely to play a role in the development of LP in HCV infection (Nagao et al. 1996).

Interestingly HCV-associated oral LP can undergo malignant transformation (Carrozzo et al. 1997); but it seems doubtful that HCV infection alone is a risk factor for the development of oral squamous cell carcinoma as hypothesised by Nagao et colleagues presenting the case of an HCV-positive patients with chronic hepatitis C and no oral lesions who developed oral cancer (Nagao et al. 1996), as a matter of fact the patient had been smoking 20 cigarettes and drinking 360 ml of sake a day for 40 years.

As with primary biliary cirrhosis (Seehafer et al. 1981; Graham-Brown et al. 1982; Powell et al. 1982; Oleaga et al. 1995), the development of LP in HCV infection could represent an immunologically-mediated reaction to therapy - in particular to INF-α , currently the treatment of choice for HCV chronic
infection; indeed some cases of LP onset following INF-α therapy have been reported (Agner et al. 1992; Dupin et al. 1994; Papini et al. 1994; Perreard et al. 1994) as well as worsening of pre-existing LP lesions (Boccia et al. 1993; Protzer et al. 1993; Heintges et al. 1994). However this hypothesis cannot entirely explain the association of HCV infection with LP, as not all affected patients have been treated with INF-α (Carrozzo et al. 1996), there are cases of resolution of LP following INF-α therapy as mentioned above, and recently INF-α was found to be an effective treatment for mucocutaneous LP in a small group of HCV-non-infected patients (Hildebrand et al. 1995). Other drugs that have been successfully adopted in the treatment of HCV-related oral LP include cyclosporine A (Papini et al. 1994), clobetasol (Mokni et al. 1996), cyclophosphamide (Aubin et al. 1994) and glycyrrhizin, a drug widely used in Japan in the treatment of chronic hepatitis C (Nagao et al. 1996).

The underlying aetiopathogenesis of HCV-related LP is unclear. Currently there is little published data of the immunological aspects of HCV-related LP; however a number of pathogenic mechanisms are possible. There may be a cell-mediated cytotoxicity to an epitope shared by HCV and damaged keratinocytes but to date there is no information of the expression of HCV in keratinocytes of the skin or mucosa in HCV-infected persons with or without LP.

It is possible that HCV-related LP has similar pathogenic mechanisms as the idiopathic form: they share the same clinical (Figure 2.1) and microscopic (Figure 2.2) features, including a well-defined CD3+ T lymphocyte infiltrate and up-regulation of key adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), very late activation antigen 4 (VLA-4) and lymphocyte function associated antigen 3 (LFA-3) (Porter et al. 1997). There is however still a need to determine if HCV RNA is expressed within the unaffected and affected mucosa of patients with HCV-related LP, to more precisely
determine if HCV-related LP is a reaction to local HCV-expression or a non-specific immunological reaction.
Table 2.2: Prevalence of hepatitis C virus infection in patients affected by lichen planus

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lichen Planus</td>
<td>HCV seropositivity</td>
</tr>
<tr>
<td>France</td>
<td>Cribier et al. 1994.</td>
<td>52</td>
<td>3.8%</td>
</tr>
<tr>
<td></td>
<td>Dupin et al. 1997</td>
<td>102</td>
<td>4.9%</td>
</tr>
<tr>
<td>Germany</td>
<td>Imhof et al. 1997.</td>
<td>83</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>French a</td>
<td>46</td>
<td>14%</td>
</tr>
<tr>
<td>Italy</td>
<td>Divano et al. 1992.</td>
<td>46</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>Gandolfo et al. 1994.</td>
<td>105</td>
<td>9.5%</td>
</tr>
<tr>
<td></td>
<td>Favia et al. 1995.</td>
<td>82</td>
<td>20.7%</td>
</tr>
<tr>
<td></td>
<td>Mignogna et al. 1996.</td>
<td>178</td>
<td>34%</td>
</tr>
<tr>
<td></td>
<td>Carrozzo et al. 1996</td>
<td>70 a</td>
<td>27.1%</td>
</tr>
<tr>
<td>Japan</td>
<td>Nagao et al. 1995.</td>
<td>45 a</td>
<td>62%</td>
</tr>
<tr>
<td></td>
<td>Tanei et al. 1995.</td>
<td>45</td>
<td>37.8%</td>
</tr>
<tr>
<td>Spain</td>
<td>Bagan et al. 1994.</td>
<td>40 a</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Santander et al. 1994.</td>
<td>50</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>Sanchezperez et al. 1996.</td>
<td>78</td>
<td>20%</td>
</tr>
<tr>
<td>UK</td>
<td>Ingafoü et al. 1997.</td>
<td>55 a</td>
<td>0%</td>
</tr>
<tr>
<td>USA</td>
<td>Bellman et al. 1995.</td>
<td>30</td>
<td>23%</td>
</tr>
</tbody>
</table>

* all oral LP
* no significant difference
* significant difference
* patients with chronic liver disease
* with impaired transaminases
Figure 2.1: Oral lichen planus lesion of the buccal mucosa in a HCV-positive patient.
Figure 2.2: H&E stained section of HCV-associated oral lichen planus biopsy specimen.
2.1.2. Epidemiological studies of the association between oral lichen planus and infection with Hepatitis c virus

Introduction

As discussed before, the frequency of anti-HCV antibodies in European groups of unselected LP patients varies in different studies from about 4% to 34% but none of 55 English patients with oral LP were HCV seropositive. Although HCV prevalence may be higher in Southern Europe (Chiaramonte et al. 1991; Munoz-Gomez et al. 1996) than in Northern European countries such as UK (0.004% HCVAb positive subjects among blood donors) (McLindon et al. 1995), these differences alone may not explain this variation in the association of LP with HCV. Within Italy, striking differences in the frequency of HCV infection amongst patients with LP has been reported, 9.5% to 34% of examined patients being HCV-infected. In addition, few studies have considered the frequency of LP in groups of patients with HCV-associated liver disease.
Aims

Study 1  In view of the lack of data of the frequency of LP in patients with HCV-associated hepatic disease, the aim of the present study was to investigate the frequency of oral LP in a large cohort of patients from central Italy with HCV-related chronic hepatic disease, compared with a similar group of patients with non HCV-related chronic hepatic disease.

Study 2  In addition, as no data on the association of HCV infection and oral LP are available from the metropolitan area of Milan, we investigated the frequency of HCV infection in the sera of a group of oral LP patients from this geographical area.

Study 3  Geographical variation in the distribution of HCV genotypes has been documented, thus in view of the knowledge that HCV genotypes may influence the clinical manifestations of HCV disease and the very limited data of HCV genotype distribution in patients with HCV-related LP, the present study analysed the distribution of HCV genotypes in a cohort of Italian patients with HCV-related oral LP.
Patients and methods

Patients groups

Study 1  The study group comprised 83 Italian patients (39 males; median age 57.0 years, range 24-83), affected by chronic liver disease and with elevated hepatic transaminases for at least 6 months. All patients were HCV seropositive as determined by second generation ELISA and RIBA (Ortho Diagnostic Systems, Raritan, NJ USA and Ortho Diagnostic and Chiron Corp., Emmeryville, CA USA). 35 patients had undergone liver biopsy and thus had a histological diagnosis; this included: chronic active hepatitis (29 cases), chronic persistent hepatitis (4 cases) and cirrhosis (2 cases). The control group comprised 40 patients (24 males, mean age 51.5 years) with non-HCV-associated chronic liver disease, including chronic hepatitis type B, alcoholic cirrhosis, autoimmune chronic hepatitis and primary biliary cirrhosis.

Study 2  The study group comprised 43 consecutive new patients (27 females, mean age 58.5±11.8 range 33-76) with clinical and histopathological features of oral LP, as established by current diagnostic criteria (Scully and el-Kom 1985), attending the Oral Medicine clinic of the University of Milan from January to July 1997. Nineteen patients of this group were affected by the reticular form of oral LP, and in the remaining 24 an atrophic or ulcerative component was present. In the majority of patients (84%) the oral lesions were localised bilaterally at the buccal mucosa, occasionally with the involvement of other mucosal surfaces (mainly tongue and gingiva), while in 7 patients the main localisation was the dorsal surface of the tongue.

Study 3  The patient group comprised 39 Italian patients (24 females; median age 61.0 years, range 30-80), attending Oral Medicine clinics in the University of Turin (n= 20) and the University of Chieti (n=19). All patients had clinical and histological features of oral LP, as established by current diagnostic criteria (Scully and el-Kom 1985). Patients usually had reticular oral LP affecting the buccal mucosa bilaterally; only 5 (13%) had cutaneous
involvement, and in one case vulval lesions were also present. All patients were HCV seropositive as determined by second or third generation ELISA and RIBA (Ortho Diagnostic Systems, Raritan, NJ USA and Ortho Diagnostic and Chiron Corp., Emmeryville, CA USA). Only 19 of the patients had known risk factors for HCV acquisition (surgery-associated blood transfusion in 17 cases and injecting drug use in two cases).

Sixty five percent of the patients had at least one abnormal serological hepatic test (serum aminotransferases, bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase and total protein). Eight of 15 tested patients had cryoglobulinaemia. Sixty percent of patients had undergone liver biopsy and thus had a histological diagnosis; this included: cirrhosis (9 cases), chronic active hepatitis (8 cases), chronic persistent hepatitis (6 cases), primary biliary cirrhosis (2 cases) and primary hepatocellular carcinoma (1 case). Thirteen patients had received INF-α therapy and also in these cases the lesions were bilateral and in no case followed such treatment.
Methods

Study 1  All the subjects underwent a detailed clinical examination of the oral mucosa. The diagnosis of oral LP was based on clinical criteria and confirmed by histological examination of lesional tissue.

Study 2  All the patients were tested for the presence of anti-HCV IgG by means of a second generation ELISA according to the manufacturers’ instructions (Ortho Diagnostic System, Raritan, NJ, USA). The seropositive status of the reactive sera was confirmed by a second generation RIBA.

Study 3  The presence of HCV viraemia was determined by reverse transcription polymerase chain reaction (RT-PCR). RNA was extracted from 100µl of serum using the Amplicor specimen preparation kit according to the manufacturer’s instructions (Roche Diagnostics). The final pellet was resuspended in 50µl of nuclease-free water. cDNA was synthesized in 40µl of reaction mixture containing 22.2µl of RNA solution and 17.8µl of cDNA synthesis mix previously prepared. 3.0µl of 10X PCR buffer (Gibco BRL, Paisley, Scotland), 3.5µl of 50mM MgCl₂ (Gibco BRL, Paisley, Scotland), 3.0µl of 10mM dNTPs (Gibco BRL, Paisley, Scotland), 1.5µl of 0.1M DTT, 1.5µl of random hexamers (20U/ml Pharmacia), 0.5µl Rnasin (Promega), 0.2µl MMLV reverse transcriptase (Gibco BRL, Paisley, Scotland) and 6.8µl of nuclease-free water. The mixture was incubated at room temperature for 10 minutes, heated at 37°C for 45 minutes, at 95°C for 5 minutes and then placed on ice. The HCV nested PCR was carried out using the following primers: oligo 1 (sense) 5’ AGCGTCTAGCCATGGCGT, oligo 2 (antisense): 5’ GCACGGTCTACGAGACCT, oligo 3 (sense) 5’ GTGGTCTGCGGAAACCGG, oligo 4 (antisense): 5’ GGGCACTCGCAAGCACCC. 2ml of first round PCR mix was prepared using 200µl of 10X PCR buffer, 100µl of 50mM MgCl₂, 10µl of oligo 1 (25pmoles/µl), 25µl of oligo 2 (25pmoles/µl), 6µl of Taq polymerase (Gibco BRL, Paisley, Scotland) and 1,659µl of sterile water. 10µl of each cDNA was
added to 40μl of first round PCR mix. The PCR amplification was carried out for 35 cycles, each consisting of denaturation at 94°C for one minute, annealing at 62°C for 1 minute and primer extension at 72°C for 1 minute. 2ml of second round PCR mix was prepared using 200μl of 10X PCR buffer, 100μl of 50mM MgCl₂, 40μl of 10mM dNTPs, 40μl of oligo 1 (25pmoles/μl), 40μl of oligo 2 (25pmoles/μl), 5μl of Taq polymerase (Gibco BRL, Paisley, Scotland) and 1,575μl of sterile water. For the second PCR round the first amplification product was diluted X10 with water, in order to eliminate non-specific PCR products. Then 2μl of each first round PCR product was added to 48μl of the second round PCR mix. The PCR amplification comprised 25 cycles, each consisting of denaturation at 94°C for one minute, annealing at 68°C for 1 minute and primer extension at 72°C for 1 minute. Appropriate positive and negative controls were included. The PCR products were separated by polyacrylamide gel electrophoresis and visualised by ethidium bromide staining. HCV genotyping was carried out by nested RT-PCR, to amplify a 174 bp product from the 5’ non-coding region (NCR), followed by restriction fragment length polymorphism (RFLP) analysis of the amplicon. The restriction enzymes Scrf 1, Hinf 1, Mva 1 and BstU 1 were used to digest the amplicon, and visualised under UV light, after ethidium bromide staining. The band patterns produced by these enzymes can distinguish HCV types 1a, 1b, 2a, 2b, 3a, 3b, 4, 5, and 6 (Pohjanpelto et al. 1995).
**Statistical analyses**

**Study 1**  Comparison between the frequency of oral LP in the two groups was carried out by a chi squared exact test using Epi Info Version 5.0.

**Study 2**  The frequency of HCV seropositivity in the group of oral LP patients was compared to the known frequency of HCV infection in the general population from Northern Italy (3.2%) (Bellentani et al. 1994). Comparison between the frequency of HCV infection in the study group and that in the general population from Northern Italy was carried out by a chi squared exact test (Monte Carlo Method) using SPSS version 7.5 for Windows 97.

**Study 3**  The frequencies of HCV genotypes in the patient group were compared to those of previous studies of HCV-infected Italian patients (Pistello et al. 1994; Ravaggi et al. 1994; Cammarota et al. 1995; Pontisso et al. 1995) (Table 2.3). Comparison between the characteristics of the patients infected with the most common HCV subtypes was carried out by chi squared test using Epi Info Version 5.0 and comparison between means was carried out by t-test for equality of means using SPSS for Windows 6.1.3.
Results

Study 1  Oral LP was diagnosed in thirteen of the 83 (15.6%) patients with HCV-associated chronic liver disease and in only one patient of the control group; this difference was statistically significant by chi squared exact test (p<.05). All affected patients had asymptomatic reticular LP with bilateral involvement of the buccal mucosa; the lack of symptoms may be the reason why such lesions went unnoticed until our visit. Only four of the patients with HCV-associated oral LP had received INF-α therapy (3 millions IU three times a week for one year) and no other patients with oral LP were receiving drug therapy likely to cause lichen planus-like reactions.

Study 2  Six of the 43 patients (14%) were found to be HCV-seropositive (4 females, mean age 60.2±8.0 range 50-71). Four of the HCV infected patients were unaware of their condition and were referred for appropriate management. Three patients required INF-α therapy; this did not affect the oral lesions. The prevalence of HCV infection in this group of patients with oral LP is significantly higher than that of the Northern Italian general population by a chi squared exact test (Monte Carlo Method) (p=0.002).

Study 3  Thirty three (84.6%) of the 39 patients had evidence of viraemia (Figure 2.3). Three of the six patients without viraemia had received INF-α therapy. Seventeen (51%) of the 33 HCV viraemic patients were infected by HCV subtype 1b, 9 (27%) were infected by HCV subtype 2a, 2 (12%) by subtype 1a and 1 by subtype 2b (Figure 2.4). In four cases the gel patterns were uninterpretable.

Comparison of the characteristics of the patients infected with the main HCV subtypes (1b and 2a) did not reveal any statistically significant difference, although the erosive form of the disease was noted to be more common in patients infected with HCV 1b group (Table 2.4).
The most common genotypes found in the present group were 1b and 2a (51% and 27% respectively) and the total percentage of subjects to be infected by type 1 (1a and 1b) was 57%.
Table 2.3: Frequency of distribution of the HCV genotypes 1a, 1b and 2a in 33 Italian patients with oral lichen planus

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Present study</th>
<th>Italian previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichen planus</td>
<td></td>
<td>Cammarota et al\textsuperscript{a}</td>
</tr>
<tr>
<td>1a</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>1b</td>
<td>51%</td>
<td>63.2%</td>
</tr>
<tr>
<td>2a</td>
<td>27%</td>
<td>12%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Patients with community-acquired infection
\textsuperscript{b} Chronic hepatitis C patients
\textsuperscript{c} Acute and chronic hepatitis C patients
\textsuperscript{d} All genotyped as type 1
\textsuperscript{e} All genotyped as type 2
Table 2.4: Characteristics of patients with HCV genotypes 1b and 2a

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HCV 1b</th>
<th>HCV 2a</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Gender ratio (male/female)</td>
<td>6/11</td>
<td>3/6</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Mean age</td>
<td>60</td>
<td>65</td>
<td>ns(^b)</td>
</tr>
<tr>
<td>Erosive vs non erosive form of lichen planus</td>
<td>7/10</td>
<td>2/7</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Chieti vs. Turin</td>
<td>5/12</td>
<td>6/3</td>
<td>ns(^a)</td>
</tr>
</tbody>
</table>

\(^a\) non significant by chi-squared test.
\(^b\) non significant by t-test for equality of means.
Figure 2.3: Detection of HCV-RNA in sera from patients affected by oral lichen planus. The sera of marked with numbers 1, 2, 3, 12, 17, 19 and 22 were HCV-RNA positive.
Figure 2.4: HCV-genotyping of 3 HCV-RNA-positive patients affected by oral lichen planus. Patients 6 and 7 were infected by the 2a subtype while patient 8 by the 1b subtype.
Discussion

The present studies confirm an epidemiological link between oral lichen planus and HCV infection, previously reported by other studies from Italy and other countries.

For the first time the frequency of oral LP in Italian patients with HCV-associated liver disease has been surveyed. The results seem to confirm an association between oral LP and HCV-associated chronic hepatic disease, but not liver disease unrelated to HCV infection. Of note, all cases of oral LP found among our study group were of the reticular type and asymptomatic, while two previous studies reported a putative relationship between HCV infection and the erosive form of oral LP (Carrozzo et al. 1996; Sanchez-Perez et al. 1996). A possible explanation is that the group examined was partially selected, as patients with oral complaints, almost invariably present among subjects with the erosive form of oral LP, might have been already referred to the oral medicine clinic.

The result of the serosurvey seems to agree with the high frequency of HCV infection previously found among oral LP patients. Comparison with the general population's prevalence as performed here may generate a bias which could be compensated in further studies by inclusion of an age- and sex-matched control group. Although the precise reasons for this association remain unclear, routine testing of Italian patients with oral LP for HCV infection may be recommended as they could have hepatic disease requiring INF-α therapy. In addition it must be stressed that none of the patients who were treated with this drug experienced any change of the oral lesions, thus confirming the unpredictable behaviour of oral LP in course of INF-α therapy.

In addition, to determine the viral genetic profile of a group of HCV positive patients affected by oral LP, the viraemic individuals of a group of Italian patients with oral LP were detected and their HCV genotypes distribution analysed. The HCV genotypes distribution found in the present study was
very similar to that reported in a recent multicentre study of 495 Italian patients with chronic hepatitis C, showing 57% of subjects to be infected with HCV-1 and 31% with HCV-2 and to those of other studies of Italian HCV seropositive patients reporting genotype 1b prevalence varying from 54 to 63% (Table 2.3); besides there was no statistically significant difference in the distribution of the two major genotypes according to the area of residence of the present group of patients (Table 2.4). These observations seem to confirm the findings of two previous studies that investigated a group of Japanese oral LP patients (Nagao et al. 1996) and a small group of French patients affected by the cutaneous form of the disease (Pawlotsky et al. 1995), which were unable to detect a significant association between LP and any particular HCV genotype.

In this study it was observed that patients with symptomatic erosive oral LP tended to be infected by the subtype 1b; this variant is possibly associated with more severe liver disease and appears less responsive to INF-α therapy than other genotypes (Sanchez-Tapias and Rodes 1995; Zein et al. 1995); thus it could also be responsible for the development of a more severe form of oral LP; nevertheless a much bigger cohort of patients with HCV-related LP is necessary to determine with more certainty if subtype 1b is correlated with erosive symptomatic LP.

It can be concluded that while Italian patients with LP may be frequently infected by HCV, there is no evidence to suggest that HCV genotypic difference influences this association.
2.1.3. Hepatitis G virus co-infection among patients with hepatitis C
virus-associated oral lichen planus

Introduction

Recently two novel viruses have been isolated by independent groups, from the serum of patients with hepatitis (Simons et al. 1995; Linnen et al. 1996). Although they were designated with different names (hepatitis G virus and GB virus C), it is now believed that they were two isolates of the same virus (Zuckerman 1996), that will be indicated in this text as hepatitis G virus (HGV).

HGV is a single stranded RNA virus, with a genomic organisation similar to that of the Flaviviridae family; shortly after its identification it was suggested that HGV might be a new HCV genotype, but since the two viruses show aminoacid homology not greater than 32%, they are now considered two separate viruses (Zuckerman 1996). Although less variable than HCV (Pickering et al. 1997), HGV is present as a quasispecies in a single individual (Viazov et al. 1997); isolates cloned from different geographic areas have genetic heterogeneity (Kao et al. 1996) and at least three distinct genomic variants have been identified (Katayama et al. 1997).

HGV is transmitted parenterally although less than 50% of recipients of HGV-infected blood or blood products acquire the infection (Roth et al. 1997). This relatively low infectivity has been confirmed in a survey of HGV transmission among organ transplanted patients (Murthy et al. 1997). As for other bloodborne viruses, routes of infection include mother-to-infant (Moaven et al. 1996; Fischler et al. 1997) and maybe sexual transmission (Wu et al. 1997).

Data from blood donations world-wide indicate that HGV infection is not uncommon; 1.4% of randomly selected US blood donors are HGV-RNA positive (Alter et al. 1997); European studies have found HGV prevalences of
1.3%, 3.2% and 4.2% in Germany, the UK and France respectively (Jarvis et al. 1996; Loiseau et al. 1997; Roth et al. 1997); in Asia about 1% of Japanese and Beijing blood donors are HGV-RNA positive (Wang et al. 1997; Yoshikawa et al. 1997), while a higher frequency has been found among healthy Vietnamese (5.7%) (Brown et al. 1997) and 4% of Australian blood donors were found to be HGV-RNA positive (Moaven et al. 1996).

Similar to HCV infection, the patient groups with the highest prevalence of HGV infection include multitransfused patients (10-48%) (Loiseau et al. 1997; Sampietro et al. 1997; Skidmore et al. 1997), patients receiving maintenance hemodialysis (15-55%) (Tsuda et al. 1996; Lampe et al. 1997; Wang et al. 1997) and injecting drug users (33-75%) (Schreier et al. 1996; Stark et al. 1996; Wu et al. 1997).

At present the diagnosis of HGV infection is based on the detection of viral RNA in the serum. HGV-RNA is detectable in the serum of infected individuals 1 to 4 weeks after acquisition (Wang et al. 1996; Shimizu et al. 1997) and it may persist for up to 16 years (Masuko et al. 1996). Assays for the detection of circulating antibodies have been developed but their specificity and sensitivity are still to be confirmed (Dille et al. 1997). In addition it has been reported that detection of an HGV antibody may be associated with loss of HGV viraemia (Tacke et al. 1997).

HGV RNA has also been demonstrated in the saliva of infected individuals (Chen et al. 1997).

Hepatitis G virus may cause acute hepatic disease resembling other viral acute hepatitis (Hwang et al. 1997), indeed HGV has been found in up to 39% of patients with chronic non A-E hepatitis (Fiordalisi et al. 1996) and may be more frequent among blood donors with elevated serum levels of alanine transferase (ALT) than those with normal ALT (Dawson et al. 1996). Nevertheless definitive evidence of significant pathogenic role for HGV in liver disease is still lacking. Only small numbers of patients with acute non-A-E
hepatitis have been found to be HGV infected (Alter et al. 1997; Yashina et al. 1997). The majority of a French group of HGV-infected patients had normal ALT levels (Loiseau et al. 1997) while biochemical evidence of liver damage was absent in 75% of prospectively followed HGV-infected US patients (Alter 1996). Patients with hepatitis caused by HGV can have a mild histological grade of liver damage (Tanaka 1997). In addition a Japanese study showed that although the virus may be persistent for longer than 16 years it seems not to cause notable hepatic injury (Masuko et al. 1996).

It has been proposed that HGV may be a major aetiological agent of fulminant hepatitis (Yoshiba et al. 1995) but this has not been confirmed by other studies (Ishikawa et al. 1997); indeed it has been suggested that HGV infection in fulminant hepatitis may be the consequence of blood transfusion received by patients during their illness (Haydon et al. 1997; Kanda et al. 1997) rather than being the prime aetiological agent. It has however been suggested that some cases of fulminant hepatitis may be due to a mutant strain of HGV (Heringlake et al. 1996).

Because of similar routes of transmission, HGV is frequently found in association with other hepatitis viruses: 10-20% of patients with chronic hepatitis C may be HGV co-infected (Nakatsuji et al. 1996; Schleicher et al. 1996; Bralet et al. 1997; Colombatto et al. 1997; Kao et al. 1997), and the percentage doubles when groups of injecting drug users are considered (Goeser et al. 1997; Kojima et al. 1997); HGV has been found in 13-55% of patients with acute hepatitis C (Nakatsuji et al. 1996; Alter et al. 1997; Colombatto et al. 1997). Although a study suggested that HGV co-infection was more common with HCV genotype 3a (Martinot et al. 1997), a consistent association between HGV viraemia and HCV genotype has not been confirmed (Jarvis et al. 1996; Schleicher et al. 1996). HGV co-infection with HBV seems to be slightly less common, occurring in 3-5% of chronic hepatitis B (Nakatsuji et al. 1996; Kao et al. 1997) and 14-32% of acute HBV illness
(Nakatsuji et al. 1996; Alter et al. 1997). However it seems evident that HGV co-infection does not affect the clinical course in patients with hepatitis A, B or C (Alter et al. 1997; Goeser et al. 1997; Kao et al. 1997; Okamoto 1997; Oshita et al. 1997; Sampietro et al. 1997). HGV co-infection in patients with chronic hepatitis C is not associated with increased liver damage (Bralet et al. 1997; Goeser et al. 1997) and HGV is unlikely to affect the post-transplantation liver disease of HCV positive patients following liver transplantation or survival rates of graft and patients (Berenguer et al. 1996; Fried et al. 1997).

As discussed before, the frequency of the association between HCV infection and LP seems to show distinct geographical variations. This variable distribution cannot be explained only on the basis of the different prevalence of HCV infection. Other factors, such as co-infection with other viruses, may be involved in such association. Thus to determine whether the co-infection with HGV could be a major feature of patients with HCV-associated LP, the prevalence of HGV infection has been examined in a large group of HCV-positive patients with oral LP.
Patients and methods

Study group

The study group comprised 39 Italian patients (24 females, mean age 61.0 years, range 30-80) with a clinical and histological diagnosis of oral LP, as established by current diagnostic criteria (Scully and el-Kom 1985). All were HCV seropositive, as determined by second generation ELISA and second generation RIBA (Ortho Diagnostic Systems, Raritan, NJ USA and Ortho Diagnostic and Chiron Corp., Emmeryville, CA USA). Clinical and serological details of this group of patients have been described in a previous section. 84.6% of this group had detectable HCV RNA in the serum.

Detection of HGV RNA

RNA was extracted from 100μl of serum using the Amplicor specimen preparation kit according to the manufacturer's instructions (Roche Diagnostics). The final pellet was resuspended in 50μl of nuclease-free water. cDNA was synthesized in 30μl of reaction mixture containing 10μl of RNA solution, 3.0μl of 10X PCR buffer (Gibco BRL, Paisley, Scotland), 3.5μl of 50mM MgCl₂ (Gibco BRL, Paisley, Scotland), 3.0μl of 10mM dNTPs (Gibco BRL, Paisley, Scotland), 1.5μl of 0.1M DTT, 1.5μl of random hexamers (20U/ml Pharmacia), 0.5μl Rnasin (Promega), 0.2μl MMLV reverse transcriptase (Gibco BRL, Paisley, Scotland) and 6.8μl of nuclease-free water. The mixture was incubated at room temperature for 10 minutes, heated at 37°C for 40 minutes, at 95°C for 5 minutes and then placed on ice. The HGV nested PCR was carried out using the following primers: oligo 77F (sense): 5' CTCTTTGTGGTAGTAGCCGAGAT, oligo 211R (antisense): 5' CGAATGAGTCAGAGACCAGGGTAT and oligo AS2 (antisense): 5' GTCTTTCATCTCGAGCTGCTCT. 1.4ml of first round PCR mix was prepared using 140μl of 10X PCR buffer, 60μl of 50mM MgCl₂, 20μl of oligo 77F (5pmoles/μl), 20μl of oligo 211R (5pmoles/μl), 5μl of Taq polymerase (Gibco
BRL, Paisley, Scotland) and 1,155μl of sterile water. 15μl of cDNA was heated to 80°C and held whilst adding 35μl of PCR mix. The PCR amplification was carried out for 35 cycles, each consisting of denaturation at 94°C for one minute, annealing at 55°C for 1 minute and 15 seconds and primer extension at 72°C for 1 minute. For the second PCR round 2ml of PCR mix were made using 200μl of 10X PCR buffer, 60μl of 50mM MgCl2, 40μl of 10mM dNTPs, 40μl of oligo 77F (20pmoles/μl), 40μl of oligo AS2 (20pmoles/μl), 5μl of Taq polymerase and 1,615μl of sterile water; 2μl of the first amplification product were added to 48μl of such PCR mix. The PCR amplification comprised 25 cycles, each consisting of denaturation at 94°C for one minute, annealing at 65°C for 1 minute and primer extension at 72°C for 1 minute. Appropriate positive and negative controls were included. The PCR products were separated by polyacrylamide gel electrophoresis and visualised by ethidium bromide staining.

**Statistical analysis**

Comparison between the characteristics of the HCV-positive patients with and without HGV co-infection was carried out by chi squared test using Epi Info Version 5.0 and comparison between means was carried out by t-test for equality of means using SPSS for Windows 6.1.3.
Results

Six (4 female, mean age 61.5 years) of the 39 (15%) HCV-positive patients with oral LP had HGV RNA-positive serum. The clinical details of these patients are shown in Table 2.5. Only one patient was HCV RNA negative, of the remaining five, four were infected by HCV subtype 1b and one by subtype 2a. Of this small group of patients with a dual hepatitis virus infection, four patients underwent liver biopsy: two of them were diagnosed as having hepatic cirrhosis, one chronic active hepatitis and one hepatocellular carcinoma. Among these patients, the most common clinical presentation of oral LP was reticular (4 cases), while the erosive form was affecting the remaining two.

Comparison of the characteristics of the patients with and without HGV infection did not reveal any statistically significant differences (Table 2.6).
Table 2.5: Clinical details of the patients with HCV-associated oral lichen planus (OLP), co-infected with HGV

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Type of OLP</th>
<th>HCV RNA</th>
<th>HCV genotype</th>
<th>Liver Disease</th>
<th>Interferon-alfa</th>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>30</td>
<td>reticular</td>
<td>negative</td>
<td>・</td>
<td>cah(^a)</td>
<td>12 months</td>
<td>ex IDU(^c)</td>
</tr>
<tr>
<td>f</td>
<td>73</td>
<td>reticular</td>
<td>positive</td>
<td>1b</td>
<td>・</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>f</td>
<td>65</td>
<td>reticular</td>
<td>positive</td>
<td>1b</td>
<td>cirrhosis</td>
<td>no</td>
<td>transfusion</td>
</tr>
<tr>
<td>f</td>
<td>66</td>
<td>reticular</td>
<td>positive</td>
<td>2a</td>
<td>cirrhosis</td>
<td>no</td>
<td>surgery</td>
</tr>
<tr>
<td>m</td>
<td>74</td>
<td>erosive</td>
<td>positive</td>
<td>1b</td>
<td>cirrhosis, hcc(^b)</td>
<td>no</td>
<td>transfusion</td>
</tr>
<tr>
<td>f</td>
<td>61</td>
<td>atrophic</td>
<td>positive</td>
<td>1b</td>
<td>・</td>
<td>no</td>
<td>surgery</td>
</tr>
</tbody>
</table>

\(^a\) chronic active hepatitis  
\(^b\) hepatocellular carcinoma  
\(^c\) injecting drug user
### Table 2.6: Characteristics of HCV-infected lichen planus patients with and without HGV co-infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HGV +</th>
<th>HGV -</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>6</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Gender ratio (male/female)</td>
<td>2/4</td>
<td>13/20</td>
<td>ns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean age</td>
<td>61.5</td>
<td>63.3</td>
<td>ns&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Erosive vs non erosive form of lichen planus</td>
<td>2/4</td>
<td>10/23</td>
<td>ns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chieti vs. Turin</td>
<td>3/3</td>
<td>15/18</td>
<td>ns&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> non significant by chi-squared test.

<sup>b</sup> non significant by t-test for equality of means.
Figure 2.5: Detection of HGV-RNA in sera from HCV-positive patients affected by oral lichen planus. Representative results of 14 cases. Sera tested in lane 4 and 12 (arrows) were HGV-RNA positive.
Discussion

As discussed before, the frequency of anti-HCV antibodies in LP patients varies in different studies from about 4% (Cribier et al. 1994) to 62% (Nagao et al. 1995). The reasons of such variations are still unclear; thus in the present study a possible role of HGV co-infection in the development of LP among HCV-positive patients has been investigated.

The proportion of HGV-positive patients in the present group of HCV-infected individuals with oral LP, is similar to those previously reported in groups of patients with chronic hepatitis C (Table 2.7). Thus it appears that HGV infection is not a peculiar feature of subjects with HCV-associated oral LP. In the present study the percentage of HGV infected patients was higher than that found in a Japanese group of oral LP patients (8.8%); however, if one considers only the HCV-positive patients of that group, the percentage of HGV co-infection is almost identical (14.3%).

HGV seems not to influence the response to INF-α in patients with chronic hepatitis C (Berg et al. 1996; Tanaka et al. 1996; Goeser et al. 1997) and HGV itself seems to be sensitive to INF-α; however the regimens employed for HBV and HCV infection seem to be unable to obtain a sustained response: up to 100% of the patients in which HGV viraemia decreased or even disappeared in course of treatment, relapsed to pre-treatment levels at the end of therapy (Baba et al. 1997; Fujikawa et al. 1997; Karayiannis et al. 1997). Of this small group of patients with dual HGV and HCV infection only one patient was HCV RNA negative, a former injecting drug user who had been treated for twelve months with INF-α; it is likely that he acquired both HCV and HGV before the INF-α treatment. This might confirm that such therapy has no efficacy in eradicating HGV.

Of the five HCV RNA positive patients, four were infected by the genotype 1b, the most common among Italian HCV positive patients with and without oral LP, as discussed in the previous chapter.
Most of the HGV co-infected patients were affected by the reticular form, the mildest of the possible clinical presentation of oral LP, thus indicating that HGV co-infection may not be an aggravating factor for the clinical features of oral LP in HCV-positive patients.

HGV and HCV are likely to share similar routes of transmission, for this reason they may frequently co-infect patients at risk for blood-borne infections. However, it seems that, as for other viral factors including HCV genotype and HCV-RNA serum level, co-infection with HGV does not seem to play a relevant role in the pathogenesis of HCV-associated oral LP.
Table 2.7: Frequency of HGV co-infection in patients with chronic hepatitis C

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Number of patients</th>
<th>HGV-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schleicher et al. 1996</td>
<td>Germany</td>
<td>100</td>
<td>19 (19%)</td>
</tr>
<tr>
<td>Nakatsuji et al. 1996</td>
<td>Japan</td>
<td>126</td>
<td>15 (12%)</td>
</tr>
<tr>
<td>Bralet et al. 1997</td>
<td>France</td>
<td>105</td>
<td>17 (15%)</td>
</tr>
<tr>
<td>Kao et al. 1997</td>
<td>Taiwan</td>
<td>100</td>
<td>10 (10%)</td>
</tr>
<tr>
<td>Colombatto et al. 1997</td>
<td>Italy</td>
<td>117</td>
<td>13 (11.1%)</td>
</tr>
<tr>
<td>Kojima et al. 1997</td>
<td>Japan</td>
<td>189</td>
<td>22 (11.6%)</td>
</tr>
<tr>
<td>Goeser et al. 1997</td>
<td>Germany</td>
<td>70 *</td>
<td>18 (27.5%)</td>
</tr>
</tbody>
</table>

* all intravenous drug users
2.1.4. Antibodies to epithelial components in lichen planus associated with hepatitis C Virus infection

Introduction

Cell-mediated immunity is considered to play a major part in LP pathogenesis (Eversole 1994) and while the role of humoral immunity may not be of primary importance, patients with oral lichenoid lesions have been reported to have circulating antibodies to nuclear (Parodi and Cardo 1989) and basal membrane zone (Lamey et al. 1995) antigens of stratified epithelial substrates. In addition, an oral mucosal disease, clinically similar to LP but characterised by epithelial depositions of anti-nuclear antibodies, has been described in a small group of patients of unknown HCV status. Moreover, patients with HCV infection frequently have autoimmune disease (Pawlotsky et al. 1994) and circulating tissue-specific and non-specific autoantibodies (Clifford et al. 1995). In the present study, in order to assess the possible role of humoral immunity in the development of LP in HCV infection, the sera of HCV-positive patients with and without LP have been examined for the presence of circulating antibodies to epithelial antigens.
Materials and Methods

Study groups

Four groups of patients were included in this study:

1. Patients with HCV infection and oral LP (HCV+ve/LP+ve): 14 caucasian patients known to be HCV-infected, as determined by second generation HCV ELISA and 4-antigen RIBA assays. Six had liver biopsies showing histological features of chronic active hepatitis and had been treated with different regimes of IFN-α. Previous blood transfusions and previous injecting drug use were identified as HCV risk activities in 3 and 1 of these patients, respectively. All of these 14 had clinical and histological features of non-erosive oral LP.

2. Patients without HCV infection affected by oral LP (HCV-ve/LP+ve): 14 anti-HCV seronegative caucasian individuals with clinical and histological features of non-erosive oral LP.

3. Patients with HCV infection without oral LP (HCV+ve/LP-ve): 21 HCV-seropositive caucasian patients who had no mucocutaneous disease. These sera were taken at the time of the diagnosis and thus none of these patients had received IFN-α therapy.

4. Patients without HCV infection and without oral LP (HCV-ve/LP-ve): 18 healthy caucasians who were HCV-seronegative and had no mucocutaneous disease.

All of the above clinical and histological diagnoses of oral LP were made on the basis of World Health Organization criteria (Kramer et al. 1978).

None of the above subjects had received any drug therapy (other than IFN-α, as noted in the text) likely to induce lichenoid reactions (Thompson and Skaehill 1994).
Indirect Immunofluorescence

Circulating antibodies against epithelial antigens in the sera were detected by an indirect immunofluorescence technique, using pre-fixed sections of monkey oesophagus (BioDiagnostics Ltd. England) as substrate. Sections were washed with phosphate-buffered saline (PBS) and incubated with 20% normal goat serum (NGS) (Dako Ltd. England) in PBS for 30 min to block non-specific binding. All incubations were carried out at room temperature in a humidified chamber. Sera were diluted as indicated in PBS containing 20% NGS and incubated on the sections for 1 hour, and then washed with PBS 3 times for 15 min each. Serum antibodies reactive with mucosal antigens were detected using FITC-conjugated goat anti-human IgG (Fc-specific) (Sigma Ltd.) diluted 1:60 in PBS containing 20% NGS. After 45 min of incubation, the sections were washed 3 times with PBS and mounted with FITC Mounting Medium (BioDiagnostics Ltd.). Fluorescence was visualized using a Zeiss Axioskop microscope. Sera were scored as positive for each particular staining pattern when they produced fluorescence at a dilution of 1:100 or more. End-point titres were determined for each of the positive serum. Negative controls were sections incubated with sera from healthy individuals (HCV-ve/LP-ve group) and sections incubated with NGS/PBS only.

Statistical analysis

Statistical analysis was carried out with the Fisher two-tailed exact test, employing the Nanostat (Alpha Bridge Ltd 1992) computer analysis program. Three tests were carried out comparing the following groups: HCV+ve/LP+ve vs HCV-ve/LP+ve; HCV+ve/LP+ve vs HCV+ve/LP-ve; HCV+ve/LP+ve vs HCV-ve/LP-ve.
Results

As shown in Table 2.8, antibodies which recognised antigens expressed in stratified epithelium were detected in the serum of a total of 8 of the 14 patients in the HCV+ve/LP+ve group. However, the pattern of staining was not uniform for all sera, three different fluorescence staining profiles being readily detected. Figure 2.6 shows the reactivity against epithelial cell nuclei (pattern I). This punctate staining was observed with the sera of 6 of the patients, having titres from 1:100 to more than 1:1000. Two of these patients' sera also exhibited staining pattern II, a marked pericellular or membrane-associated reactivity against epithelial cells in the suprabasal region (Figure 2.6). One other serum of this group gave only the latter intense surface staining, with no anti-nuclear reactivity (not shown). A third pattern was also produced by the serum of another HCV-positive/LP-positive patient. As shown in Figure 2.7, fluorescence staining pattern III was characterised by punctate granules distributed throughout the cytoplasm of all epithelial cells, frequently being excluded from the nuclear region.

Despite the marked differences in specific staining profiles produced by the sera of these patients, none of the other 53 sera samples examined was found to give a positive reaction using the same standard mucosal assay specimen (monkey oesophagus). The results in Table 2.8 show that none of the 14 patients with LP only, and none of the 21 with HCV only, produced any detectable immunofluorescence when tested at a dilution of 1:100 or greater. The concomitance between LP and HCV infection and the presence of circulating antibodies to epithelial antigens was found to be statistically significant when compared with the other two patient groups (HCV-ve/LP+ve and HCV+ve/LP-ve) or with the normal controls (HCV-ve/LP-ve group) (p<0.01). It was not possible to assess whether there was any significant association between the extent of the oral lesions and the presence of circulating antibodies.
In view of the well-known propensity of certain drugs to elicit lichenoid reactions (Thompson and Skaehill 1994), which recent reports have suggested are associated with the concurrent presence of anti-epithelial antibodies (Parodi and Cardo 1989), in the present study we also examined the relationship between IFN-α therapy and immunoreactive sera. Although 5 of the 8 patients in the HCV+ve/LP+ve group who had circulating antibodies against epithelial antigens had been treated with IFN-α (for at least six months), 3 patients in the same antibody-positive patient group had received no drug therapy. Conversely, while 5 of the 6 HCV+ve/LP+ve group of patients whose sera produced no reaction against antigenic determinants expressed by epithelial tissue had not been treated with IFN-α, it is notable that there was one patient who had received long-term IFN-α therapy but was nevertheless in this antibody-negative group. Statistical analysis of this data showed that the presence of anti-epithelial antibodies was not significantly associated with IFN-α therapy (p=0.24).
Table 2.8: Immunofluorescence staining patterns of sera of patients with HCV-related and non-related oral lichen planus, patients with HCV infection and healthy controls

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Number of positive sera (% of total)</th>
<th>Staining pattern</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>HCV</td>
<td>LP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Staining patterns were anti-nuclear (I), anti-membrane (II) and anti-cytoplasmic (III), as described in the text.

*b* Patterns I and II were produced together by the sera of 2 patients.
Figure 2.6: Immunostaining profile of serum from one patient of the HCV+ve/LP+ve group against monkey oesophagus, showing reactivity against epithelial nuclei (pattern I; arrowheads) and against epithelial antigen in the basal layer (pattern II; arrows) (original magnification x 400).
Figure 2.7: Punctate cytoplasmic staining (pattern III) produced by serum from a different patient in HCV+ve/LP+ve group (original magnification x 400). Note the apparent absence of anti-nuclear reactivity in many cells (arrowheads).
Discussion

HCV infection has been associated with a number of immunologically-mediated disorders including mixed cryoglobulinaemia, membranoproliferative glomerulonephritis, autoimmune hepatitis, autoimmune thyroiditis and Sjögren's syndrome (Gumber and Chopra 1995). Moreover, tissue-specific and non-specific autoantibodies are frequently present in the sera of some groups of patients with chronic HCV infection (Clifford et al. 1995).

In the present study we have found that patients with HCV who develop oral LP have relatively high titres of circulating antibodies directed against antigens expressed in oral mucosa. Notably, this type of immune reactivity was not detected in the sera of patients who have HCV but not LP; nor was there anti-epithelial activity in the sera of oral LP patients without HCV. While the aetiopathogenic role of antibodies directed against epithelial antigens in systemic autoimmune pathologies such as pemphigus and pemphigoid is well documented (Eversole 1994), the presence of anti-epithelial immunoglobulins in some LP patients and in drug-induced lichenoid reactions (DLR) has not yet been explained. In one of the previous studies describing circulating antibodies in LP (Parodi and Cardo 1989), no data were presented about the HCV status of the two patients described, most probably because serodiagnostic tests for HCV were not readily available at that time. It is notable, however, that one of the patients was affected by chronic hepatitis and the other was seropositive for antibodies against hepatitis B virus surface antigens. In addition, although antibodies producing a pattern similar to the type II described in the present study have previously been associated with DLR (Lamey et al. 1995) and IFN-α therapy (Fleischmann et al. 1996), we have not been able to confirm the relationship between IFN-α therapy and the appearance of similar antibodies since none of the patients in the three control groups had received this drug. The type II staining pattern was,
however found in one of the HCV+ve/LP+ve patients who did not receive IFN-α therapy, showing that the presence of this type of circulating antibodies is not necessarily linked to IFN-α therapy. Whether these abnormal humoral responses we have described is directly elicited by viral presence in the epithelium of HCV-infected patients or reflects the expression of cross-reactivity between HCV determinants and mucosal epitopes is not yet known. However, despite our finding that there is a highly diverse immunological response between different patient groups, there is nevertheless an apparent similarity in the clinical outcome. This suggests that a number of multiple factors, including certain drugs, may act by a similar molecular mechanism to elicit the development of LP and LP-like lesions.
2.1.5. Detection of the hepatitis C virus genome and antigens in the oral mucosa of patients with hepatitis C virus-associated oral lichen planus

Introduction

Although a number of studies have investigated possible aetiological agents for oral LP, including host and environmental factors as well as infectious agents, no definitive aetiology for this disease has thus far been identified. There is, nevertheless, substantial evidence suggesting an immunological basis, mainly cellular, for the pathogenesis of oral LP. Histologically, oral LP is characterized by a dense sub-epithelial mononuclear cell infiltrate, consisting mainly of T lymphocyte, damage to basal epithelial cells and alterations in epithelial architecture (atrophy, acanthosis, hyperkeratosis). It has been suggested that keratinocytes, particularly the cells of the basal membrane zone, are the main target of a cytotoxic lymphocyte-mediated process. This immune process is also likely to involve antigen-presenting Langerhans cells and a complex network of cytokines and adhesion molecules and may be triggered by an as yet unknown modification of an epithelial cell antigen.

The exact mechanisms underlying oral LP are still to be elucidated, but a possible role for viral agents has previously been suggested (Boyd and Neldner 1991), despite the lack of definitive aetiological evidence. Human papillomavirus has been detected in the oral tissue of a high proportion of patients with oral LP (Syrjanen et al. 1986; Maitland et al. 1987; Jontell et al. 1990; Kashima et al. 1990), although its presence has also been demonstrated in normal oral mucosa and other oral conditions (Maitland et al. 1987; Kashima et al. 1990). In addition, a recent report has suggested a possible association between oral LP and Epstein-Barr virus (EBV) on the basis of up-regulated expression of EBV receptors (CD21) (Walsh et al. 93
1990) and abnormal humoral response to EBV in oral LP patients (Pedersen 1996).

On the basis of epidemiological evidence of a link between LP and HCV infection (see previous section), an aetiopathogenetic role for HCV has also been suggested. The characteristic features of the cell-mediated immune response seen in LP may be a direct result of the presence of a viral epitope expressed on the surface of infected keratinocytes or other antigenic alterations induced by the presence of HCV in oral tissue. Alternatively, it is also possible that oral mucosal epithelial cells may share a common epitope with antigens expressed by hepatocytes damaged by HCV infection (Rebora 1994; Porter 1995) and thereby become a target of immune reaction.

Aims

The presence of HCV in oral LP tissue in HCV-positive patients could indicate a direct involvement of the virus in the mechanisms leading to epithelial damage. Thus, the aim of the present study was to examine the presence of HCV in samples of HCV-associated oral LP. Since the presence of HCV in body fluids or tissue is currently assessed by the demonstration of the viral genome and/or viral antigens, molecular biological and immunohistochemical techniques have been selected in order to detect the possible presence of HCV in samples of oral HCV-associated LP.
Materials and Methods

Detection of HCV RNA

**Tissue specimens:** Lesional tissue was obtained from 16 patients with clinical and histological features of oral LP affecting the oral mucosa, based on current diagnostic criteria (Scully and el-Kom 1985). In addition, two samples from patients with oral carcinoma developed from malignant change of oral LP lesions were included in the study. All of these patients were HCV positive as determined by second generation ELISA and second generation RIBA (Ortho Diagnostic Systems, Raritan, NJ USA and Ortho Diagnostic and Chiron Corp., Emmeryville, CA USA). Tissue samples were repeatedly washed in physiological saline and then mounted in Tissue-Tek medium (Raymond A Lamb, London, UK), immediately frozen in liquid nitrogen and stored at -70°C until required.

**RNA extraction:** For each of the biopsy samples, 10 cryostat sections of 20μm were cut, added to 800μl of Trizol (Gibco BRL, Paisley, Scotland) and vortexed vigorously. 160μl of chloroform were added and after 5min at room temperature, the samples were centrifuged at 10,000 x g for 15min at 4°C. The aqueous upper phase containing the RNA was then removed, added to 400μl of isopropanol, kept at -70°C for 10min and spun at 10,000 x g for 10min at 4°C. The RNA pellet was washed with 75% ethanol, dried and resuspended in 50μl of 0.1% diethyl pyrocarbonate (DEPC) treated water. A nested RT-PCR reaction for HCV was then carried out as previously described. The HCV genotype was also determined, as described in a previous section.

**Immunohistochemistry**

**Frozen samples:** Specimens of healthy buccal mucosa were obtained from 6 HCV-seronegative patients undergoing routine oral surgery in the Eastman Dental Hospital, London, UK. Samples of OLP buccal mucosa were obtained from the lesional sites of 10 HCV-seropositive patients of the Dental School,
University of Turin, Italy. All tissues were stored at -70°C until required. The day preceding the staining procedure, cryostat sections of each tissue sample were cut to 7μm thickness, mounted on Superfrost slides (Merck Ltd., Poole, UK) and left to dry overnight. Sections were fixed in ice-cold acetone-chloroform (1:1) for 10min, washed with Tris-buffered saline (TBS), placed in 2.5% hydrogen peroxide in methanol to block endogenous peroxidase activity and incubated with 20% normal goat serum (NGS) in TBS (TBS/NGS) for 30min to block non-specific binding.

**Paraffin-embedded samples:** Sections cut from formalin-fixed, paraffin-embedded samples of lesional tissue were obtained from 12 oral LP patients attending the Oral Medicine Clinic of the Eastman Dental Institute (n=8) and the Oral Medicine Clinic of the Dental School, University of Turin, Italy (n=4). A total of 8 of these oral LP patients were also HCV-positive for anti-HCV antibodies by ELISA and for HCV-RNA by RT-PCR. In addition, sections of liver specimens of 6 patients (3 HCV-positive and 3 HCV-negative) were kindly provided by the Department of Histopathology, Addenbrooke’s NHS Trust, Cambridge, UK. The liver specimens were obtained from needle biopsy or hepatectomy at transplantation. Before the staining procedure, the paraffin-embedded sections were deparaffinized in xylene for 10min, rehydrated in descending concentrations of alcohol, washed with TBS and placed in 2.5% hydrogen peroxide in methanol to block endogenous peroxidase activity. Based on the results of previous studies the visualization of low-expressed HCV antigens in paraffin-embedded sections is best carried out after "retrieval". The sections were treated for 10min at 37°C with 0.2% chymotrypsin (Sigma Ltd., Poole, UK) in 0.1% calcium chloride (pH7.8). After washing with TBS, the sections were incubated with 20% TBS/NGS for 30 min to block non-specific binding.
**Immuno-staining procedure:** All incubations were carried out in a humid chamber. The following anti-HCV primary murine monoclonal antibodies (mAbs) were employed:

1. anti-c22-0 mAbs recognizing the core protein (mAb 1);
2. anti-c100-3 mAbs recognizing amino acids (AA) 1690-1696 (mAb 2);
3. anti-c100-3 mAbs recognizing AA 1694-1711 (mAb 3);
4. anti-c100-3 antibody recognizing a linker sequence at the junction of superoxide dismutase and HCV sequences in the NS4-encoded protein (SOD/NS4) (mAb 4);
5. anti c33-c recognizing a structural determinant in the NS3-encoded protein (mAb 5).

All mAbs were provided by Dr Mitchell J Nelles (Ortho Diagnostic System, Raritan, NJ, USA) and were produced in mice by using recombinant antigens as immunogens. All primary mAbs were applied for 18h at 4°C at a working dilution of 5μg/ml. After incubation with the primary mAbs, the sections were then washed thoroughly with several changes of TBS, incubated for 2h with biotin-conjugated goat anti-mouse Ig (Dako Ltd., High Wycombe, UK) diluted 1:200 in TBS/NGS, washed thoroughly with PBS and incubated for 1h with peroxidase-conjugated Extravidin (Sigma Ltd., Poole, UK). The Vector DAB Peroxidase Substrate Kit (Vector, Peterborough, UK) was prepared and incubated on the sections for 5 min to visualize the antigens. The AEC Peroxidase Substrate Kit (Vector, Peterborough, UK) was used on the formalin-fixed sections. The reaction was terminated by washing the sections with TBS and the sections counterstained with Mayers haematoxylin and mounted in Aquamount (Merck Ltd., Poole, UK). Isotype matched irrelevant mouse IgG were used as negative controls.

Examination and photography of sections was carried out using an Olympus BX50 microscope fitted with appropriate filters and PM30 control unit.
Results

HCV RNA in oral LP tissue

HCV RNA sequences were detected by RT-PCR in 3 of the 16 HCV-associated oral LP specimens (19%) and in one of the two oral carcinoma which developed from malignant change of HCV-associated oral LP lesions. HCV genotype analysis of the positive samples showed infection with subtype 1b in two of the oral LP and subtype 2a in the remaining one. The genotype of the oral carcinomata tissue was 1b.

Expression of HCV antigens in oral LP tissue

Table 2.9 summarizes the results of the immunohistochemical detection of HCV-associated antigens in the lesional tissue of patients with oral LP, with and without HCV infection.

Frozen samples: Six out of 10 specimens of HCV-positive patients expressed HCV-associated antigens compared with none of the specimens from HCV-negative patients. However, the HCV antigens in 3 of these samples were detected by 3 different mAbs, while one other mAb detected HCV antigens in 3 separate patient biopsies. Thus, as shown in Table 2.9 A, while mAbs 2, 4 and 5 each visualized a different sample, mAb 1 detected HCV antigens in 3 other patients. Moreover antigen expression in HCV-associated oral LP occurred in three distinct patterns. In the first, which was the most common, there was staining of a small proportion of scattered inflammatory cells localized in the subepithelial infiltrate (Figure 2.8), as shown by mAb 1 (2 patients) and by mAb 4 and 5 in one sample each. mAb 1 also stained the upper epithelial layers of one biopsy; in particular the layers where the keratinocytes have lost nuclei, as shown in Figure 2.9. In another specimen mAb 2 stained most of the epithelial nuclei of some intermediate cellular layers (Figure 2.10).

Paraffin-embedded samples: Three out of 8 paraffin-embedded specimens from HCV-positive patients reacted with anti-HCV mAbs and, in all three
cases, the putative HCV-associated antigens were detected by the same mAb (mAb 2). In one of these the positive staining was limited to some scattered cells within the inflammatory infiltrate (Figure 2.11), while in the other two it was observed in epithelial cells of the suprabasal region (Figure 2.12 a + b). Notably, none of the specimens from HCV-negative patients showed positive staining for HCV antigens, although in one of this group a weak, diffuse and possibly background epithelial staining was noted with the same mAb 2. In addition, mAb 2 gave positive staining in 2 of 3 liver samples from HCV-positive patients, with foci of intensely positive hepatocytes scattered over much of the sections (Figure 2.13). These 2 patient samples also gave positive staining with three of the other mAbs (3, 4 and 5), although it is not clear whether this was due to possible cross-reaction with bile and bile ducts. In two of the liver samples from HCV-negative patients a strong but diffuse background staining was evident with mAb 2 only.
Table 2.9: Immunohistochemical staining observed in samples of oral and liver tissues

(a) Unfixed frozen substrates

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Patient HCV status</th>
<th>mAb 1 anti c22-0</th>
<th>mAb 2 anti c100-3 (1690-1696)</th>
<th>mAb 3 anti c100-3 (1694-1711)</th>
<th>mAb 4 anti c100-3 (SOD/NS4)</th>
<th>mAb 5 anti c33-c (NS3)</th>
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</thead>
<tbody>
<tr>
<td>OLP1</td>
<td>pos neg</td>
<td></td>
<td>positive epithelial nuclei</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP2</td>
<td>pos neg</td>
<td></td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP3</td>
<td>pos positive upper epithelial layers</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP4</td>
<td>pos scattered positive inflammatory cells</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP5</td>
<td>pos neg</td>
<td></td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP6</td>
<td>pos neg</td>
<td></td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
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<td></td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP8</td>
<td>pos neg</td>
<td></td>
<td>neg</td>
<td>neg scattered positive inflammatory cells</td>
<td>neg</td>
<td>neg</td>
</tr>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
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<td></td>
<td>neg</td>
<td>neg scattered positive inflammatory cells</td>
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<td>neg</td>
</tr>
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<td>neg neg</td>
<td></td>
<td>neg</td>
<td>neg</td>
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<td>neg</td>
</tr>
<tr>
<td>OLP13</td>
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<td>neg</td>
</tr>
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<td>neg</td>
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</table>
(b). Fixed, paraffin-embedded substrate

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Patient HCV status</th>
<th>mAb 1 anti c22-0 (1690-1696)</th>
<th>mAb 2 anti c100-3 (1694-1711)</th>
<th>mAb 3 anti c100-3 (SOD/NS4)</th>
<th>mAb 4 anti c100-3 (NS3)</th>
<th>mAb 5 anti c33-c (NS3)</th>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP19</td>
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<td>neg</td>
<td>positive epithelial cells</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP20</td>
<td>pos</td>
<td>neg</td>
<td>scattered positive inflammatory cells</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP22</td>
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<td>neg</td>
<td>positive epithelial cells</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>OLP23</td>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
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<td>neg</td>
<td>neg</td>
<td>weak background</td>
<td>neg</td>
<td>neg</td>
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<td>neg</td>
<td>neg</td>
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<td>neg</td>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>liver1</td>
<td>pos</td>
<td>neg</td>
<td>groups of positive hepatocytes</td>
<td>cross reaction with bile ducts (?)</td>
<td>cross reaction with bile ducts (?)</td>
<td>cross reaction with bile ducts (?)</td>
</tr>
<tr>
<td>liver2</td>
<td>pos</td>
<td>neg</td>
<td>groups of positive hepatocytes</td>
<td>cross reaction with bile ducts (?)</td>
<td>cross reaction with bile ducts (?)</td>
<td>cross reaction with bile ducts (?)</td>
</tr>
<tr>
<td>liver3</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
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</tr>
<tr>
<td>liver4</td>
<td>neg</td>
<td>neg</td>
<td>strong background</td>
<td>neg</td>
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<td>neg</td>
</tr>
<tr>
<td>liver5</td>
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<td>strong background</td>
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<td>liver6</td>
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</table>
Figure 2.8: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Positive cell of the inflammatory infiltrate. Antibody used: anti-c22-0 (mAb 1) (original magnification x 400).
Figure 2.9: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Intense staining of the keratinized layers. Antibody used: anti-c22-0 (mAb 1) (original magnification x 80).
Figure 2.10: Expression of putative HCV antigens in oral lichen planus lesional tissue (frozen sample). Intense nuclear staining of numerous keratinocytes. Antibody used: anti-c100-3 (mAb 2) (original magnification x 160).
Figure 2.11: Expression of putative HCV antigens in oral lichen planus lesional tissue (paraffin-embedded sample). Positive cells of the inflammatory infiltrate. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400).
Figure 2.12: Expression of putative HCV antigens in oral lichen planus lesional tissue (paraffin-embedded sample). (a) staining of suprabasal keratinocytes (original magnification x 160), (b) cytoplasmic staining of suprabasal keratinocytes, characterised by a granular appearance. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400).
Figure 2.13: Expression of putative HCV antigens in HCV-positive liver tissue (paraffin-embedded sample). (a) group of positive hepatocytes (original magnification x 160), (b) cytoplasmic staining of hepatocytes, characterised by a coarse granular appearance. Antibody used: anti-c100-3 (mAb 2) (original magnification x 400).
Discussion

Detectable levels of HCV-RNA have, for the first time, been demonstrated in tissue samples of oral lesions from HCV-positive patients (two oral LP and one oral carcinoma). This is in contrast with a previous report, in which HCV-RNA was not detected in tissues from 6 cases of oral cancer and also from tumor cell lines derived from oral cancer (Nagao et al. 1995). Inability to detect HCV RNA may have been due to the RT-PCR primers or conservation and preparation of the samples or possibly to the different nature of the lesions examined. In the present study the HCV RNA-positive oral carcinoma originated from a malignant change of HCV-associated oral LP. However, HCV-RNA was not detected in all oral LP samples, suggesting either that (1) the presence of the viral genome in the tissue at the time of biopsy is not necessarily related to the clinical presence of the HCV-associated oral LP mucosal lesions or that (2) in many cases the level of HCV-RNA is below the detectability limits. Indeed, HCV RNA levels in affected liver are known to be very low and it can be expected to be even lower in possible extrahepatic localizations.

The amplified HCV RNA, extracted from the three positive samples, was also analysed in order to ascertain if the presence of HCV in oral tissues was linked to any specific HCV genotype. Of the four positive specimens, two of the oral LP samples and the oral cancer tissue were infected by HCV subtype 1b; the remaining oral LP specimen showed HCV subtype 2a infection. However, because of the small number of positive samples it is no possible to draw conclusions on the influence of genotype in the epithelial localization of HCV.

Because RT-PCR, when applied to tissue samples, does not provide information about the specific localization of the genome in the tissue under investigation, a number of attempts have been made to develop a reliable technique for the detection and localization of the HCV genome and HCV-
gene products in liver. Such procedures would provide valuable information about the cellular tropism, site of replication and virus-host interactions of HCV and help to clarify the pathogenic mechanisms responsible for hepatocellular damage in the HCV-associated liver disease. *In situ* hybridization (ISH) and immunohistochemistry have therefore been used to detect and localise HCV RNA and proteins in infected tissues. However, these studies have thus far provided conflicting data on the type and proportion of HCV-infected cells, their topographical association with cell damage (Blight and Gowans 1995; Lau et al. 1996), as well as on methodological aspects of the staining procedures. According to these various studies based on ISH and immunohistochemistry, the number of positive cells in a liver sample can vary between 1 and 100%, (Blight and Gowans 1995). With both techniques, hepatocytes have been identified as the most commonly infected cells in the liver, although the presence of HCV proteins and/or genome in mononuclear cells, bile duct epithelium and sinusoidal cells has also been described (Nouri-Aria et al. 1993). Cytoplasmic localization of HCV products has been the most consistent observation, but nuclear and perinuclear signals have also been detected in a few studies (Blight et al. 1992; Nouri-Aria et al. 1993; Chamlian et al. 1996).

A number of extrahepatic localizations of putative HCV antigens have also been reported, including a malignant lymphoma lesion (De-Vita et al. 1995) and skin and lymph nodes of patients with mixed cryoglobulinaemia (Sansonno et al. 1995 (b); Sansonno et al. 1996).

To date, no studies of oral tissue have been carried out. In the present study a immunohistochemical technique has been chosen because it is a relatively simple method compared with ISH, the mAbs for HCV antigens are available and also because HCV proteins may be more stable than the RNA or may accumulate to more detectable levels. The mAbs used in the present study have previously been successfully employed for the detection of HCV
antigens in hepatic and extrahepatic tissues (Sansonno et al. 1995 (a); Sansonno et al. 1995 (b)).

Expression of putative HCV-associated antigens have been detected in sections of HCV-associated oral LP tissues of both frozen and paraffin-embedded specimens. In the unfixed frozen tissue six out of ten oral LP samples showed expression of putative HCV antigens, characterized by three distinct staining patterns as shown in Figures 2.8, 2.9 and 2.10. The demonstration of HCV-infected inflammatory cells is consistent with other reports and it could imply a role for such cells as vectors of infection from HCV reservoirs, such as liver or lymph nodes (Sansonno et al. 1996), to other tissues (i.e. oral mucosa). The other staining patterns visualized in the tissues of two different patients are more difficult to explain. One case showed intense, diffuse staining of the upper keratinized layers of the whole section (Figure 2.9). Although these results may indicate the presence of HCV antigens in the epithelial tissue, it is also possible that some cross-reacting epitopes may have been detected since such high expression was not detected by any of the other mAbs that were able to detect putative HCV antigens on section obtained from frozen samples. The third staining pattern showed intense nuclear staining in a high proportion of epithelial cells of a different specimen (Figure 2.10). Again, despite the high signal expressed by the epithelial nuclei, one mAb only detected such staining; in addition only a minority of studies reported nuclear expression of HCV-antigens in infected cells. However, because of the lack of frozen HCV-positive and HCV-negative liver, it was not possible to verify the actual specificity and sensitivity of the mAbs employed in this work, although previous studies have suggested that they are reliable tools for the detection of HCV-associated antigens (Sansonno et al. 1995 (a); Sansonno et al. 1995 (b)).

The staining patterns obtained with paraffin-embedded tissues differed from that of the frozen sections. This is not entirely unexpected since mAbs have
different reactivity as well as sensitivity according to the substrate used and the effects of fixation and embedding on antigenicity. Indeed, one mAb only (mAb 2) was able to visualise putative HCV antigens in formalin-fixed, paraffin-embedded tissue. The positive samples of oral LP showed intense staining of the epithelial cells of the suprabasal layers, which extended to the whole specimen in one case (Figure 2.12 a) and was limited to only some areas in another (Figure 2.12 b). In addition, one other specimen confirmed the presence of cells expressing HCV-associated antigens in the inflammatory infiltrate, as also observed in the frozen sections, although one of the HCV-negative oral LP samples showed a weak, diffuse staining of epithelial cells. In control paraffin-embedded liver tissues, expression of putative HCV antigens was detected in groups of hepatocytes as a coarse granular cytoplasmic staining, consistent with other immunohistochemistry studies (Figure 2.13), although a distribution in the tissue as foci of positive cells has not been reported previously. However, because the uncertainty of the reactivity of this panel of mAbs, the present results must be interpreted as only preliminary.

Thus, while no definitive conclusion can be drawn thus far on a causative role of HCV in the aetiology of HCV-associated oral LP, the results of the present study have nevertheless demonstrated the presence of HCV genome and suggested that putative HCV-associated structural and non-structural proteins might be expressed in this tissue.

Introduction

As discussed before, oral LP is considered to be T lymphocyte driven disorder (Walsh et al. 1990; Eversole et al. 1994; Porter et al. 1997), with lesions demonstrating a dense lymphocytic infiltrate within the dermis. Altered adhesion molecule expression has previously been noted in oral LP lesional tissue, including members of the β1 integrin family of very late activation antigens (VLA-1, -3 and -6) (Konter et al. 1990) and of intercellular adhesion molecule-1 (ICAM-1) and its ligand lymphocyte function-associated antigen-1 (LFA-1) (Verdickt et al. 1992). Moreover, it has been recently shown that expression of lymphocyte function-associated antigen-3 (LFA-3), the natural ligand for the T cell surface antigen CD2, is also altered in idiopathic oral LP (Kirby et al. 1995). The CD2/LFA-3 pathway is a major antigen-independent mediator of T cell activation as well as adhesion (Selvaraj et al. 1987), and it was observed that the oral LP lesional tissue expressed this adhesion molecule at a high level within the inflammatory infiltrate, with a substantial proportion of the antigen apparently associated with the extracellular matrix (ECM) as a ‘soluble’ form (sLFA-3) (Kirby et al. 1995). LFA-3 expression has also been shown to be elevated in the liver and sera of patients with chronic active hepatitis (Autschbach et al. 1991; Hoffmann et al. 1993; Mosnier et al. 1994).

The above studies suggest that a number of adhesion molecules play an important role in oral LP, but it is not yet known whether they are similarly involved in the HCV-associated form of this disease. Thus, the present study has examined the expression of these surface antigens in the lesional tissue and in the sera of oral LP patients with and without HCV infection.
Immunoglobulin (IgG) deposition in these tissues and serum IgG levels have also been examined since they have previously been implicated in the pathology of oral LP (Baart de la Faille-Kuyper and Baart de la Faille 1974). The possible influence of anti-HCV therapy on a number of these molecular features of HCV-associated oral LP pathology has also been considered.
Materials and methods

Tissue Samples

Healthy buccal mucosa was obtained from 8 HCV-seronegative patients undergoing routine oral surgery in the Eastman Dental Hospital, London, UK. Samples of oral LP buccal mucosa were obtained from the lesional sites of 7 HCV-seronegative patients at the same Institute and of six HCV-seropositive patients of the Dental School, University ‘G. D’Annunzio’, Chieti, Italy. The HCV-seropositive oral LP patients were affected with the reticular form of the disease and the HCV-seronegative oral LP group were selected to concur with this clinical presentation.

All tissues were mounted in OCT compound (Bright Ltd, Huntingdon, UK), immediately frozen in liquid nitrogen and stored at -70°C until required.

Immunohistochemistry

Cryostat sections of each tissue sample were cut to 7 μm thickness and mounted on Superfrost slides (Merck Ltd., Poole, UK). Sections were fixed in ice-cold acetone for 10 min, washed with phosphate-buffered saline (PBS) and incubated with 20% normal goat serum (NGS) in PBS (PBS/NGS) for 30 min to block non-specific binding. All incubations were carried out at room temperature in a humid chamber. The primary murine monoclonal antibodies (mAbs) described in Table 2.10 were applied for 1h and the sections were washed thoroughly with several changes of PBS. Sections were then incubated for 1h with horseradish peroxidase (HRP)-conjugated goat anti-mouse Ig (Dako Ltd., High Wycombe, UK) diluted 1:100 in PBS/NGS and washed thoroughly with PBS. The SigmaFast DAB Peroxidase Substrate Set (Sigma Ltd., Poole, UK) was prepared and incubated on the sections for 5 min to visualize the antigens. Reactions were terminated by washing the sections in PBS before counterstaining with Mayers haematoxylin, dehydrating through ascending concentrations of alcohol and mounting in
DePeX slides (Merck Ltd., Poole, UK). Isotype matched irrelevant mouse IgG (Dako Ltd., High Wycombe, UK) were used as negative controls.

Examination and photography of sections was carried out using an Olympus BX50 microscope fitted with appropriate filters and PM30 control unit.

**Direct Immunofluorescence**

The control, oral LP and HCV-associated oral LP tissues were sectioned, fixed and blocked as described above, before being probed for tissue IgG. Fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (Fc specific) (Sigma Ltd., Poole, UK), diluted 1:40 in PBS/NGS, was incubated on the sections for 1 h at room temperature in the dark. Sections were then washed extensively in PBS and mounted in fluorescence mounting medium (Sigma Ltd., Poole, UK). Fluorescent staining was viewed using a Zeiss Axioskop with fluorescence attachments.

Serum Samples: sera were obtained from 40 healthy HCV-seronegative subjects with no clinical evidence of mucocutaneous LP. Test groups consisted of 17 patients with oral LP and no associated HCV, 14 patients with HCV-associated oral LP and 11 HCV-seropositive patients with no associated oral LP. Healthy controls and oral LP sera were collected at the Eastman Dental Institute. HCV sera and HCV-associated oral LP sera were kindly provided by Professor Piattelli. Within the HCV-associated oral LP group, 4 patients had received IFN-a therapy for at least 12 months and a further 2 patients had undergone IFN-a therapy for 9 months or less.

**ELISA for sLFA-3**

Serum levels of sLFA-3 were measured using a 'sandwich' ELISA technique. Immulon 2 96-well ELISA plates (Dynatech Ltd., Virginia, U. S. A.) were coated with 50 ml/well of anti-human LFA-3 mAb 1E6 (10 mg/ml in 50 mM sodium carbonate/bicarbonate buffer, pH 9.6) and incubated overnight at 4°C. The plates were washed six times with PBS and blocked with 250
ml/well of PBS containing 5% foetal calf serum (FCS) in PBS (PBS/FCS) by incubation for 2h at room temperature. The plates were washed once with PBS. All subsequent washes were with PBS containing 0.1% Tween 20 (Sigma Ltd., Poole, UK) (PBS-T). All subsequent incubations were carried out at room temperature. Test samples, diluted in PBS/FCS as appropriate, were added at 50 ml/well, and all samples were analysed in duplicate. After incubating for 1h, the plates were washed six times and again incubated for 1h, with 50 ml/well of rabbit anti-human LFA-3 diluted 1:5000 in PBS/FCS. The plates were again washed six times and incubated for 1 h with HRP-conjugated goat anti-rabbit IgG (Dako Ltd., High Wycombe, UK) diluted 1:2000 in PBS/FCS. After six more washes the colour was developed by the addition of 50 ml/well substrate solution (42 mM tetramethylbenzidine in dimethyl sulphoxide diluted 1:100 with 0.1 M sodium acetate/citrate buffer, pH4.9; 7.3 µl of 30% H2O2 was added per 50 ml of substrate solution immediately before use). The reaction was allowed to proceed for 10 min and terminated by the addition of 50 ml/well of 2N H2SO4. Absorbance was measured at 450 nm using a Titertek Multiskan Plus plate reader. The sLFA-3 concentrations in the serum samples were determined by comparison with a standard curve included on each plate, which was prepared using an LFA-3/Ig fusion protein (LFA-3 TIP) diluted in PBS/FCS in the range 1-50 ng/ml, again with each concentration tested in duplicate.

MAb 1E6, rabbit polyclonal anti-LFA-3 serum and LFA-3 TIP were all generously provided by Dr. Paula Hochman, Biogen Corporation, Boston, U.S.A.

The significance of the results for each group versus control values was analysed statistically by the Student’s t-test.
ELISA for sICAM-1

The levels of soluble ICAM-1 in the serum samples was measured using a Cytoscreen immunoassay kit for human ICAM-1, kindly provided by Dr. R. Rothlein, Boehringer Ingelheim, Conn., U.S.A. The assay was carried out according to the manufacturers instructions. Each serum sample was again tested in duplicate, using 20 randomly selected control sera and those of the patient groups described above.

The significance of the results for each group versus control values was analysed statistically by the Student's t-test.

Serum Immunoglobulin Levels

Quantitative assessment of serum IgG levels was determined for 18 of the healthy control sera and for the oral LP, HCV-associated oral LP and HCV groups described above. This was carried out by immunodiffusion using NOR-Partigen IgG-HC plates (Behring Diagnostics Ltd., Milton Keynes, U. K.) according to the manufacturers instructions. Diffusion was allowed to proceed for 48 h before measurement. Test sera IgG levels were calculated by comparison with a standard curve derived using solutions containing known concentrations of human IgG included in each immunodiffusion plate. The results were compared using a two-tailed Student’s t-test.
Results

Adhesion Molecule Expression

The expression patterns of a number of adhesion molecules were examined using a panel of mAbs, as described in Table 2.10, with cell phenotypes being assessed on serial tissue sections of buccal mucosa from healthy individuals, oral LP patients and HCV-associated oral LP patients. The distribution of the different cell types was noted and the staining patterns for each adhesion molecule determined visually, by one observer, over the whole of each section and also graded from negative (-) to strongly positive (+++) for each of the cell phenotypes (Table 2.11). Gradings were made relative to the control tissues within each experiment, allowing comparison to be made between experiments carried out at different times. A mean grade was obtained for each cell type in each tissue group. In control experiments, no endogenous peroxidase activity was observed in any of the tissues used in this study and no staining was observed for any specimen when an irrelevant mouse Ig was used instead of primary antibody.

The surface antigen CD29 is the β1 integrin sub-unit common to all members of the VLA family and was found to be similarly expressed in all three tissue groups, as shown in Table 2.11.

The integrin VLA-1, a receptor for laminin, was detected only on endothelium in normal mucosa, whereas a small subset of T lymphocytes also appeared weakly positive for this adhesion molecule in both the oral LP and HCV-associated oral LP tissues. These T cells were restricted mainly to the epithelium and comprised less than approximately 10% of the total of infiltrating T cells.

Two other β1 integrins, VLA-2 and VLA-3 (receptors for collagen and fibronectin/laminin/collagen respectively), had similar epithelial distributions within all three groups. Unlike VLA-2, however, keratinocyte expression of VLA-3 was limited to those cells in the suprabasal region and was not
expressed in the higher layers of the epithelium. Notably, approximately 50% of the infiltrating lymphocytes in both oral LP groups were observed to be weakly positive for VLA-2, but did not express VLA-3 (Table 2.11).

VLA-4, a receptor for both vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, was readily detected on T lymphocytes in both disease groups, although it was particularly prominent in the oral LP group, including on T lymphocytes infiltrating the epithelium. Increased VLA-4 expression also appeared to be up-regulated on endothelial cells and on epithelial dendritic cells in the oral LP group only. Moreover, in these samples the relative numbers of Langerhans cells (CD1a-positive dendritic cells) in the epithelium adjacent to the dense infiltrate were much greater than those adjacent to sparsely infiltrated areas. In addition, the dendritic cells were consistently detected below the basal layer and in the inflammatory infiltrate at sites where damage to the basement membrane was apparent and the infiltrate most dense. This feature, observed only in the oral LP group, was not present in either the control or HCV-associated oral LP samples, in which the CD1a-positive cells were more evenly distributed.

The expression of VLA-6, a laminin receptor, was very similar on the keratinocytes and endothelial cells of both the control and disease groups. However, there was a low level of VLA-6 expression on some infiltrating T lymphocytes in 3 of the 7 oral LP samples and 2 of the 6 HCV-associated oral LP tissues.

In both the oral LP and HCV-associated oral LP groups, the expression of the adhesion molecule LFA-3, a member of the immunoglobulin superfamily, was also clearly distinct from that observed in normal mucosal tissue. In both disease groups, macrophages and T cells were positive for LFA-3, the lymphocytes staining with particularly notable intensity. The apparently elevated expression of LFA-3 was, however, restricted to the lesional site. The most intense staining of keratinocytes and basal cells coincided with
areas adjacent to the most dense regions of inflammatory infiltration and was more pronounced in the oral LP patients compared with the HCV-associated oral LP group.

In both oral LP groups staining of ICAM-1, also an immunoglobulin-like adhesion molecule, was observed on keratinocytes, basal cells, infiltrating T lymphocytes and macrophages. Keratinocyte and basal cell ICAM-1 expression in the oral LP and HCV-associated oral LP tissues occurred in patches, with the strongest staining coinciding with the most dense areas of inflammatory infiltrate, whilst uninvolved epithelium was negative for ICAM-1. Moreover, fibroblasts within the band of infiltration were observed to be weakly positive for ICAM-1 expression, whereas fibroblasts in the deeper connective tissue appeared to be ICAM-1 negative. A similar pattern of expression was also observed for macrophages, which were intensely ICAM-1-positive and densely-localized within the inflammatory infiltrate, compared with their lack of ICAM-1 expression and sparse distribution in the deeper tissue. However, despite similar staining patterns, ICAM-1 levels appeared to be consistently elevated in the oral LP samples compared with that in the HCV-associated oral LP group.

**Tissue Deposition of Immunoglobulin**

Immunofluorescence analysis was carried out to detect putative autoantibodies in the control, oral LP and HCV-associated oral LP oral mucosa. The presence of such antibodies could reflect the humoral immune status of each group and thus illuminate possible differences related to specific features of disease pathology. However, no positive staining pattern was observed for any of the samples tested (data not shown).
Serum sLFA-3 Levels

Quantitative measurement of serum LFA-3 was carried out for the healthy controls, oral LP, HCV and HCV-associated oral LP patient groups, as described in the Materials and Methods. The results in Table 2.13 and Figure 2.14 show that serum LFA-3 levels in the oral LP group did not differ significantly from those measured in the healthy control group. Similar levels of circulating sLFA-3 were obtained for the HCV-associated oral LP group, in which the mean sLFA-3 value was again close to the control value. However, the average serum sLFA-3 level in the HCV group was clearly elevated compared with both the oral LP and control groups, and statistical analysis showed that these differences were significant.

The clinical therapeutic data available for the HCV-associated oral LP sera group showed that no statistical associations could be made between IFN-a therapy and sLFA-3 levels.

Serum ICAM-1 Levels

The levels of sICAM-1 were also measured in the sera of the control, oral LP, HCV and HCV-associated oral LP groups, as shown in Table 2.12. The sera of the idiopathic oral LP group were found to have significantly increased sICAM-1 compared with controls. This difference was more pronounced in the HCV-associated oral LP group and especially in the HCV group, which were both statistically significantly higher compared with controls. Furthermore, these two HCV groups also exhibited extended ranges in addition to highly significantly increased mean values of sICAM-1 compared with the oral LP group.
As with serum sLFA-3 levels, no statistical associations could be made between IFN-a therapy and sICAM-1 concentration in the HCV-associated oral LP sera group.

**Serum IgG Levels**

The levels of serum IgG were determined for the control, oral LP, HCV and HCV-associated oral LP groups, as shown in Table 2.12. For the oral LP group the mean value was close to the accepted normal healthy average of 12.5 g/l (Nor-Partigen IgG-HC Kit; Behring Diagnostics Ltd), as was the level of the control group. Moreover, while the HCV group exhibited a somewhat elevated mean serum IgG level, below the 'high-normal' value of 17 g/l, the HCV-associated oral LP group had markedly higher serum IgG levels, with 10 of the 14 sera exceeding the 'high-normal' value. Analysis by Students two-tailed t-test showed this result to be significant when compared with the control group and highly significant compared with the HCV-seronegative oral LP group.
Table 2.10: Monoclonal antibodies used in immunohistochemical studies

<table>
<thead>
<tr>
<th>Antibody Clone</th>
<th>Antigen Specificity</th>
<th>Main Cellular Distribution</th>
<th>Source</th>
<th>Working Dilution or Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA1/34</td>
<td>CD1a</td>
<td>Langerhans cells, cortical thymocytes</td>
<td>Dako, High Wycombe, UK</td>
<td>1:200</td>
</tr>
<tr>
<td>UCHT1</td>
<td>CD3</td>
<td>T lymphocytes</td>
<td>Dako</td>
<td>1:200</td>
</tr>
<tr>
<td>UCHM1</td>
<td>CD14</td>
<td>Monocytes, macrophages</td>
<td>Cymbus Bioscience, Southampton, UK</td>
<td>1:10</td>
</tr>
<tr>
<td>3S3</td>
<td>CD29 (Integrin β1)</td>
<td>Ubiquitous except erythrocytes</td>
<td>Serotec Ltd., Oxford, UK.</td>
<td>1:100</td>
</tr>
<tr>
<td>TS2/7</td>
<td>VLA-1 (CD49a)</td>
<td>Endothelial cells, activated T cells</td>
<td>Serotec</td>
<td>2 μg/ml</td>
</tr>
<tr>
<td>HAS-4</td>
<td>VLA-2 (CD49b)</td>
<td>Endothelial cells, T and B lymphocytes</td>
<td>Dr. F. M. Watt ICRF, London, UK.</td>
<td>1:10</td>
</tr>
<tr>
<td>11G5</td>
<td>VLA-3 (CD49c)</td>
<td>B lymphocytes</td>
<td>Cymbus Bioscience</td>
<td>1:10</td>
</tr>
<tr>
<td>B-5G10</td>
<td>VLA-4 (CD49d)</td>
<td>Lymphocytes, monocytes</td>
<td>Dr. M. E. Hemler</td>
<td>1:100</td>
</tr>
<tr>
<td>4F10</td>
<td>VLA-6 (CD49f)</td>
<td>Lymphocytes, monocytes</td>
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<td>1:10</td>
</tr>
<tr>
<td>TS2/9</td>
<td>LFA-3 (CD58)</td>
<td>Widely expressed, including T cells, endothelial cells, and keratinocytes</td>
<td>Hybridoma HB205, ATCC</td>
<td>5 μg/ml</td>
</tr>
<tr>
<td>RR1.1</td>
<td>ICAM-1 (CD54)</td>
<td>Endothelial cells, wide range of activated cells</td>
<td>Dr. R. Rothlein Boehringer Ingelheim, Conn., USA.</td>
<td>5 μg/ml</td>
</tr>
</tbody>
</table>
Table 2.11: Immunohistochemical staining patterns observed in buccal mucosal tissues

(a) Normal buccal mucosa

<table>
<thead>
<tr>
<th></th>
<th>CD29</th>
<th>VLA-1</th>
<th>VLA-2</th>
<th>VLA-3</th>
<th>VLA-4</th>
<th>VLA-6</th>
<th>LFA-3</th>
<th>ICAM-1</th>
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<tbody>
<tr>
<td>LC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KC</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>+/</td>
<td>++</td>
<td>++</td>
<td>-</td>
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<tr>
<td>BC</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
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<td>++</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>EC</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+/-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Macrophages</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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</table>

*Positive (+) in 3 of 8 samples

(b) oral LP lesional buccal mucosa

<table>
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<tr>
<th></th>
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<th>VLA-1</th>
<th>VLA-2</th>
<th>VLA-3</th>
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<th>VLA-6</th>
<th>LFA-3</th>
<th>ICAM-1</th>
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<td>-</td>
<td>-</td>
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<tr>
<td>Fibroblasts</td>
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<td>-</td>
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<td>++</td>
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<tr>
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<td>+</td>
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*<10% of total T cells
*bPositive in 5 of 7 samples
*cPositive in 3 of 7 samples

(c) HCV-associated oral LP lesional buccal mucosa

<table>
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<th></th>
<th>CD29</th>
<th>VLA-1</th>
<th>VLA-2</th>
<th>VLA-3</th>
<th>VLA-4</th>
<th>VLA-6</th>
<th>LFA-3</th>
<th>ICAM-1</th>
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<tbody>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
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<td>-</td>
</tr>
<tr>
<td>KC</td>
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<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Fibroblasts</td>
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</table>

*a<5% of total T cells
*bPositive in 2 of 6 samples

LC, Langerhans cells; KC, suprabasal keratinocytes; BC, basal layer cells; EC, endothelial cells
Table 2.12: Circulating adhesion molecules and IgG levels

(a) Serum sLFA-3 levels

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>n</th>
<th>Mean sLFA-3</th>
<th>Range</th>
<th>Significance (p) versus Control</th>
<th>Significance (p) versus oral LP</th>
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<tbody>
<tr>
<td>Control</td>
<td>40</td>
<td>118.2</td>
<td>67.5 - 162</td>
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<tr>
<td>oral LP</td>
<td>17</td>
<td>107.8</td>
<td>62.9 - 220</td>
<td>p=0.72</td>
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<tr>
<td>HCV+oral LP</td>
<td>14</td>
<td>113.4</td>
<td>42.7 - 201</td>
<td>p=0.89</td>
<td>0.74</td>
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<td>HCV</td>
<td>11</td>
<td>157.2</td>
<td>108 - 241</td>
<td>p=0.015*</td>
<td>0.037*</td>
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</table>

(b) Serum sICAM-1 levels

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>n</th>
<th>Mean sICAM-1</th>
<th>Range</th>
<th>Significance (p) versus Control</th>
<th>Significance (p) versus oral LP</th>
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<tr>
<td>Control</td>
<td>20</td>
<td>10.3</td>
<td>6.4 - 14.7</td>
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<td>oral LP</td>
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<td>14.1</td>
<td>7.3 - 19.1</td>
<td>0.003**</td>
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<tr>
<td>HCV+oral LP</td>
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<td>7.5 - 44.4</td>
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<td>0.006**</td>
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<tr>
<td>HCV</td>
<td>11</td>
<td>27.9</td>
<td>17.2 - 48.5</td>
<td>0.0003**</td>
<td>0.002**</td>
</tr>
</tbody>
</table>

(c) Serum IgG levels

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>n</th>
<th>Mean IgG Level (g/l)</th>
<th>Range (g/l)</th>
<th>Significance (p) versus control</th>
<th>Significance (p) versus oral LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>13.9</td>
<td>9.9 - 9.8</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>oral LP</td>
<td>17</td>
<td>12.4</td>
<td>5.5 - 16.0</td>
<td>0.086</td>
<td>---</td>
</tr>
<tr>
<td>HCV+oral LP</td>
<td>14</td>
<td>19.9</td>
<td>11.8 - 37.5</td>
<td>0.004**</td>
<td>0.0007**</td>
</tr>
<tr>
<td>HCV</td>
<td>11</td>
<td>15.2</td>
<td>6.9 - 20.2</td>
<td>0.405</td>
<td>0.034**</td>
</tr>
</tbody>
</table>

*Significant (p<0.05) and **highly significant (p<0.01) results using two-tailed t-test.
Figure 2.14: Graphs showing the distribution of (a) sLFA-3, (b) sICAM-1 and (c) serum IgG levels within each patient group.
Figure 2.14.1: Detection of LFA3 antigen in idiopathic (a) and HCV-associated (b) oral lichen planus lesional tissue. In both sections LFA-3 is expressed primarily within the inflammatory infiltrate, with lower levels in adjacent regions of the epithelium and connective tissue.
Discussion

This study has compared certain lesional and systemic features of two epidemiologically and possibly aetiologically distinct forms of oral LP. A number of previous studies have demonstrated altered adhesion molecule expression in idiopathic oral LP (Konter et al. 1990; Verdickt et al. 1992; Kirby et al. 1995), and the present study, for the first time, investigated the expression of these antigens in the HCV-associated form of the disease.

Both types of oral LP exhibit very similar pathology, dominated by a prominent T lymphocyte infiltrate below the basement membrane. The present study showed that such T cells express VLA-1, a laminin receptor, suggesting that this antigen could be involved in their migration into the epithelium. It also showed weak expression of VLA-2, a collagen receptor, on a large proportion of the infiltrating T lymphocytes in both idiopathic and HCV-associated oral LP, indicating that these cells are likely to be chronically activated in both types of oral LP (Springer 1990). In the present study the profile of VLA-3 differed in some aspects from a previous report of integrin expression in oral LP (Konter et al. 1990): increased VLA-3 expression was observed on keratinocytes, but no T lymphocytes expressed this antigen in oral LP, consistent with other reports that VLA-3 is more commonly associated with B lymphocytes (Hemler 1990).

T lymphocytes expressing VLA-4 in oral LP were observed to be mainly intra-epithelial. Moreover, this integrin, which is both a fibronectin receptor and the natural ligand for VCAM-1 (Hemler 1990), is also expressed in oral LP tissue by macrophages, endothelial cells and Langerhans cells (Walton et al. 1994). The present findings thus suggest the possibility that VLA-4 could facilitate T lymphocyte interactions with VCAM-1-expressing Langerhans cells in oral LP. Such antigen-presenting cell (APC)/T cell interactions may be due to an oral LP-specific immune response, consistent with previous reports that infiltrating T lymphocytes in oral LP are mainly of the memory phenotype.
Thus, these activated T cells may have already been primed with 'oral LP-specific' antigen and may be re-exposed within the lesion, although the precise role of intra-epithelial T lymphocytes in oral LP remains to be clearly defined. However, the apparently elevated level of VLA-4 observed in oral LP was not evident in the sections examined from patients with HCV-associated oral LP, suggesting that VLA-4-mediated interactions may be of lesser importance in this form of the disease.

Further evidence of a prominent role for APCs in the pathogenesis of oral LP is the distribution of CD1a-positive Langerhans cells in lesional tissue. In idiopathic oral LP tissues these cells were accumulated in areas of the epithelium adjacent to the inflammatory infiltrate. In addition, in areas of severe basement membrane damage, CD1a-positive cells were localized in the inflammatory infiltrate, where they were apparently in direct contact with inflammatory cells. This distribution has been noted previously (Hirota and Osaki 1992) and strongly suggests that highly activated Langerhans cells present in oral LP lesions (Farthing et al. 1990) may play a major part in the immune pathology of oral LP by presenting antigen to, and activating, antigen-specific T lymphocytes. It is notable, however, that this distribution was not observed in the HCV-associated oral LP tissues, again suggesting different pathogenic mechanisms may be involved in these two forms of oral LP.

Very similar patterns of expression were observed in both oral LP groups for the adhesion molecules LFA-3 and ICAM-1, which were found to be localized primarily within the inflammatory infiltrate, with lower levels in adjacent regions of the epithelium and connective tissue. Interactions of LFA-3 and ICAM-1 with their counter-structures on T lymphocytes, CD2 and LFA-1 respectively, constitute two major antigen-independent T lymphocyte activation pathways (Springer 1990). Both ligands have previously been
implicated in inflammatory pathologies, including that of oral LP (Konter et al. 1990; Kirby et al. 1995). The results of the present study have thus shown that these antigens are also potentially important in the HCV-associated form of the disease. Moreover, the relatively elevated levels of these antigens in the oral LP patients compared with the HCV-associated group suggest, as with VLA-4, that they may have distinct pathogenic roles in the two types of the disease.

In addition to certain differences in adhesion molecule expression at lesional sites, in the present study some systemic differences between the two disease groups were also noted. Circulating adhesion molecules have been considered to be useful markers of inflammatory reactions in a number of immune pathologies (Gearing and Newman 1993), although little is known about these 'soluble' forms in oral LP. However, despite previous finding that LFA-3 was elevated in the lesional tissue and accumulated in the extracellular matrix of oral LP lesions (Kirby et al. 1995), the present study showed serum levels of sLFA-3 both in the idiopathic and HCV-associated oral LP groups did not differ from each other or from those of healthy control individuals. However, in HCV-positive patients with no evidence of oral LP there was an increase in both the average and the range of sLFA-3 levels, and these differences were statistically significant. Although it is not clear why the HCV patient group has a higher serum level of sLFA-3 than the group with oral LP as well as HCV, elevated levels of serum LFA-3 have previously been reported to occur in patients with hepatitis, possibly due to cytokine-induced shedding of LFA-3 in chronic inflammatory liver disease, as is also observed in vitro (Hoffmann et al. 1993).

In contrast to sLFA-3, significantly elevated levels of sICAM-1 were observed in the oral LP group compared with controls. This finding suggests that the localized inflammation observed in oral LP is associated with a systemic change in sICAM-1 levels. In addition, both the average and the
range of serum levels of sICAM-1 were found to be even more markedly increased in the HCV-associated oral LP patients, and these differences were statistically significant compared with the oral LP group as well as with the control group. sICAM-1 levels in the HCV patients were very similar to those in the HCV-associated oral LP group, again possibly indicating the modulating effects of the inflammation which accompanies HCV infection.

As with sICAM-1, serum IgG levels were also found to be significantly increased in the HCV-associated oral LP group compared with the oral LP group as well as with normal individuals, although the average level of the HCV group was also elevated compared with controls. These results indicate that while increased serum IgG is apparently associated with HCV infection, IgG levels are nevertheless higher in patients with oral LP as well as HCV. The finding that serum IgG levels are normal in oral LP patients has been reported previously (Sklavounou et al. 1983), although other studies have observed increased levels of IgG (Scully 1982). Using direct immunofluorescence, however, no deposition of antibodies within any of the oral LP and HCV-associated oral LP lesional tissues examined was detected, suggesting that a localised humoral immune response is unlikely to play a major role in the pathogenesis of either form of the disease.

In conclusion, the results of this study provide evidence that the role of a number of adhesion molecules, particularly VLA-4, LFA-3 and ICAM-1, could be functionally different in the pathogenesis of the idiopathic and the HCV-associated forms of oral LP. Additionally, the two patient groups were also distinguished by the circulating levels of the adhesion molecule sICAM-1 and IgG. Despite these apparent differences, however, it remains to be determined whether these features are directly related to the onset or progression of HCV infection alone or associated with the accompanying development of oral LP.
2.1.7. Development of squamous cell carcinoma in hepatitis C virus-associated oral lichen planus

Introduction

The most serious complication of oral LP is the possible development of oral squamous cell carcinoma as reported by numerous case reports and follow-up studies (Table 2.13) (Willinger 1924; Montgomery and Culver 1929; Schuermann 1939; Deschaume et al. 1957; Sugar and Banoczy 1959; Warin 1960; Altman and Perry 1961; Andreasen and Pindborg 1963; Janner et al. 1967; Abramova 1968; Cawson 1968; Shklar 1972; Fulling 1973; Kovesi and Banoczy 1973; Silverman et al. 1985; Murty et al. 1986; Holmstrup et al. 1988; Salem 1989; Sigurgeirsson and Lindelof 1991; Silverman et al. 1991; Voute et al. 1992; Barnard et al. 1993; Markopoulos et al. 1997). Although some controversies due to the diagnostic criteria to differentiate oral LP from premalignant dysplasia with lichenoid appearances (Krutchkoff and Eisenberg 1985; Eisenberg 1992). The results of the follow-up studies on the development of squamous cell carcinoma in lesions diagnosed as oral LP are surprisingly uniform. The studies involving more than 200 cases showed a frequency of malignant change ranging from 0.4 to 3.3%, as shown on Table 2.13. According to some studies atrophic, ulcerative, erosive oral LP lesions have increased tendency to malignant change (Kovesi and Banoczy 1973; Silverman et al. 1985; Murty et al. 1986; Silverman et al. 1991; Blacker et al. 1993; Markopoulos et al. 1997); in addition a location preponderance was also recognised, most carcinomas developing in either the tongue, gingiva or buccal mucosa (Silverman et al. 1991; Blacker et al. 1993). Thus it appears that persons with oral LP may be at 20 to 100-fold greater risk of developing oral cancer than in the general population. The reason for such increased risk are unknown; smoking, alcohol (Holmstrup et al. 1988) and infection with Candida albicans could be involved (Holmstrup and Dabelsteen 1974; Simon...
and Hornstein 1980; Krogh et al. 1987). Infection of epithelial cells with viruses (i.e. herpes viruses or papilloma viruses) have been proposed as potential aetiological factors for oral LP and oral cancer but detailed objective supporting data are still lacking (Walsh et al. 1990; Cox et al. 1993).

HCV infection is strongly associated with hepatocellular carcinoma and it may have a direct aetiological role in the development of this cancer; in addition epidemiological and experimental findings have also suggested a relationship between liver cirrhosis (a common consequence of HCV infection) and oral cancer (Lekholm and Stenman 1989; Gerson 1990), and a Japanese study found a significantly increased prevalence of HCV antibodies in a group of oral cancer patients (Nagao et al. 1995).

However, there is no information concerning the premalignant potential of HCV-associated LP. We now report the features of a patient with long-standing HCV-associated oral LP who developed a squamous cell carcinoma of the oral cavity.
Table 2.13: Studies of malignant change in groups of oral lichen planus patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Number of subjects</th>
<th>Follow-up period (years)</th>
<th>Frequency of malignant change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williger 1924</td>
<td>Germany</td>
<td>20</td>
<td>•</td>
<td>10%</td>
</tr>
<tr>
<td>Montgomery &amp; Culver 1929</td>
<td>UK</td>
<td>17</td>
<td>1-9</td>
<td>6%</td>
</tr>
<tr>
<td>Schuermann 1939</td>
<td>Germany</td>
<td>310</td>
<td>•</td>
<td>0.6%</td>
</tr>
<tr>
<td>Deschaume et al. 1957</td>
<td>France</td>
<td>50</td>
<td>•</td>
<td>10%</td>
</tr>
<tr>
<td>Sugar &amp; Banoczy 1959</td>
<td>Hungary</td>
<td>36</td>
<td>11</td>
<td>3%</td>
</tr>
<tr>
<td>Warin, 1960</td>
<td>UK</td>
<td>53</td>
<td>1-10</td>
<td>9%</td>
</tr>
<tr>
<td>Altman &amp; Perry 1961</td>
<td>USA</td>
<td>128</td>
<td>6-10</td>
<td>0.8%</td>
</tr>
<tr>
<td>Andreasen &amp; Pindborg 1963</td>
<td>Denmark</td>
<td>115</td>
<td>2-5</td>
<td>0%</td>
</tr>
<tr>
<td>Janner et al. 1967</td>
<td>Germany</td>
<td>585</td>
<td>1-24</td>
<td>1.7%</td>
</tr>
<tr>
<td>Abramova 1968</td>
<td>USSR</td>
<td>436</td>
<td>5-8</td>
<td>1.1%</td>
</tr>
<tr>
<td>Cawson 1968</td>
<td>UK</td>
<td>138</td>
<td>•</td>
<td>0.7%</td>
</tr>
<tr>
<td>Shklar 1972</td>
<td>USA</td>
<td>600</td>
<td>1-15</td>
<td>0.5%</td>
</tr>
<tr>
<td>Fulling 1973</td>
<td>Denmark</td>
<td>225</td>
<td>3.66</td>
<td>0.4%</td>
</tr>
<tr>
<td>Kovessi &amp; Banoczy 1973</td>
<td>Hungary</td>
<td>274</td>
<td>1-10</td>
<td>0.4%</td>
</tr>
<tr>
<td>Silverman et al. 1985</td>
<td>USA</td>
<td>570</td>
<td>5.6</td>
<td>1.2%</td>
</tr>
<tr>
<td>Murti et al. 1986</td>
<td>India</td>
<td>702</td>
<td>5.1</td>
<td>0.4%</td>
</tr>
<tr>
<td>Holmstrup et al. 1988</td>
<td>Denmark</td>
<td>611</td>
<td>1-26</td>
<td>1.5%</td>
</tr>
<tr>
<td>Salem 1989</td>
<td>Saudi Arabia</td>
<td>72</td>
<td>3.2</td>
<td>0.4%</td>
</tr>
<tr>
<td>Sigurgeirsson &amp; Lidelo, 1991</td>
<td>Sweden</td>
<td>2071</td>
<td>9.9</td>
<td>0.4%</td>
</tr>
<tr>
<td>Silverman et al. 1991</td>
<td>USA</td>
<td>214</td>
<td>0.6-10</td>
<td>2.3%</td>
</tr>
<tr>
<td>Voute et al. 1992</td>
<td>Holland</td>
<td>113</td>
<td>7.8</td>
<td>2.7%</td>
</tr>
<tr>
<td>Barnard et al. 1993</td>
<td>UK</td>
<td>241</td>
<td>10</td>
<td>3.3%</td>
</tr>
<tr>
<td>Markopoulos et al. 1997</td>
<td>Greece</td>
<td>326</td>
<td>0.6-10</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
Case Study

A 61 year old Indian male was referred to the Department of Oral Medicine of the Eastman Dental Institute for investigation and treatment of generalised oral mucosal soreness that had been present for 3 years. The patient had previously been under the care of oral surgeons who had undertaken 2 biopsies of lesional tissue and had established the cause of the oral discomfort to be erosive lichen planus. Previous management of the erosive lichen planus had included hydrocortisone hemisuccinate mouth rinses, triamcinolone acetonide in carboxymethylcellulose paste, betamethasone mouth rinse, benzydamine hydrochloride and chlorhexidine gluconate mouth rinses and sprays, systemic prednisolone and topical cyclosporin suspension. Each agent had provided only transient relief of his oral symptoms.

The patient was otherwise well and review of systems revealed no notable abnormalities aside from mild hesitancy of micturition due to known prostatism. The patient had had non-insulin dependent diabetes mellitus for 12 years, was mildly hypertensive and had had hepatitis C virus- associated chronic active hepatitis for at least 1 year. The source of the HCV infection was not known.

The patient's current medication comprised glibenclamide and bendrofluazide. He had no known allergies. The patient was heterosexual, married with 5 adult children and had been resident in the UK for the previous 20 years. He did not smoke tobacco, drink alcohol and denied any tobacco-betel type habits or injecting drug use.

Extra-oral examination revealed no cervical lymphadenopathy or orofacial stigmata of chronic hepatic disease. Intra-orally there was extensive erosive lichen planus affecting the buccal mucosae, ventral and dorsal aspects of the tongue, the labial and palatal/lingual free and attached gingivae, and the upper and lower labial mucosae (Figure 2.15). There was a 1.5 cm diameter raised, speckled mass on the posterior aspect of the anterior two-thirds of the
left side of the dorsum of the tongue (Figure 2.16). No trigeminal or hypoglossal nerve abnormalities were detected. The likely clinical diagnosis was a squamous cell carcinoma in association with pre-existing lichen planus.

Haematological investigations prior to biopsy revealed mild thrombocytopenia, elevated serum alanine transaminase, normal prothrombin and activated partial thromboplastin times and hepatitis C virus seropositivity. Biopsy from the tongue confirmed the lesion to be a moderately well-differentiated squamous cell carcinoma. The patient has now undergone laser-excision of the squamous cell carcinoma and is under regular clinical review.
Figure 2.15: Severe oral lichen planus affecting the palatal mucosa.
Figure 2.16: Squamous cell carcinoma with associated lichen planus of the dorsum of the tongue.
Discussion

Lichen planus may occur in up to 12% of patients with HCV infection. The precise cause of this association remains unclear, but it need not be due to concomitant drug therapy. To date there have been no reports of oral squamous cell carcinoma developing in patients with HCV-associated LP.

The present report indicates that squamous cell carcinoma can occur in patients with HCV-infection and oral lichen planus. It is noteworthy that the patient denied the best known risk factors for oral cancer development (tobacco use and alcohol consumption); however, this must be interpreted cautiously. It is not known how long the patient had had Hepatitis C virus, indeed his mode of acquisition of the virus is quite unclear, particularly as he denied any risk activities for HCV transmission, nevertheless as HCV-associated hepatic disease is generally a long-term problem giving rise to morbidity it is possible that the patient may have had HCV infection for many years. It is possible that the oral LP may have been associated with glibenclamide therapy (Lamey et al. 1990) but the patient had only been receiving this therapy for the past 2 years and thus after the development of oral LP.

Liver cirrhosis may be associated with oral cancer (Gerson 1990) and it is a common finding in certain groups of oral LP patients (del Olmo et al. 1989; Gandolfo et al. 1994). In addition an association between HCV-infection and oral squamous cell carcinoma has been recently suggested as 24% of a group of Japanese patients with oral squamous cell carcinoma were HCV seropositive, 17% having HCV RNA in serum. It is not know if any of these HCV-infected persons with oral cancer had previously had oral LP, and it is unclear if the concurrence of HCV with oral malignancy is of any aetiological significance - especially as HCV RNA has not been found in any of the squamous cell carcinoma tissue of HCV-infected patients (Nagao et al. 1995).
The risk of oral malignancy and premalignancy in long-standing LP is relatively small, nevertheless the present report has highlighted the possibility of HCV-associated LP being a risk factor for the development of oral squamous cell carcinoma.
2.2. Hepatitis C virus infection and Sjögren’s syndrome. 

a British study

Introduction

Sjögren’s syndrome (SS) comprises the combination of dry mouth (xerostomia), dry eyes (xerophthalmia) and rheumatoid arthritis or another connective-tissue disease. Sjögren’s syndrome is most commonly associated with rheumatoid arthritis but it can develop in any connective-tissue disease. Such association is now defined “secondary SS”, while “primary SS” indicates xerostomia and xerophtalmia in absence of other connective-tissue disease. The progressive reduction of salivary and lacrimal flow seen in patients with SS is due to a progressive destruction of glandular tissue characterized by lymphocytic infiltration where CD4-positive cells predominate (Daniels 1996). Presence of circulating multiple autoantibodies is a common finding in patients with SS, in particular SS-A antibodies (anti RO) may be found in 50-80% of patients with secondary SS and in a smaller proportion of patients with primary SS (5-10%) while SS-B antibodies (anti LA) are much more common in the primary form of SS (50-75%) than in the secondary (2-5%).

Because of these immunological features SS is generally thought to be an autoimmune disease, although the factors triggering SS immunopathological reaction are still unknown a possible viral aetiology has been suggested (Mariette 1995). Coronavirus in rats and HTLV-1 in mice may cause a similar syndrome (Green et al. 1989), while in human the viruses possibly involved are members of the herpes virus family, particularly human cytomegalovirus (HCMV) and Epstein-Bar virus (EBV) but so far no conclusive results on a infective causative agent of SS have been reported (Maitland et al. 1995).

In 1992 a French study found that 16 out of 28 patients with HCV-associated chronic liver disease had grade 3 or 4 sialadenitis (according to Chisholm and Mason’s classification) (Chisholm and Mason 1968), compared
with only one in 20 controls (Haddad et al. 1992). The authors stressed the
similarities between the nodular pattern of lymphocyte infiltrate in salivary
glands and in liver and suggested a possible link between HCV infection and
sialadenitis on the basis of the frequent presence of HCV in the saliva; in
addition antibodies against host derived epitopes are a frequent finding in
HCV-infected patients (Mishiro et al. 1990; Clifford et al. 1995). Following this
report, groups from different geographical areas studied the possible
association of HCV infection and SS, investigating the prevalence of HCV
infection in groups of patients with SS and, conversely, the frequency of
glandular abnormalities in HCV-infected patients. As shown in Table 2.14,
between 0 to 40% of patients with SS can be HCV-infected, the frequency
varying with geographical region and inclusion criteria. Some studies may
have been biased by the use of early generation tests that could have given
false positive results due to hyperglobulinemia, a condition frequently found in
SS patients (Vitali et al. 1992); however many latter studies adopted suitable
confirmatory assays or detected serum HCV-RNA and thus their serological
diagnosis must be considered free from methodological errors.

Up to 77% of HCV-infected individuals may have some salivary or lacrimal
abnormality. However many studies seem to indicate that the sialadenitis
affecting patients with HCV infection may be different from that of SS. In fact
the histopathological picture of HCV-related sialadenitis shows a less
profound lymphocytic infiltrate compared with that of SS (Scott et al. 1997)
and often characterised by a CD8+ T cell predominance (while in SS CD4+
cells predominate) (Boscaglio et al. 1996); in addition in most of the HCV-
positive cases there is lack of specific SS autoantibodies and milder

Improvement in lacrimal and salivary function may occur following INF-α
therapy (Durand et al. 1995), an unlikely event in case of primary or
secondary SS.
Hepatitis C virus may be present in the saliva of 83% of patients with HCV-associated sialadenitis (Jorgensen et al. 1996), is also detectable in tears in concentrations higher than serum (Feucht et al. 1994), and HCV genome has been detected in minor salivary glands of patients with SS and chronic hepatitis C (Durand et al. 1995), thus a direct action of the virus upon exocrine glandular tissue is possible although a virus-induced immune mechanism may also be at work. It has been suggested that the association between HCV infection and SS is indirect, being due to cryoglobulinaemia (King et al. 1994), however half of a large group of HCV-positive patients with salivary gland abnormalities were cryoglobulinaemia free (Pawlotsky et al. 1994).
Table 2.14: Association of HCV infection with sialadenitis or Sjögren’s syndrome

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>HCV infection in SS* patients</th>
<th>Lacrimal or salivary abnormality in HCV infected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haddad et al. 1992</td>
<td>France</td>
<td>•</td>
<td>57% with histological changes characteristic of SS</td>
</tr>
<tr>
<td>Aceti et al. 1992</td>
<td>Italy</td>
<td>0%</td>
<td>•</td>
</tr>
<tr>
<td>Almasio et al. 1992</td>
<td>Italy</td>
<td>•</td>
<td>45% lacrimal dysfunction in patients with HCV-related chronic liver disease</td>
</tr>
<tr>
<td>Guisset et al. 1993</td>
<td>France</td>
<td>•</td>
<td>50% with abnormal lacrimal secretion</td>
</tr>
<tr>
<td>Mariette et al. 1993</td>
<td>France</td>
<td>10%</td>
<td>•</td>
</tr>
<tr>
<td>Pawlotsky et al. 1994</td>
<td>France</td>
<td>•</td>
<td>49% with histological alterations of the salivary glands (14% with histological changes characteristic of SS)</td>
</tr>
<tr>
<td>Poet et al. 1994</td>
<td>France</td>
<td>•</td>
<td>9% with positive Schirmer’s test and very mild histological alterations of the salivary glands</td>
</tr>
<tr>
<td>Pirisi et al. 1994</td>
<td>Italy</td>
<td>•</td>
<td>77% with sialadenitis</td>
</tr>
<tr>
<td>Rodriguez-Cuartero et al. 1994</td>
<td>Spain</td>
<td>40%</td>
<td>•</td>
</tr>
<tr>
<td>King et al. 1994</td>
<td>USA</td>
<td>0% (subjects without cryoglobulinaemia)</td>
<td>•</td>
</tr>
<tr>
<td>Wattiaux et al. 1995</td>
<td>France</td>
<td>2.75%</td>
<td>42% with SS</td>
</tr>
<tr>
<td>Masaki et al. 1995</td>
<td>Japan</td>
<td>11%</td>
<td>•</td>
</tr>
<tr>
<td>Marrone et al. 1995</td>
<td>USA</td>
<td>0%</td>
<td>•</td>
</tr>
<tr>
<td>Boscagli et al. 1996</td>
<td>France</td>
<td>4.7%</td>
<td>17% with histological alterations of the salivary glands</td>
</tr>
<tr>
<td>Jorgensen et al. 1996</td>
<td>France</td>
<td>19%</td>
<td>•</td>
</tr>
<tr>
<td>Garcia et al. 1997</td>
<td>Spain</td>
<td>14%</td>
<td>•</td>
</tr>
</tbody>
</table>

* SS Sjögren’s syndrome
Patients and methods

To assess the frequency of the association between HCV-infection and SS in a geographical area at low HCV prevalence, the present study has examined the HCV serostatus of a group of British patients with SS, in which hyperglobulinaemia was excluded.

The study group comprised 18 patients (17 female, median age 54 years, range 45-75 years) with clinical, labial gland histopathological, and serological features of SS (Vitali et al. 1993) in the absence of cryoglobulinaemia. Eleven patients had primary SS while seven had secondary disease.

Serum samples were tested for the presence of IgG antibodies to HCV using two second-generation enzyme-linked immunosorbent assays (Ortho Diagnostic System, Raritan, NJ, USA; Murex Diagnostics, Dartford, UK). Additional confirmatory assays were not required.
Results and discussion

None of the eighteen patients with primary or secondary SS had serum IgG antibodies to HCV.

Thus, while some cohorts of patients with HCV infection may have clinical and histological evidence of Sjögren's syndrome-like salivary gland disease, there is no evidence that British patients with well-defined SS have HCV-infection. It is of course possible that variation in HCV genotype distribution, and perhaps clinical expression, may explain some of the contrasting data shown in Table 2.14. The present results might also be explained by the existence of a separate HCV-related sialadenitis, with clinical, histological and aetiological features different from those of the "true" SS; in this case it would not be surprising that in a country with a very low prevalence of HCV infection, such UK, none of a relatively small group of SS patients is HCV-positive. But until a study of the frequency of well-defined salivary gland disease in UK patients with HCV-infection is undertaken, this will remain unclear.
CHAPTER 3.

HEPATITIS C VIRUS INFECTION
AND THE DENTAL HEALTH CARE WORKER
3.1. Hepatitis C virus infection as an occupational hazard for the health care workers

3.1.1. Review of literature

Introduction

Transmission of microbial pathogens in the health care setting is a serious occupational hazard for the health care workers (HCW); in particular HCW involved in surgical procedures are at increased risk of infection by blood-borne viruses through needlestick injuries and other blood exposures. Until 1982, when vaccine against hepatitis B virus (HBV) became available, the dentist and other members of the dental staff were among the categories at highest risk of infection with HBV, having a risk ratio 3-5 times higher than that of the general population. Because of its parenteral transmission, high infectivity and presence in high titres in oral fluids, HBV is still a major occupational hazard for non-immune dental health care workers (DHCW) and in the United States 125 to 190 HCW die every year for occupationally acquired hepatitis B (Sepkowitz 1996). In addition numerous cases of transmission from infected HCW to patients have been documented (Heptonstall 1996). Thus, when in 1989 a new parenteral transmitted hepatitis virus (hepatitis C virus) was identified, it was assumed that, since HBV and HCV share similar routes of transmission, groups at increased risk for HBV infection also would be at risk for HCV infection.

Modes of HCV transmission in the health care setting

Since the first report of a surgeon becoming infected with HCV following an injury with a needlestick contaminated with HCV (Vaglia et al. 1990) several further episodes of nosocomial transmission of HCV have been documented and some cases have been confirmed by molecular confirmatory analysis; the majority of such incidents were sharp accidents (Cariani et al. 1991; Tsude et al. 1992). Hollow-bore needles are involved in most of the sharp
accidents leading to seroconversion, and a history of accidental needlestick injury was independently associated with anti-HCV in a group of HCW (Polish et al. 1993). Suture needles, scalpels or other sharp instruments have also been involved in occupational transmission of HCV. In addition to sharp injuries, exposure by other routes, such as conjunctival contact with HCV infected blood (Sartori et al. 1993), has led to HCV acquisition in the health care setting. The Public Health Laboratory Service (PHLS) Hepatitis Subcommittee described the possible occupational exposures to HCV (Table 3.1) and defined as significant “all the percutaneous exposures and any mucocutaneous exposure to blood and bloody body fluids (but not mucocutaneous exposure to other body fluids)” (Anonymous 1993). Although to date no dental HCW has knowingly acquired HCV via an occupational route, the high frequency of sharp injuries occurring in the dental setting (Felix et al. 1994; Siew et al. 1995) places the dental HCW at genuine risk of HCV acquisition. Hepatitis C virus RNA has been detected from a number of different surfaces of a dental clinic after treatment of a HCV-positive patient (Piazza et al. 1995) and can remain stable at room temperature for more than 5 days (Fong et al. 1993). Hepatitis C virus RNA is detectable in the saliva in 0 to 100% of infected individuals (Hsu et al. 1991; Wang et al. 1991; Couzigou et al. 1993; Roy et al. 1995), detection possibly being dependent upon the severity and duration of the HCV disease (Sugimura et al. 1995). Salivary HCV RNA may originate from the cell fraction or contaminating blood (Chen et al. 1995), and oral surgery seems to increase the occurrence of HCV in the oral fluid (Chen et al. 1995). Inoculation of HCV RNA positive saliva caused seroconversion in a recipient chimpanzee (Abe and Inchauspe 1991) and transmission of HCV via human bite has been reported, although the status of the source was unknown (Dusheiko et al. 1990).

Unfortunately at present no vaccine is available to prevent HCV infection and post-exposure prophylaxis with immune globulin is not recommended;
however a recent report by the Centre for Diseases Control (Anonymous 1997) suggested a number of procedures that should be included in the protocols for the follow-up of HCWs who sustained percutaneous or permucosal exposure to blood (Table 3.2).
Table 3.1: Hepatitis C virus: definition of occupational exposure according to UK Public Health Laboratory Service Hepatitis Subcommittee

Percutaneous exposure:
where the skin of the health care worker is cut or penetrated by a needle or other sharp object (e.g., scalpel blade, trochar, bone fragment or tooth) which is contaminated by blood or other body fluid

Mucocutaneous exposure:
where the eye(s), the inside the nose or mouth, or an area of non-intact skin of the health care worker are contaminated by blood or other body fluid

Significant exposure*:
includes all percutaneous exposure and any mucocutaneous exposure to blood or bloody body fluids (but not mucocutaneous exposure to other body fluids).

*significant exposures must be regarded as being of sufficient potential hazard to justify follow up, regardless of whether the source is identifiable or unknown.
Table 3.2: Recommendations of the Centers for Disease Control for the follow-up of health care workers, after percutaneous or permucosal exposure to potentially HCV-infected blood

- for the source, baseline testing for antibody to HCV (anti-HCV);
- for the person exposed to an anti-HCV-positive source, baseline and follow-up (e.g., 6-month) testing for anti-HCV and alanine aminotransferase activity;
- confirmation by supplemental anti-HCV testing of all anti-HCV results reported as repeatedly reactive by enzyme immunoassay (EIA);
- recommending against postexposure prophylaxis with immune globulin or antiviral agents (e.g., interferon); and
- education of HCWs about the risk for and prevention of bloodborne infections, including hepatitis C, in occupational settings, with the information routinely updated to ensure accuracy
Hepatitis C virus Epidemiology among the Health Care Workers

To assess the efficacy of HCV transmission following occupational accidents, a number of studies investigated the frequency of HCV seroconversion in groups of HCW who had experienced exposure to blood in the health care setting; in many of such studies all the patients involved in the accident were known to be HCV-positive (Table 3.3) (Kiyosawa et al. 1991; Polywka and Laufs 1991; Wormser et al. 1991; Francavilla et al. 1992; Hernandez et al. 1992; Marranconi et al. 1992; Mitsui et al. 1992; Bowden et al. 1993; Sodeyama et al. 1993; Lanphear et al. 1994; Perez-Trallero et al. 1994; Petroisillo et al. 1994; Zuckerman et al. 1994; Huertas et al. 1995; Puro et al. 1995; Puro et al. 1995; Arai et al. 1996; Mizuno et al. 1997).

With the exception of the results from two studies (Mitsui et al. 1992; Huertas et al. 1995), one of which included a very small group of HCW, the risk of HCV acquisition following occupational exposure is no higher than 6% and in many studies none of the large number of subjects exposed become infected. This low efficacy in the transmission of the HCV, particularly if compared with that of hepatitis B virus (Figure 3.1), is probably consistent with its low viral titer, although seroconversion rate does not increase when the source of infection are HIV/HCV co-infected patients in which enhanced replication is likely (Wormser et al. 1991).

It is noteworthy that with a single exception, all the seroconversion occurred after needlestick injuries and in a study following 646 occupational accidents 51% of which were hollow-bore needlesticks, 16.5% suture needle or sharp object injuries, 19.5% skin contaminations and 13% mucous membrane contaminations, the seroconversions occurred exclusively after hollow-bore needlesticks, while no seroconversions were observed after other routes of exposure (Puro et al. 1995).

This low efficiency is indirectly confirmed by numerous seroprevalence studies investigating the frequency of HCV infection in groups of HCW from
different geographical areas (Table 3.4) (Hoffman and Kunz 1990; Abb 1991; Arguillas et al. 1991; Chiaramonte et al. 1991; Jaqueti et al. 1991; Miyasaka 1991; Polywka and Laufs 1991; Besso et al. 1992; Blackmore et al. 1992; Cagatay et al. 1992; Campello et al. 1992; Cooper et al. 1992; De-Luca et al. 1992; Francavilla et al. 1992; Fujiyama et al. 1992; Jochen 1992; Libanore et al. 1992; Malaguti et al. 1992; Norrgren et al. 1992; Perez-Trallero et al. 1992; Petrarulo et al. 1992; Forseter et al. 1993; Polish et al. 1993; Stellini et al. 1993; Thomas et al. 1993; Tokars et al. 1993; Villate et al. 1993; Amarapurkar 1994; De-Brouwer and Lecomte 1994; Di-Nardo et al. 1994; Frider et al. 1994; Gerberding 1994; Germanaud et al. 1994; Jadoul et al. 1994; Jankovic et al. 1994; Petrosillo et al. 1994; Shakhgil'dian et al. 1994; Struve et al. 1994; Zuckerman et al. 1994; al-Sohaibani et al. 1995; Fujiyama et al. 1995; Panlilio et al. 1995; Puro et al. 1995; Neal et al. 1997; Olubuyide et al. 1997). However, with the exception of a study from an area at high prevalence in which the HCV prevalence was higher in the control group (12%) than in the HCW (11%) (Olubuyide et al. 1997), the frequency of HCV infection in the health care personnel is generally low, varying from 0% to 4.8%; such variability is probably due to a number of factors including prevalence of HCV infection in the geographical area, characteristics of the study group (number of subjects, age, speciality), and serological test used. However, although HCV infection may be more common among the HCW groups than the control groups or the general population, this difference is rarely significant, indicating a relatively low risk of HCV occupational acquisition for the HCW. In addition it must be noted that blood donors, often used as controls, are a highly selected group and thus the HCV-prevalence in HCW might be even closer to that of the general population.

Little risk of HCV transmission has also been indicated by studies on DHCW (Table 3.5) (Schiff et al. 1990; Klein et al. 1991; Herbert et al. 1992; Kuo et al. 1993; Gerberding 1994; Thomas et al. 1996; Olubuyide et al. 1997). In 1990
1% of a group of US dental health care workers were found to be HCV seropositive; auxiliary dental personnel being at a higher risk for HCV infection (1.4%) than dental surgeons (Schiff et al. 1990). In a second study eight of 456 (1.75%) dental health care workers in the New York City metropolitan area were anti-HCV seropositive compared with one of 723 controls, oral surgeons were at higher risk than other dentists (Odd Ratio 10.5; 95% Confidence Interval: 1.9 to 58) (Klein et al. 1991). In a third study none of 94 dental surgeons in Wales were found to be HCV-infected even though 68% of the group had sustained inoculation injuries (Herbert et al. 1992). Another study found that only three of 481 (0.65%) dentists from Taiwan were infected, the seroprevalence rate being similar to that of local blood donors (0.95%) and pregnant women (0.63%) (Kuo et al. 1993). None of the 90 dentists providing dental care at the San Francisco General Hospital were seropositive for anti-HCV antibodies (Gerberding 1994). More recently the low risk of HCV carriage amongst dental health care staff was confirmed by the finding of 2% of 343 US oral surgeons and 0.7% of 305 dentists being HCV seropositive (Thomas et al. 1996). In addition, a study of a group of Nigerian HCW formed at 30% by dentists, found a high prevalence of infection (11%) although lower than that of the local unpaid blood donors (12%) (Olubuyide et al. 1997).

The existence of an only slightly increased risk of HCV occupational acquisition has been further confirmed by few incidence studies undertaken in large groups of HCW (Table 3.6) (Di-Nardo et al. 1994; Gerberding 1994; Lanphear et al. 1994; Puro et al. 1995; Stroffolini et al. 1996). A large Italian study enrolling 40% of all the hospital workers, found that since 1988 the incidence of non A, non B hepatitis (NANBH) in HCW has fallen from 12.3 x 100,000 subjects per year, to 4.3 x 100,000 subjects per year; since more than 70% of NANBH cases in this study were HCV-positive, it is likely that this decline involved HCV infection as well. The authors explain this data with
the implementation of blood donors screening that almost eliminated the risk of transfusion-associated NANBH and the educational campaign against HIV infection that may have affected also other blood-borne infections (Stroffolini et al. 1996).

It is evident that the risk of HCV acquisition through an occupational route is generally small. Nevertheless in the absence of an effective therapy and any passive or active vaccination there is still a significant risk that HCV-infected health care workers will develop chronic hepatic disease (Manian 1992).

As aforementioned in a previous section, nosocomial patient acquisition of HCV infection is possible and in some geographic areas the use of non-disposable needles may potentially have played a role in the spread of the infection (Chen et al. 1995). Recently, a study investigating HCV incidence among a group of low risk blood donors, described a patient that seroconverted whose only identifiable possible risk factor was a gingivectomy by a dental surgeon of unknown HCV status (Prati et al. 1997); in addition one case-control study found a strong association between dental treatment and HCV-seropositivity (Mele et al. 1994).

Hepatitis C virus infection can cause profound morbidity, thus all dental health care staff must take appropriate steps to minimise transmission of HCV during dental health care. Fortuitously few DHCW have became infected with HCV via occupational routes and thus hopefully the maintenance of effective cross-infection procedures should minimise further DHCW becoming infected by these routes. Current cross-infection control measures are sufficient to prevent HCV transmission however good standards of cross-infection control must be maintained and regularly reviewed in the absence of an effective vaccine program for HCV.
### Table 3.3: Hepatitis C virus seroconversion rates in health care workers occupationally exposed to blood

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>N° of Subjects</th>
<th>Serological tests</th>
<th>Sero conversions n° (%)</th>
<th>Clinical exposure</th>
<th>FU† months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiyosawa et al. 1991</td>
<td>Japan</td>
<td>110*</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
<td>3 (2.7%)</td>
<td>needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Poliwka et al. 1991</td>
<td>Germany</td>
<td>18</td>
<td>HCV 1st generation ELISA</td>
<td>0</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Wormser et al. 1991</td>
<td>USA</td>
<td>98**</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
<td>0</td>
<td>•</td>
<td>17.6</td>
</tr>
<tr>
<td>Francavilla et al. 1992</td>
<td>Italy</td>
<td>173</td>
<td>HCV 2nd generation ELISA</td>
<td>0</td>
<td>•</td>
<td>12</td>
</tr>
<tr>
<td>Hernandez et al. 1992</td>
<td>Spain</td>
<td>81*</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0</td>
<td>•</td>
<td>12</td>
</tr>
<tr>
<td>Mitsui et al. 1992</td>
<td>Japan</td>
<td>68*</td>
<td>anti cp9 ELISA or anti cp10 ELISA or anti GOR ELISA or HCV 1st generation ELISA or PCR</td>
<td>7 (10%)</td>
<td>needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Marranconi et al. 1992</td>
<td>Italy</td>
<td>117*</td>
<td>HCV ? generation ELISA and HCV ? generation RIBA</td>
<td>3 (2.5%)</td>
<td>needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Bowden et al. 1993</td>
<td>Australia</td>
<td>230</td>
<td>•</td>
<td>0</td>
<td>•</td>
<td>12</td>
</tr>
<tr>
<td>Sodeyama et al. 1993</td>
<td>Japan</td>
<td>88*</td>
<td>anti cp9 ELISA or anti cp10 ELISA or anti GOR ELISA or HCV 1st generation ELISA or PCR</td>
<td>2 (2.3%)</td>
<td>needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Langheer et al. 1994</td>
<td>USA</td>
<td>50*</td>
<td>HCV 2nd generation ELISA</td>
<td>3 (6.0%)</td>
<td>needlestick</td>
<td>5</td>
</tr>
<tr>
<td>Perez-Trallero et al. 1994</td>
<td>Spain</td>
<td>53</td>
<td>HCV 2nd generation ELISA or HCV 3rd generation ELISA</td>
<td>1 (1.8%)</td>
<td>biopsy forceps</td>
<td>6</td>
</tr>
<tr>
<td>Petrozillo et al. 1994</td>
<td>Italy</td>
<td>140</td>
<td>•</td>
<td>0</td>
<td>•</td>
<td>10</td>
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<tr>
<td>Zuckerman et al. 1994</td>
<td>UK</td>
<td>24*</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0</td>
<td>•</td>
<td>12</td>
</tr>
<tr>
<td>Puro et al. 1995</td>
<td>Italy</td>
<td>133*</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>1 (0.75%)</td>
<td>73% needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Puro et al. 1995</td>
<td>Italy</td>
<td>646*</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>4 (0.8%)</td>
<td>51% needlestick</td>
<td>6</td>
</tr>
<tr>
<td>Huertas et al. 1995</td>
<td>Mexico</td>
<td>9*</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>1 (11%)</td>
<td>needlestick</td>
<td></td>
</tr>
<tr>
<td>Arai et al. 1996</td>
<td>Japan</td>
<td>56*</td>
<td>HCV 1st generation ELISA and HCV 2nd generation ELISA and RT-PCR</td>
<td>3 (5.4%)</td>
<td>needlestick</td>
<td></td>
</tr>
<tr>
<td>Mizuno et al. 1997</td>
<td>Japan</td>
<td>37*</td>
<td>HCV 2nd generation ELISA and RT-PCR</td>
<td>2 (5.4%)</td>
<td>needlestick</td>
<td></td>
</tr>
</tbody>
</table>

† FU follow up
* 12.6% of donors were HCV+  †† seroconversion among needlestick only was 1.2%
* All donors were HCV+  ** All donors were HIV+
HCV 1st generation ELISA tests C100-3 antibody
HCV 2nd generation ELISA tests C100-3, C33-c, C22-3 antibodies
HCV 1st generation RIBA tests C100-3, 5-1-1 antibodies
HCV 2nd generation RIBA tests C100-3, C33-c, C22-3, 5-1-1 antibodies

156
Table 3.4: Prevalence of HCV infection among health care workers
(a) 1990-1992

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Subjects</th>
<th>Tests</th>
<th>Study Group</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td>Hofmann et al. 1990</td>
<td>Austria</td>
<td>294</td>
<td>HCV 1st generation ELISA</td>
<td>2.0</td>
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<tr>
<td>Abb et al. 1991</td>
<td>Germany</td>
<td>738</td>
<td>HCV 1st generation ELISA</td>
<td>1.1</td>
<td>•</td>
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<tr>
<td>Polywka et al. 1991</td>
<td>Germany</td>
<td>217</td>
<td>HCV 1st generation ELISA</td>
<td>2.8</td>
<td>0.4*</td>
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<tr>
<td>Chiaramonte et al. 1991</td>
<td>Italy</td>
<td>1519</td>
<td>HCV 1st generation ELISA</td>
<td>1.57</td>
<td>1.31§</td>
</tr>
<tr>
<td>Miyasaka et al. 1991</td>
<td>Japan</td>
<td>145</td>
<td>HCV 2nd generation ELISA</td>
<td>2</td>
<td>?</td>
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<td>Arguillas et al. 1991</td>
<td>Philippines</td>
<td>123</td>
<td>HCV 1st generation ELISA</td>
<td>1.6</td>
<td>2.2</td>
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<tr>
<td>Jaqueti et al. 1991</td>
<td>Spain</td>
<td>413</td>
<td>•</td>
<td>1.7</td>
<td>•</td>
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<td>Norrgren et al. 1992</td>
<td>Sweden</td>
<td>311</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.96</td>
<td>•</td>
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<td>Blackmore et al. 1992</td>
<td>New Zealand</td>
<td>20</td>
<td>ELISA</td>
<td>0</td>
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<tr>
<td>Cagatay et al. 1992</td>
<td>Turkey</td>
<td>416</td>
<td>•</td>
<td>0.9</td>
<td>1.6</td>
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<td>Jochen et al. 1992</td>
<td>Germany</td>
<td>1033</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.58</td>
<td>0.24</td>
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<tr>
<td>Besso et al. 1992</td>
<td>Italy</td>
<td>55</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>3.6</td>
<td>•</td>
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<tr>
<td>Campello et al. 1992</td>
<td>Italy</td>
<td>407</td>
<td>HCV 1st generation ELISA and neutralization test or HCV 2nd generation ELISA</td>
<td>1.2</td>
<td>0.8§</td>
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<td>DeLuca et al. 1992</td>
<td>Italy</td>
<td>945</td>
<td>HCV 1st generation ELISA</td>
<td>4.8</td>
<td>1.1‡</td>
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<td>Francavilla et al. 1992</td>
<td>Italy</td>
<td>635</td>
<td>HCV 2nd generation ELISA</td>
<td>0.8</td>
<td>•</td>
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<td>Libanore et al. 1992</td>
<td>Italy</td>
<td>1008</td>
<td>HCV ?? generation ELISA</td>
<td>4.07</td>
<td>0.95‡</td>
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<tr>
<td>Malaguti et al. 1992</td>
<td>Italy</td>
<td>20</td>
<td>ELISA</td>
<td>0</td>
<td>0.46</td>
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<td>Petrarulo et al. 1992</td>
<td>Italy</td>
<td>122</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
<td>2.45</td>
<td>•</td>
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<td>Fujiyama et al. 1992</td>
<td>Japan</td>
<td>152</td>
<td>HCV 1st generation ELISA</td>
<td>0.7</td>
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<td>Perez Trallero et al. 1992</td>
<td>Spain</td>
<td>349</td>
<td>•</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Cooper et al. 1992</td>
<td>USA</td>
<td>243</td>
<td>HCV 1st generation ELISA and HCV 2nd generation RIBA</td>
<td>1.6</td>
<td>•</td>
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<tr>
<td>Tokars et al. 1992</td>
<td>USA</td>
<td>3267</td>
<td>•</td>
<td>0.8</td>
<td>•</td>
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<td>Tests</td>
<td>Study Group</td>
<td>Control Group</td>
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<td>-----------</td>
<td>--------------------------------------------</td>
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<td>---------------</td>
</tr>
<tr>
<td>Stellini et al. 1993</td>
<td>Italy</td>
<td>1357</td>
<td>HCV 1st generation ELISA and HCV ? generation RIBA</td>
<td>1.47</td>
<td>0.86*</td>
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<tr>
<td>Nakashima et al. 1993</td>
<td>Japan</td>
<td>1097</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA or anti GOR ELISA</td>
<td>3.0</td>
<td>1.1§</td>
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<td>Villate et al. 1993</td>
<td>Spain</td>
<td>874</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>1.6</td>
<td>0.4</td>
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<td>Struve et al. 1993</td>
<td>Sweden</td>
<td>880</td>
<td>HCV 1st generation ELISA and supplement test</td>
<td>0.7</td>
<td>0.6*</td>
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<tr>
<td>Forseter et al. 1993</td>
<td>USA</td>
<td>518*</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
<td>1.9</td>
<td>•</td>
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<tr>
<td>Polish et al. 1993</td>
<td>USA</td>
<td>1677</td>
<td>HCV 1st generation ELISA and neutralization test</td>
<td>1.4</td>
<td>•</td>
</tr>
<tr>
<td>Thomas et al. 1993</td>
<td>USA</td>
<td>943</td>
<td>HCV 1st generation ELISA and HCV 2nd generation RIBA or HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.7</td>
<td>0.4*§</td>
</tr>
<tr>
<td>Shakhgild'yan et al. 1994</td>
<td>Russia</td>
<td>1581</td>
<td>•</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Frider et al. 1994</td>
<td>Argentina</td>
<td>439</td>
<td>HCV 2nd generation ELISA and Line Immunoassay</td>
<td>1.59</td>
<td>•</td>
</tr>
<tr>
<td>De Brouwer et al. 1994</td>
<td>Belgium</td>
<td>2031</td>
<td>HCV 3rd generation ELISA and HCV 3rd generation RIBA</td>
<td>1.48</td>
<td>0.32*§</td>
</tr>
<tr>
<td>Jadoul et al. 1994</td>
<td>Belgium</td>
<td>120</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>4.1</td>
<td>0.6*</td>
</tr>
<tr>
<td>Jankovic et al. 1994</td>
<td>Croatia</td>
<td>75</td>
<td>HCV 2nd generation ELISA</td>
<td>0</td>
<td>•</td>
</tr>
<tr>
<td>Germainaud et al. 1994</td>
<td>France</td>
<td>610</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>1.14</td>
<td>•</td>
</tr>
<tr>
<td>Amarapurkar et al. 1994</td>
<td>India</td>
<td>90</td>
<td>•</td>
<td>0</td>
<td>•</td>
</tr>
<tr>
<td>Di Nardo et al. 1994</td>
<td>Italy</td>
<td>937</td>
<td>HCV 2nd generation ELISA and HCV ? generation RIBA</td>
<td>0.85</td>
<td>0.53*§</td>
</tr>
<tr>
<td>Zuckerman et al. 1994</td>
<td>U.K.</td>
<td>1053</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.28</td>
<td>•</td>
</tr>
<tr>
<td>Gerberding et al. 1994</td>
<td>USA</td>
<td>851</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>1.4</td>
<td>•</td>
</tr>
</tbody>
</table>
### (c) 1995-1997

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Subject</th>
<th>Tests</th>
<th>Study Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panlilio et al. 1995</td>
<td>USA</td>
<td>770</td>
<td>•</td>
<td>0.9</td>
<td>*</td>
</tr>
<tr>
<td>Petrosillo et al. 1995</td>
<td>Italy</td>
<td>5813</td>
<td>various</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>Puro et al. 1995</td>
<td>Italy</td>
<td>3073</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>2.2</td>
<td>*</td>
</tr>
<tr>
<td>Fujimura et al. 1995</td>
<td>Japan</td>
<td>216</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>2.3</td>
<td>1.3§</td>
</tr>
<tr>
<td>al-Sohaibani et al. 1995</td>
<td>Saudi Arabia</td>
<td>46</td>
<td>ELISA and RIBA</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Olubuyide et al. 1997</td>
<td>Nigeria</td>
<td>75</td>
<td>HCV 3rd generation ELISA</td>
<td>11</td>
<td>12*</td>
</tr>
<tr>
<td>Neal et al. 1997</td>
<td>UK</td>
<td>1949</td>
<td>HCV 2nd generation ELISA and HCV 3rd generation ELISA</td>
<td>0.2</td>
<td>*</td>
</tr>
</tbody>
</table>

*Local blood donors        † All orthopaedic surgeons;  ††† HCV of a dialysis unit
++++ All surgeons; ‡‡‡‡ (28 physicians, 25 surgeons, 22 dentists)
§ New workers   not significant difference   significant difference

HCV 1st generation ELISA tests C100-3 antibody;
HCV 2nd generation ELISA tests C100-3, C33-c, C22-3 antibodies;
HCV 1st generation RIBA tests C100-3, 5-1-1 antibodies;
HCV 2nd generation RIBA tests C100-3, C33-c, C22-3, 5-1-1 antibodies.
Table 3.5: Seroprevalence of HCV in groups of dental health care workers

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Subjects</th>
<th>Study Group</th>
<th>Control Group</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schiff et al. 1990</td>
<td>USA</td>
<td>960 dental health care workers</td>
<td>1%</td>
<td>•</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
</tr>
<tr>
<td>Klein et al. 1991</td>
<td>USA</td>
<td>456 dentists</td>
<td>1.75%</td>
<td>0.14%</td>
<td>HCV 1st generation ELISA and HCV 1st generation RIBA</td>
</tr>
<tr>
<td>Herbert et al. 1992</td>
<td>U.K.</td>
<td>94 oral surgeons</td>
<td>0%</td>
<td>0.3%*</td>
<td>HCV 2nd generation ELISA</td>
</tr>
<tr>
<td>Kuo et al. 1993</td>
<td>Taiwan</td>
<td>461 dentists</td>
<td>0.65%</td>
<td>0.95%*</td>
<td>HCV 2nd generation ELISA</td>
</tr>
<tr>
<td>Gerberding 1994</td>
<td>USA</td>
<td>90 dentists</td>
<td>0%</td>
<td>•</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
</tr>
<tr>
<td>Thomas et al. 1996</td>
<td>USA</td>
<td>343 oral surgeons 305 dentists</td>
<td>2.0%</td>
<td>0.7%</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
</tr>
<tr>
<td>Olubuyide et al. 1997</td>
<td>Nigeria</td>
<td>75 health care workers (30% of whom dentists)</td>
<td>11%</td>
<td>12%*</td>
<td>HCV 3rd generation ELISA</td>
</tr>
</tbody>
</table>

*Local blood donors
Table 3.6: Hepatitis C virus incidence among health care workers

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>N° of Subjects</th>
<th>Tests</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di Nardo et al. 1994</td>
<td>Italy</td>
<td>765</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.03 x100 subjects-year</td>
</tr>
<tr>
<td>Gerberding et al. 1994</td>
<td>USA</td>
<td>851</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.08 x100 subjects-year</td>
</tr>
<tr>
<td>Lanphear et al. 1994</td>
<td>USA</td>
<td>2882</td>
<td>HCV 2nd generation ELISA and Matrix Immunoblot</td>
<td>0.054 x100 subjects-year</td>
</tr>
<tr>
<td>Puro et al. 1995</td>
<td>Italy</td>
<td>2622</td>
<td>HCV 2nd generation ELISA and HCV 2nd generation RIBA</td>
<td>0.1 x100 subjects -year</td>
</tr>
<tr>
<td>Stroffolini et al. 1996</td>
<td>Italy</td>
<td>&gt;250,000$</td>
<td>various</td>
<td>0.0031 x100 subjects-year*</td>
</tr>
</tbody>
</table>

$ 40\%$ of the Italian hospital workers

* vs 0.0018 x100 subjects-year among the general population
Figure 3.1: Risk of seroconversion after occupational exposure with different blood-borne viruses
3.1.2. Prevalence of HCV infection in health care workers of a UK dental hospital

Introduction

As discussed before, to date there have been few published reports of the frequency of HCV infection in DHCW (see Table 3.5), only one considering UK DHCW - a small group in Wales (Herbert et al. 1992). Thus, the aim of the present investigation is to determine the frequency of HCV seropositivity in a large cohort of DHCW working inside UK, using currently available specific serological assays.

Materials and Methods

Study group (Table 3.7 and 3.8)

The study group comprised 167 apparently healthy DHCW who had worked in a UK Dental Hospital sometime between 1992 and 1995. Details of the occupational status, gender and age of the DHCW are provided in Table 3.7 and the specialities of the DHCW are indicated in Table 3.8. As part of a yearly occupational health assessment each DHCW underwent venepuncture for hepatitis B virus serological assessment. An aliquot of this serum was stored at -20°C for subsequent Hepatitis C virus antibody estimation. At the time of venepuncture staff gave written consent for their blood to be used for additional research investigations; local ethical approval was obtained prior to commencement of the study.

Serum IgG antibodies to HCV were tested in January 1996 using two third-generation enzyme-linked immunosorbent assays (ELISA) (Ortho Diagnostic Systems, Emmeryville, California, US; Sanofi Diagnostic Pasteur, Marnes la Coquette, France). Confirmation of HCV seropositivity was based on polymerase chain reaction (PCR) analysis.
Table 3.7: Occupational status, gender and age of 167 UK dental health care staff

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Gender</th>
<th>Mean age (years)</th>
<th>Range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Dentists</td>
<td>39</td>
<td>52</td>
<td>31.6</td>
</tr>
<tr>
<td>Dental nurses</td>
<td>23</td>
<td>0</td>
<td>31.6</td>
</tr>
<tr>
<td>Student dental nurses</td>
<td>21</td>
<td>1</td>
<td>22.4</td>
</tr>
<tr>
<td>Other auxiliary staff*</td>
<td>6</td>
<td>9</td>
<td>27.7</td>
</tr>
<tr>
<td>Office staff</td>
<td>10</td>
<td>6</td>
<td>31.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>67</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* Including scientific laboratory research staff and dental laboratory technical staff
### Table 3.8: Speciality of 167 UK dental health care staff

<table>
<thead>
<tr>
<th>Speciality</th>
<th>Dentists</th>
<th>Nurses</th>
<th>Other</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Conservation</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Endodontics</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Oral Medicine</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Oral Surgery</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Orthodontics</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Periodontology</td>
<td>19</td>
<td>6</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Prosthetics</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Student dental nurses*</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>0</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Offices</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>91</td>
<td>45</td>
<td>31</td>
<td>167</td>
</tr>
</tbody>
</table>

* various departments
Results

Two of the 167 (1.2%) DHCW had detectable IgG antibodies to hepatitis C virus - a qualified dental nurse and a student dental nurse who had just commenced dental duties. The mode of acquisition of the HCV of the two asymptomatic DHCW was not known. No other DHCW had serological evidence of HCV infection (Table 3.9).
Table 3.9: Frequency of HCV seropositivity in 167 UK dental health care staff

<table>
<thead>
<tr>
<th>Occupation</th>
<th>HCV serpositive subjects</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>percentage</td>
<td></td>
</tr>
<tr>
<td>Dentists</td>
<td>0/91</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Dental nurses</td>
<td>1/23</td>
<td>4.34%</td>
<td></td>
</tr>
<tr>
<td>Student dental nurses</td>
<td>1/22†</td>
<td>4.54%</td>
<td></td>
</tr>
<tr>
<td>Other auxiliary staff*</td>
<td>0/15</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Office staff</td>
<td>0/16</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2/167</td>
<td>1.19%</td>
<td></td>
</tr>
</tbody>
</table>

† also HCV-viremic
* including scientific laboratory research staff and dental laboratory technical staff
Discussion

Since the prevalence of HCV infection in the UK general population is less than 0.2%, the present result of 2 out of 167 DHCW (1.2%), together with those of the previous studies (Table 3.5), suggests a slightly increased risk of HCV infection in DHCW. However these data must be interpreted with caution. The sources of HCV-infection of the present two HCV-infected DHCW are not known. It is possible that they have become infected via routes such as blood transfusion or, possibly, injecting drugs use. Our data differ from the seroprevalence observed by Zuckerman and co-authors among non dental health care workers of the same metropolitan area (0.28%) (Zuckerman et al. 1994). This difference could be due to the higher number of surgical procedures performed by DHCW in comparison with most of the other health care workers as well as the high incidence of sharp injuries occurring among dental staff (Felix et al. 1994; Siew et al. 1995). Similar to the data of the previous studies, the present results would suggest that dental auxiliary staff may be at slightly increased risk of HCV infection. It is possible that this may reflect their liability to needlestick injuries during routine dental healthcare (Porter et al. 1990), but, as noted above, we cannot be sure about the source of HCV acquisition of the previous two DHCW.

While there is no evidence that infected HCW can easily transmit HCV to patients or other staff workers during dental treatment, the current data highlights the need for all DHCW to maintain high standards of cross-infection control measures to avoid nosocomial transmission of HCV.

In conclusion a small number of DHCW in the UK may be infected with HCV. Risk of HCV infection may to be higher in dental nurses than in other groups of DHCW, however, this may not reflect occupational risk but activities outwith dental practice.
3.2. **Knowledge of hepatitis C virus (HCV) infection among dental students from three European countries.**

Introduction

Appropriate training of dental undergraduates on aspects of blood-borne pathogens such as HIV and hepatitis B virus (HBV) is important, not only because of their possible nosocomial transmission in the dental environment, but also because they can give rise to oral manifestations that may be diagnosed and/or managed by dentists. A number of studies have investigated dental students' knowledge and attitude regarding HIV and HBV infection (Ranta and Tuominen 1991; Gilbert and Nuttall 1994; Chehaitly and Alary 1995), but to our knowledge there is no such study on hepatitis C virus (HCV) infection.

As mentioned in previous sections, the HCV-infected dental patient may have oral manifestations, such as oral lichen planus and xerostomia and request special dental management (Wisnom and Kelly 1993). Furthermore there is a potential, albeit small, risk of HCV transmission during routine dental therapy.

In view of the lack of data on the knowledge or attitudes of dental students regarding HCV infection, the aim of the present investigation was to investigate the knowledge of dental undergraduates from three European countries concerning aspects of HCV infection relevant to the practice of dentistry; in addition a group of dentists from different countries, attending postgraduate programmes at a UK dental post-graduate education and training institute, has been surveyed.
Subjects and methods

A self-administered structured questionnaire in English, Italian or Spanish was given to dental students in their fourth and fifth year of undergraduate training in three European countries: United Kingdom (n=63), Italy (n=93) and Spain (n=200). The samples surveyed comprised all the students attending lectures of the day the survey was undertaken. The questionnaire was also given to all the dentists attending postgraduate programmes at the Eastman Dental Institute, a UK postgraduate education and training institute. The dentists of this group received undergraduate education from many different dental schools world-wide.

The anonymous questionnaire was based on data on HCV available at the time of its devising and comprised three parts. The preliminary section included details of the demographics of the respondents, who were also asked if they had been tested for HCV infection and vaccinated against HBV infection. In the second section the knowledge of the respondents with respect to general aspects of HCV infection was assessed, this included questions on likely routes of transmission of the virus, and the natural history, diagnosis and treatment of HCV infection. In the third section the respondents were questioned on the dental implications of the infection, including the risk of transmission during dental health care and the possible associated oral manifestations.
Results

All the subjects completed the questionnaire.

Demographic details

Table 3.10 provides the demographic and personal details of the four groups. As expected the postgraduates have a mean age higher than the undergraduate groups. Whereas only 6.0% of UK undergraduates had been tested for HCV infection, almost 25.0% of Italian students had been tested. In contrast more than 90% of all the groups of respondents had been vaccinated against HBV.

Knowledge of HCV transmission

The respondents' knowledge of HCV routes of transmission is summarized in Table 3.11. The majority of respondents were aware that risk of HCV acquisition is mainly associated with exposure to infected blood (including blood transfusion and use of blood derivatives) and sharing needles in injecting drug use; however a significant proportion, particularly students, believed that HCV transmission was likely via social contact, cutlery sharing and sex between females, thus demonstrating some uncertainty on the understanding of modes of transmission of HCV.

Knowledge of the natural history, diagnosis and treatment of HCV infection.

Forty percent of the Italian, and nearly half of the Spanish undergraduates believing HCV infection to be likely to cause fulminant hepatitis (a rare event), and about half of all respondents suggested that severe acute hepatitis is a common consequence of HCV acquisition (Table 3.12), again an unusual feature of HCV infection. Furthermore a considerable proportion of the respondents overestimated the significance of the patient medical history and clinical assessment in the diagnosis of HCV infection.

Oral and dental aspects of HCV

Only a small percentage of the four groups was aware of having treated a patient with HCV infection, an activity considered a likely occupational hazard
by most of the respondents and that for almost all of the Italian undergraduates requires additional precautions against nosocomial transmission of HCV. All the four groups considered surgical treatment as the most likely activity to result in HCV transmission during dentistry (Table 3.14). Respondents showed little knowledge of the possible oral manifestations of HCV disease. About half of the Italian undergraduates knew of the possible association between HCV infection and lichen planus, nevertheless 60% of the respondents were not able to answer many of the questions of this section of the questionnaire (Table 3.15).
Discussion

Hepatitis C virus infection is a significant health problem of which dentists should have some knowledge. This is the first study investigating the knowledge of HCV infection among dental students.

Although HCV infection is present in most countries world-wide, its prevalence has high geographic variability; in Western Europe the frequency of HCV infection in blood donors may vary from one in 1400 in the UK (MacLennan et al. 1994) to ten times greater figures in the Mediterranean area (Chiaramonte et al. 1991). The geographic variations in HCV prevalence may underlie the different proportion of respondents tested for HCV infection, this being higher among the Italian and Spanish than UK respondents (Table 3.10). Furthermore it must be noted that Italian undergraduates, despite the highest HCV testing, have the lowest percentage of HBV vaccination, one in ten students not being vaccinated.

Hepatitis C virus is principally transmitted via parenteral routes and as a consequence the main patient groups liable to HCV carriage are injecting drug users and recipients of blood and blood products. The respondents had a fairly good knowledge of HCV transmission, particularly the most common routes such as blood transfusions or needle sharing in injecting-drug use; however it is noteworthy that 25% or more of postgraduates were unable to answer 9 out of the 13 questions related to route of HCV infection (Table 3.11). The postgraduates may have a poorer knowledge than the undergraduates as a consequence of HCV being identified only recently; indeed over 50% of the postgraduates had obtained their dental qualification prior to 1989, when HCV was first described (Choo et al. 1989) and were thus very unlikely to have adequate information on this subject. These findings could also reflect insufficient continuing education - particularly worrying as the present group of responding dentists were attending postgraduate courses and thus likely to be motivated as regards updating their knowledge
of dental education. Previous studies on the knowledge of HIV infection and AIDS among dental health care workers have pointed out the relevance of the mass media as source of information, (Nair et al. 1995; Kitaura et al. 1997) thus the more limited media attention on HCV-related health problems may also have influenced the knowledge of this group of postgraduates.

Respondents from all the four groups showed an incomplete knowledge of the natural history of HCV-associated liver disease; the ability of HCV infection to induce a fulminant or severe acute hepatitis was overestimated by large proportions of all the groups, in addition 17.5% of the Spanish undergraduates and nearly 10% of postgraduates thought that a vaccine against HCV infection is available. The postgraduates again had a rather greater inadequate understanding of natural history than the undergraduates, diagnosis and treatment of HCV infection, indeed more than 30% of them were unable to answer 7 out of 8 relevant questions (Table 3.12).

In contrast to available data regarding HCV infection in dental health care workers, many of the respondents believed that HCV infection was possible, if not likely, during dental treatment (Table 3.13). Italian students in particular considered additional protective precautions to be necessary when providing dental treatment to HCV-infected patients. This may reflect local teaching, or suggest a lack of understanding of the principles of universal precautions, or fear of infection with HCV. Due to the known parenteral spread of HCV, and the high frequency of needlestick/sharp injuries in dental practice (Felix et al. 1994; Siew et al. 1995) and among clinical dental students (de Vries and Cossart 1994), the undergraduate and postgraduate students were probably correct in regarding surgical treatment as a more likely cause of HCV transmission than clinical examination alone (Table 3.14). Nevertheless the actual frequency of nosocomial HCV acquisition following a needlestick injury with an HCV contaminated needle is probably low (Table 3.3).
As previously discussed, a number of associations between HCV infection and oral diseases has been proposed but not all of them are consistent. There may be a significantly increased frequency of HCV infection in some groups of patients with lichen planus; some HCV-positive patients may be affected by lymphocytic sialadenitis, sometimes manifesting as xerostomia, and an association between HCV infection and oral cancer has been reported in Japanese patients. However these oral features may be absent in some groups of patients with HCV infection, thus a lack of knowledge of such aspects might be expected. Nevertheless while the other groups of respondents had no knowledge of such disease associations, half of the Italian undergraduates did know that lichen planus could be an oral feature of HCV infection; possibly reflecting the higher prevalence of HCV infection in Italy, the common finding of HCV infection among Italian lichen planus patients, or local educational policies. Conversely the lack of knowledge of the UK respondents may reflect the low prevalence of HCV in the UK, while the poor knowledge of the Spanish undergraduates is unlikely to be due to this factor and may reflect other influences.

The results of this “snap-shot” of European dental undergraduates and postgraduates suggest that they have a limited knowledge of the relevant aspects of HCV infection and thus there is a need to inform them more adequately of the potential risks of transmission of HCV during dental treatment, and the possible oral manifestations of HCV disease.
Table 3.10: Demographics of 343 dental European undergraduate students and 63 postgraduates students

<table>
<thead>
<tr>
<th></th>
<th>Italian Undergraduates (n=93)</th>
<th>UK Undergraduates (n=50)</th>
<th>Spanish Undergraduates (n=200)</th>
<th>Postgraduates (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ±SD (range)</td>
<td>24.7±4.7 (21-52)</td>
<td>23.0±2.9 (20-32)</td>
<td>22.8±3.8 (22-38)</td>
<td>30.4±6.1 (22-51)</td>
</tr>
<tr>
<td>Female/male</td>
<td>37/56</td>
<td>24/26</td>
<td>126/74</td>
<td>27/36</td>
</tr>
<tr>
<td>Years of dental practice</td>
<td>na*</td>
<td>na*</td>
<td>na*</td>
<td>7.5±5.0 (1-28)</td>
</tr>
<tr>
<td>Tested for HCV infection</td>
<td>24.5%</td>
<td>6.0%</td>
<td>11.0%</td>
<td>12.7%</td>
</tr>
<tr>
<td>Vaccinated against hepatitis B virus (HBV)</td>
<td>90.4%</td>
<td>96.0%</td>
<td>94.0%</td>
<td>93.4%</td>
</tr>
</tbody>
</table>

* not applicable
Table 3.11: Knowledge of dental undergraduates and postgraduates of routes of transmission of HCV

<table>
<thead>
<tr>
<th>Proposed route of transmission of HCV</th>
<th>Italian Undergraduates (%)</th>
<th>UK Undergraduate (%)</th>
<th>Spanish Undergraduates (%)</th>
<th>Postgraduates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yes</td>
<td>no</td>
<td>do not know</td>
<td>yes</td>
</tr>
<tr>
<td>Sexual intercourse between males and females</td>
<td>92.6</td>
<td>6.4</td>
<td>1.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Sexual intercourse between males</td>
<td>94.6</td>
<td>2.2</td>
<td>3.2</td>
<td>89.6</td>
</tr>
<tr>
<td>Sexual contact between females</td>
<td>71.4</td>
<td>11.0</td>
<td>17.6</td>
<td>54.2</td>
</tr>
<tr>
<td>Social contact</td>
<td>13.2</td>
<td>83.5</td>
<td>3.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Contact with blood</td>
<td>98.9</td>
<td>1.1</td>
<td>0</td>
<td>98.0</td>
</tr>
<tr>
<td>Receipt of blood transfusion</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>94.0</td>
</tr>
<tr>
<td>Receipt of blood products</td>
<td>86.0</td>
<td>2.2</td>
<td>11.8</td>
<td>59.2</td>
</tr>
<tr>
<td>Tattooing</td>
<td>73.4</td>
<td>10.8</td>
<td>16.0</td>
<td>68.0</td>
</tr>
<tr>
<td>Body piercing (ie ears, nipples, etc.)</td>
<td>60.4</td>
<td>16.5</td>
<td>23.1</td>
<td>62.0</td>
</tr>
<tr>
<td>Sharing needles in injecting-drug use</td>
<td>98.9</td>
<td>1.1</td>
<td>0</td>
<td>98.0</td>
</tr>
<tr>
<td>Sharing cutlery of HCV-infected individuals</td>
<td>25.5</td>
<td>58.5</td>
<td>16.0</td>
<td>23.4</td>
</tr>
<tr>
<td>Carriage from an infected mother to a newborn child (ie vertical transmission)</td>
<td>78.7</td>
<td>3.2</td>
<td>18.1</td>
<td>73.5</td>
</tr>
<tr>
<td>Breast feeding by an HCV-infected mother</td>
<td>44.1</td>
<td>20.4</td>
<td>35.5</td>
<td>46.9</td>
</tr>
</tbody>
</table>
Table 3.12: Respondents' knowledge of natural history, diagnosis and treatment of HCV infection

<table>
<thead>
<tr>
<th>HCV infection is likely to cause:</th>
<th>Italian Undergraduates (%)</th>
<th>UK Undergraduates (%)</th>
<th>Spanish Undergraduates (%)</th>
<th>Postgraduates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yes</td>
<td>no</td>
<td>do not know</td>
<td>yes</td>
</tr>
<tr>
<td>Fulminant hepatitis (i.e. rapid-onset hepatitis and death)</td>
<td>39.8</td>
<td>44.1</td>
<td>16.1</td>
<td>15.9</td>
</tr>
<tr>
<td>Severe acute hepatitis</td>
<td>44.4</td>
<td>34.4</td>
<td>21.2</td>
<td>54.8</td>
</tr>
<tr>
<td>Mild acute hepatitis</td>
<td>40.4</td>
<td>29.2</td>
<td>30.4</td>
<td>45.2</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>84.9</td>
<td>6.5</td>
<td>8.6</td>
<td>89.1</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>72.3</td>
<td>8.5</td>
<td>19.2</td>
<td>61.7</td>
</tr>
<tr>
<td>Is it possible to identify an HCV-infected patient on the basis</td>
<td>21.5</td>
<td>63.4</td>
<td>15.1</td>
<td>6.4</td>
</tr>
<tr>
<td>of the medical history and clinical assessment?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is there any effective treatment for HCV infection?</td>
<td>7.5</td>
<td>71.0</td>
<td>21.5</td>
<td>12.2</td>
</tr>
<tr>
<td>Is any form of effective vaccination available?</td>
<td>0</td>
<td>90.3</td>
<td>9.7</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Table 3.13: Respondents' knowledge of HCV transmission during dental health care

<table>
<thead>
<tr>
<th></th>
<th>Italian Undergraduates (%)</th>
<th>UK Undergraduates (%)</th>
<th>Spanish Undergraduates (%)</th>
<th>Postgraduates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you knowingly ever treated a patient with HCV infection?</td>
<td>yes 25.0</td>
<td>no 65.2</td>
<td>do not know 9.8</td>
<td>yes 16.1</td>
</tr>
<tr>
<td></td>
<td>yes 8.0</td>
<td>no 34.0</td>
<td>do not know 58.0</td>
<td>no 29.0</td>
</tr>
<tr>
<td></td>
<td>yes 20.0</td>
<td>no 79.0</td>
<td>do not know 1.0</td>
<td>do not know 54.9</td>
</tr>
<tr>
<td>Do you believe acquisition of HCV infection to be a likely</td>
<td>yes 100</td>
<td>no 0</td>
<td>do not know 0</td>
<td>yes 83.6</td>
</tr>
<tr>
<td>occupational hazard for dental health care staff?</td>
<td>yes 94.0</td>
<td>no 4.0</td>
<td>do not know 2.0</td>
<td>no 3.3</td>
</tr>
<tr>
<td></td>
<td>yes 85.0</td>
<td>no 8.0</td>
<td>do not know 7.0</td>
<td>do not know 13.1</td>
</tr>
<tr>
<td></td>
<td>yes 35.6</td>
<td>no 37.3</td>
<td>do not know 27.1</td>
<td></td>
</tr>
<tr>
<td>Are additional precautions against cross-infection required</td>
<td>yes 95.6</td>
<td>no 2.2</td>
<td>do not know 2.2</td>
<td></td>
</tr>
<tr>
<td>during the dental care of HCV-infected patients?</td>
<td>yes 17.0</td>
<td>no 63.8</td>
<td>do not know 19.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yes 77.0</td>
<td>no 17.0</td>
<td>do not know 6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yes 35.6</td>
<td>no 37.3</td>
<td>do not know 27.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.14: Respondents' average score* of perceived risk of HCV transmission during clinical activity

<table>
<thead>
<tr>
<th>How would you rate your risk of becoming infected with HCV during:</th>
<th>Italian Undergraduates (mean)</th>
<th>UK Undergraduates (mean)</th>
<th>Spanish Undergraduates (mean)</th>
<th>Postgraduates (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>an oral examination of an HCV-infected patient?</td>
<td>1.27</td>
<td>1.15</td>
<td>1.23</td>
<td>1.20</td>
</tr>
<tr>
<td>restorative treatment of an HCV-infected patient?</td>
<td>1.60</td>
<td>1.45</td>
<td>1.51</td>
<td>1.33</td>
</tr>
<tr>
<td>surgical treatment of an HCV-infected patient?</td>
<td>2.83</td>
<td>2.12</td>
<td>2.68</td>
<td>2.38</td>
</tr>
<tr>
<td>and after a sharp injury with an HCV-contaminated needle?</td>
<td>2.79</td>
<td>2.56</td>
<td>2.77</td>
<td>2.77</td>
</tr>
</tbody>
</table>

* Scoring: 1=low, 2=medium, 3=high.
Table 3.15: Respondents' knowledge of potential oral manifestations of HCV infection

<table>
<thead>
<tr>
<th>Do the oral manifestations of HCV infection include:</th>
<th>Italian Undergraduates (%)</th>
<th>UK Undergraduates (%)</th>
<th>Spanish Undergraduates (%)</th>
<th>Postgraduates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yes</td>
<td>no</td>
<td>do not know</td>
<td>yes</td>
</tr>
<tr>
<td>Lichen planus?</td>
<td>49.5</td>
<td>16.5</td>
<td>34.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Leukoplakia?</td>
<td>8.9</td>
<td>41.1</td>
<td>50.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Oral malignancy?</td>
<td>3.3</td>
<td>53.3</td>
<td>43.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Dry mouth (xerostomia)?</td>
<td>1.1</td>
<td>51.1</td>
<td>47.8</td>
<td>0</td>
</tr>
<tr>
<td>Periodontal disease?</td>
<td>22.0</td>
<td>37.4</td>
<td>40.7</td>
<td>6.8</td>
</tr>
</tbody>
</table>
CONCLUSIONS AND FURTHER STUDIES

Scientific information published in the current literature, together with the results of the studies presented in this thesis, affirms that hepatitis C virus infection, is not only a major medical problem world-wide, but also exhibits characteristics that make it a matter of great interest for the clinical dental health care worker and the oral science researcher: With some geographical variations, HCV infection can be associated with oral diseases such as oral lichen planus and salivary gland sialoadenitis; parenteral transmission makes this virus a potential occupational hazard for the dental staff, although serosurveys seem to indicate that the risk is relatively low; in addition HCV-infected patients may need special care for their dental management. Unfortunately, as indicated in this thesis, a large proportion of dental health care workers may not be aware of the oral implications of HCV infection. Thus a co-ordinated effort may be necessary in order to spread available information to dental health care workers on such a relevant issue. This difficult task may be accomplished by modifying the formative curricula of dental schools and by publishing scientific and informative papers in widespread professional publications.

The data exposed in the present text not only provide new information on the aspect of HCV infection which are of relevance for the oral science worker and researcher, but they also represent the basis for future epidemiological, clinical and laboratory-based studies. Many areas of research are worthy of investigation either with new and different approaches or by enrolling populations that have not yet been studied. This is the case for dentists and dental staff of countries with high prevalence of HCV infection; so far the HCV infection rate among dental health care workers has been studied in countries with relatively low prevalence while data from geographic areas with medium or high prevalence remain to be determined.
As mentioned before, numerous studies have shown that the association between oral lichen planus and HCV-infection is not consistent world-wide. A large multicentric case-control study, carried out in two countries, one where the association is present and one where it is absent, could elucidate the reasons why oral lichen planus and HCV infection are so variably associated. In addition, cohort studies of oral lichen planus patients, with and without HCV infection, should be started in order to establish if such groups of patients have similar clinical features in terms of natural history of the disease, response to treatment and oral cancer incidence.

A clinical study assessing the salivary function of HCV-positive patients would help to establish if the sialoadenitis that may occur in association with HCV infection has also a clinical significance.

Further laboratory-based studies should include experiments directed towards the detection of the HCV genome in samples of split mucosa of patients affected by oral lichen planus. Furthermore, the detection in the same oral tissues of the negative strand of the HCV genome may indicate the active replication of the virus in the oral mucosa. Positive results in either of these studies would provide strong evidence for the putative role of HCV in the aetiopathogenesis of HCV-associated oral lichen planus.
REFERENCES


Hepatitis C virus infection and lichen planus: a short review

G Lodi, SR Porter

Department of Oral Medicine, Eastman Dental Institute for Oral Health Care Sciences, University of London, 256 Gray’s Inn Road, London WCIX 8LD, UK

OBJECTIVE: To review the current literature regarding the association of lichen planus (LP) and liver disease, with particular attention to the association of the oral variant of the disease with hepatitis C virus (HCV) infection.

MATERIALS AND METHODS: Available literature of the possible association of LP with systemic disorders, in particular chronic hepatic disease, has been reviewed.

RESULTS: LP is sometimes associated with infectious or autoimmune disease and/or neoplasia, however an aetiological association between LP and these disorders seems unlikely. A more consistent association exists between LP and chronic hepatic disease. The precise cause of this association is not known. However, in the last 6 years a notable association between HCV infection and LP has been observed, particularly in patients in Spain, Italy and Japan. The pathogenesis of this possible HCV-associated LP is not known, but it may involve a cell mediated response to an altered epithelial antigen.

CONCLUSION: There is now evidence to suggest a significant association between HCV infection and LP in some groups of patients.

Keywords: hepatitis C; hepatitis C virus; lichen planus; lichen planus, oral

Introduction

Lichen planus (LP) is a common mucocutaneous inflammatory disorder of uncertain aetiology (Scully and El-Kom, 1985; Scully et al, 1997) that can arise in association with a number of systemic diseases. In some instances these associations probably reflect reactions to drug therapy or represent coincidental co-occurrence because of the relatively high prevalence of LP, and affected patients being in middle to late life and thus liable to systemic diseases.

Associations between LP and immunologically-mediated diseases, infections and malignancies have long been suggested, but often the evidence is equivocal. For example while sulphonylurea-like agents can cause lichenoid eruptions (Thompson and Skaehill, 1994) there is no definitive evidence of a significant increased frequency of diabetes mellitus in patients with LP and vice versa. Associations with a wide range of autoimmune disorders have been reported but most reports have included only small groups of patients and thus few helpful conclusions can be drawn of their relationship with the pathogenesis of LP. Occasionally LP, often bullous in presentation, can arise in patients affected by malignancies (Scully et al, 1997).

LP is unlikely to have a strong autoimmune basis; there is no association with HLA-DR4 haplotypes and a lack of frequent circulating autoantibodies in high titre in affected individuals (Porter et al, 1997a). The lesions of LP have a predominantly CD8+ T lymphocyte infiltrate, probably mediated by local upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3). In addition there is an accumulation of antigen presenting cells such as langerhans cells that may mediate local lymphocyte activation. LP thus probably represents a cell-mediated response to an antigenic trigger (Porter et al, 1997a); nevertheless the identity of this trigger remain unknown.

LP has been observed in patients with syphilis (Lochner and Pomeranz, 1974), chronic bladder infection (Shelley and Shelley, 1984), intestinal amoebiasis (Wahba-Yahav, 1989), herpes simplex virus type 2 (HSV-2) (Kürkçüoglu and Öz, 1995) and human immunodeficiency virus (HIV) (Ficarra et al, 1993), but to date there is no evidence of LP having a specific infectious aetiology.

LP and liver disease

In the last 15 years an increasingly strong association between LP and chronic hepatic disease has been suggested (Table 1). Since the first report of erosive LP in a small group of patients with chronic active hepatitis (Rebora et al, 1978), as many as 64% of some groups of patients with LP in Spain and Italy have been reported to have chronic hepatic disease (Ayala et al, 1986; Cottoni et al, 1988; Bagan et al, 1994) and the result of a large case-control study indicated that patients with a history of liver disease had an increased risk of developing LP (GISED, 1990).

An association between LP and hepatitis B virus (HBV) infection has been suggested as hepatitis B surface antigen (HBsAg)-positive patients may have double the risk of...
Hepatitis C virus infection and lichen planus
G Lodé and SR Porter

Table 1 Previous data of the possible association between LP and chronic liver disease

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects no. cutaneous/oral</th>
<th>Abnormal liver function (%)</th>
<th>Chronic hepatitis (%)</th>
<th>Hepatic cirrhosis (%)</th>
<th>Liver disease overall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rebora et al, 1982</td>
<td>37 mucocutaneous LP</td>
<td>40.9</td>
<td>13.5</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Rebora and Rongioletti, 1984</td>
<td>44 mucocutaneous LP</td>
<td>11.3</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Powell et al, 1984</td>
<td>3897 LP</td>
<td>11.1</td>
<td>3.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Mobacken et al, 1984</td>
<td>54 oral LP (70.7% erosive)</td>
<td>52.0</td>
<td>4.0</td>
<td>4.0</td>
<td>12a</td>
</tr>
<tr>
<td>Korkji et al, 1984</td>
<td>73 LP</td>
<td>13.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katz and Pisani, 1985</td>
<td>15 oral LP (100% erosive)</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monk 1985</td>
<td>55 LP</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scully et al, 1985</td>
<td>115 oral LP (22.1% erosive)</td>
<td>9.5</td>
<td>53.3</td>
<td>61.9</td>
<td></td>
</tr>
<tr>
<td>Ayala et al, 1986</td>
<td>21 oral LP (100% erosive)</td>
<td>9.6</td>
<td>14.5</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Cottoni et al, 1988</td>
<td>62 LP (17.7% erosive)</td>
<td>7.6</td>
<td>10.7</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>del Olmo et al, 1989</td>
<td>65 oral LP</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GISED, 1990</td>
<td>577 LP (3.1% erosive)</td>
<td>5.2</td>
<td>7.2</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>Gandolfo et al, 1992</td>
<td>96 oral LP</td>
<td>25.0a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El Kabli et al, 1993</td>
<td>180 oral LP (33.3% erosive)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bagas et al, 1994</td>
<td>187 oral LP (42.7% erosive)</td>
<td>21.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Precise characteristics of LP not stated

+Significantly higher than the control group

*62.9% mucosal involvement

*No significant difference with the control groups

developing LP compared with HBV-Ag-negative patients (GISED, 1990). In addition there are reports of anti-HBV antibodies in LP patients (Divano et al, 1992; Rebora, 1994), of lichenoid eruption following administration of different HBV vaccines (Ciaccio and Rebora, 1990; Trevisan and Stinco, 1993; Aubin, 1994), and of an association of LP with hepatocellular carcinoma – an HBV/HCV linked malignancy (Virgili et al, 1992). Nevertheless the majority patients with both LP and chronic hepatic disease are not HBV-infected.

A chronic liver disease sometimes described in association with LP is primary biliary cirrhosis (PBC) but, although some cases of oral lichenoid lesions have occurred in PBC in the absence of penicillamine treatment (Graham-Brown et al, 1982; Powell et al, 1982; Oleaga et al, 1995), the association of LP and PBC is mostly due to the administration of this agent (Powell and Rogers, 1981; Powell et al, 1982).

LP and hepatitis C virus infection

Recently an association between hepatitis C virus (HCV) infection and LP has been suggested that perhaps explains some aspects of the association of LP with chronic hepatic disease.

HCV is an RNA virus comprising six different types and at least 40 subtypes. HCV is principally transmitted by percutaneous routes although sexual transmission is possible and more than one-third of the infected individuals have no history of any risk factors. The vast majority of subjects infected by HCV develop chronic liver disease, cirrhosis complicates more than 20% of these individuals and some of these patients develop hepatocellular carcinoma (Iwarson, 1994; Dusheiko et al, 1996). HCV infection can also give rise to a wide variety of extrahepatic immunologically-mediated abnormalities including the generation of a number of tissue specific and non-specific autoantibodies (Clifford et al, 1995), cryoglobulinaemia (Gumber and Chopra, 1995), autoimmune thyroiditis (Tran et al, 1993), lymphocytic sialadenitis resembling Sjogren's syndrome (Haddad et al, 1992), diabetes (Allison et al, 1994) and possibly non-Hodgkin's lymphoma (Ferri et al, 1995).

Interferon alpha is the drug of choice in the treatment of chronic hepatitis C; this may inhibit virus replication and in some 15–20% of the patients may eradicate the infection. In addition to this limited efficacy, this therapy is very expensive and can cause serious side effects such as thrombocytopenia, leucopenia, hyper- or hypothyroidism and diabetes (Hoofnagle and Di Bisceglie, 1997).

LP may accompany the development of HCV-associated hepatic disease (Mokj et al, 1991; Amichai et al, 1994; Benchikhi et al, 1994; Cecchi et al, 1994; Jubert et al, 1994; Pereyo et al, 1996) and there can be coincident resolution of cutaneous and oral LP with interferon alpha therapy of chronic HCV-associated hepatic disease (Doutre et al, 1992).

Five to 8% of patients with HCV-related chronic hepatic disease may have LP (Pawlotsky et al, 1994; Sata et al, 1996). The frequency of anti-HCV antibodies in European groups of unselected LP patients varies in different studies from about 4% (Rebora et al, 1992; Cribier et al, 1994) to 34% (Mignogna et al, 1996) but none of 55 English patients with oral LP were HCV-seropositive (Ingafou et al, 1997).

Bocytopenia, leucopenia, hyper- or hypothyroidism and diabetes (Hoofnagle and Di Bisceglie, 1997).
that the authors recommended that patients affected by LP should be systematically screened for the presence of HCV infection (Carrozzo et al., 1996). In the same study a significant association was found between HCV infection and the erosive form of oral LP, confirming a previous study that found a tendency in patients with erosive oral LP to have chronic hepatic disease (Bagan et al., 1992).

It thus appears that there may be geographical differences in the association between HCV infection and LP. Antibodies to the GOR epitope (GRRGQKAKSNPNRPL) (Mishiro et al., 1990) are found almost exclusively in a subgroup of autoimmune hepatitis type 2 patients who are also anti-HCV positive and who represent the majority of autoimmune type 2 hepatitis patients in Italy (80%) but not in the UK (10%) (Michel et al., 1992). While two of 36 Italian patients with LP without chronic hepatic disease were HCV-seropositive, none were anti-GOR positive, whereas 11 of 20 patients with LP and chronic hepatic disease were HCV-positive and eight were also anti-GOR positive (Divano et al., 1994). This, considered with the aforementioned data, suggests that the association of LP and chronic hepatic disease may reflect infection with a particular form of HCV infection, although even the great variability of prevalence of HCV infection within countries may have a role. Nevertheless recent, albeit limited, data of French, Japanese and Italian patients, do not suggest any association between a specific HCV genotype and LP (Pawlotsky et al., 1995; Nagao et al., 1996; Lodi et al., 1997a). In addition it has been shown that there are no significant differences in serum levels of HCV RNA in infected patients with or without oral LP. Similarly co-infection with hepatitis GBV-C virus, a newly described hepatitis virus sometimes also designated hepatitis G virus (HGV), seems unlikely to play a role in the development of LP in HCV infection (Nagao et al., 1997). Interestingly, HCV-associated oral LP can undergo malignant transformation (Porter et al., 1997b), but it seems doubtful that HCV infection alone is a risk factor for the development of oral squamous cell carcinoma (Nagao et al., 1995b).

As with primary biliary cirrhosis (Seehafer et al., 1981; Graham-Brown and Sarkany, 1982; Powell et al., 1982; Oleaga et al., 1995), the development of LP in HCV infection could represent an immunologically-mediated reaction to therapy – in particular to interferon alpha (Protzer et al., 1993; Dupin et al., 1994; Heintges et al., 1994; Papini et al., 1994; Perreard et al., 1994). However this hypothesis cannot entirely explain the association of HCV infection with LP, as not all affected patients have been treated with interferon (Carrozzo et al., 1996) and recently interferon alpha was found to be an effective treatment for mucocutaneous LP in a small group of HCV non-infected patients (Hildebrand et al., 1995).

The underlying aetiopathogenesis of HCV-related LP is unclear. Currently there is little published data of the immunological aspects of HCV-related LP; however, a number of pathogenic mechanisms are possible. There may be a cell-mediated cytotoxicity to an epitope shared by HCV and damaged keratinocytes (Rebora, 1994) but to date there is no information of the expression of HCV in keratinocytes of the skin or mucosa in HCV-infected persons with or without LP. A possible role for autoantibodies directed towards epithelial epitopes has been suggested as some patients with HCV-related LP may have circulating anti-epithelial antibodies; in contrast patients with HCV disease without LP or those with LP unrelated to HCV do not have these antibodies (Lodi et al., 1997b). As HCV infection can give rise to tissue-specific and non-tissue-specific autoantibodies, these antibodies may represent a non-specific response whose presence induces keratinocyte antigenic changes and hence the generation of cell-mediated reaction manifesting as LP. It is, of course, possible that these antibodies are of no aetiopathological significance as their presence has been occasionally described in non-HCV-related forms of LP (Parodi and Cardo, 1989).

### Table 2 Prevalence of HCV infection in patients affected by LP

<table>
<thead>
<tr>
<th>Country</th>
<th>References</th>
<th>Population</th>
<th>HCV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCV % seropositivity</td>
</tr>
<tr>
<td>France</td>
<td>Cribier et al, 1994</td>
<td>52 LP^*</td>
<td>3.8</td>
</tr>
<tr>
<td>Italy</td>
<td>Divano et al, 1992</td>
<td>46 LP</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Rebora et al, 1992</td>
<td>29 LP^*</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Gandolfo et al, 1994</td>
<td>105 oral LP</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Favia et al, 1995</td>
<td>82 oral LP</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>Mignogna et al, 1996</td>
<td>178 oral LP</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Carrozzo et al, 1996</td>
<td>70 oral LP</td>
<td>27.1</td>
</tr>
<tr>
<td>Japan</td>
<td>Nagao et al, 1995a</td>
<td>45 oral LP</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Tanzi et al, 1995</td>
<td>45 LP</td>
<td>37.8</td>
</tr>
<tr>
<td>Spain</td>
<td>Bagan et al, 1994</td>
<td>40 oral LP^*</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Santander et al, 1994</td>
<td>50 LP</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Sanchezperez et al, 1996</td>
<td>78 LP^*</td>
<td>20</td>
</tr>
<tr>
<td>UK</td>
<td>Ingafou et al, 1997</td>
<td>55 oral LP</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td>Bellman et al, 1995</td>
<td>30 LP</td>
<td>23</td>
</tr>
</tbody>
</table>

^Four erosive oral LP
^34 mucocutaneous, 22 mucous, 22 cutaneous

---

*References: 1981; 1982; 1983*
It is possible that HCV-related LP has similar pathogenic mechanisms as the idiopathic form: they share the same microscopic features, including a well-defined CD3+ T lymphocyte infiltrate and up-regulation of key adhesion molecules including ICAM-1, very late activation antigen 4 (VLA-4) and LFA-3 (Porter et al, 1997a). There is, however, still a need to determine if HCV RNA is expressed within the unaffected and affected mucosa of patients with HCV-related LP, to more precisely determine if HCV-related LP is a reaction to local HCV-expression or a non-specific immunological reaction.

References
Hepatitis C virus infection and lichen planus
G. Loidi and S. Forfori


The authors are responsible for the accuracy of the references.
HCV genotypes in Italian patients with HCV-related oral lichen planus


Hepatitis C virus (HCV) has high genomic variability and since its discovery, six different "types" and an increasing number of "subtypes" have been reported. HCV genotype may influence viral replication, natural history of disease and response to therapy. Recently, an association between lichen planus (LP) and HCV infection has been suggested, as there is an increased frequency of HCV infection among some groups of patients with LP, in particular from Italy and Japan. These results have not been confirmed by other reports from different geographical areas. Since HCV genotypes have a heterogeneous geographical distribution, we have determined by restriction fragment length polymorphism the genotypes of 39 HCV-seropositive Italian patients with oral LP in order to establish whether the association between LP and HCV infection is influenced by HCV subtype. Of the 33 (84.6%) viraemic patients, 17 (51%) were infected by HCV subtype 1b, 9 (27%) were infected by HCV subtype 2a, 2 by subtype 1a and 1 by subtype 2b. In four cases the gel patterns were uninterpretable. This distribution of HCV genotypes is similar to that reported in recent studies of Italian HCV-seropositive patients of unknown LP status. It is concluded from this small sample that the association of lichen planus with HCV infection and its differential geographic distribution is unlikely to be due to infection by a particular HCV genotype.

A pathogenetic relationship between lichen planus (LP) and hepatitis C virus (HCV) has recently been proposed because controlled studies have demonstrated a higher prevalence of seropositivity for HCV antibodies (HCVAbs) and RNA in patients with LP compared with sex and age-matched controls (1-4). Moreover, it has been shown that, excluding cases and controls with HCV-infection, there was no difference between the two groups regarding frequency of chronic liver disease (4). Lichen planus may accompany the development of HCV-associated hepatic disease (5) and there can be coincident resolution of cutaneous and oral LP with interferon-alpha therapy of chronic HCV-associated hepatic disease (6). Five (7) to 8% (8) of patients with HCV-related chronic hepatic disease may have LP. However, the prevalence of HCVAbs among LP patients varies greatly according to the country of origin. While only 4% of a cohort of French patients with oral LB were HCVab positive (9), the prevalence of such markers was 27 to 34% in Italian oral LP patients (4, 10), 37 to 62% among Japanese patients (2, 11) and 15 to 20% in Spain (3, 12). Although HCV prevalence may be higher in Japan (13) and in southern Europe (14, 15) than in northern European countries such as the UK (0.004% HCVab positive subjects among blood donors) (16), this difference alone may not explain the apparent geographic variation in the association of LP with HCV. Geographical variation in the distribution of HCV genotypes has been well documented, so in view of the knowledge that HCV genotypes may influence the clinical manifestations of HCV disease and the very limited data of HCV genotype distribution in patients with HCV-related LP, we sought to analyse the distribution of HCV genotypes in a cohort of Italian patients with HCV-related oral LP.

Patients and methods

Patients

The patient group comprised 39 Italians patients (24 women and 15 men;
median age 61.0 years, range 30–80 years), attending Oral Medicine clinics in the University of Turin (n=20) and the University of Chieti (n=19). All patients had clinical and histological features of oral LF as established by current diagnostic criteria (17). Patients usually had reticular oral LP affecting the buccal mucosa bilaterally; only 5 (13%) had cutaneous involvement, and in one case vulval lesions were also present. All patients were HCV-seropositive, as determined by second or third generation ELISA and RIBA (Ortho Diagnostic Systems, Raritan, NJ, USA, and Ortho Diagnostic and Chiron Corp., Emeryville, CA, USA). Only 19 of the patients had known risk factors for HCV acquisition (surgery-associated blood transfusion in 17 cases and injecting drug use in two cases). Sixty-five percent of the patients had at least one abnormal serological hepatic test (serum aminotransferases, bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase and total protein). Eight of 15 tested patients had cryoglobulinaemia. Sixty percent of patients had undergone liver biopsy and thus had a histological diagnosis; these included: cirrhosis (9 cases), chronic active hepatitis (8 cases), chronic persistent hepatitis (6 cases), primary biliary cirrhosis (2 cases) and primary hepatocellular carcinoma (1 case). Thirteen patients had received interferon-alpha therapy; in these cases the lesions were bilateral and in no case followed the administration of the therapy.

HCV-RNA detection and genotype analysis

The presence of HCV viraemia was determined by reverse transcription polymerase chain reaction (RT-PCR) analysis (Roche Diagnostic Systems, Branchbury, NJ, USA). HCV genotyping was carried out by nested RT-PCR, to amplify a 174 bp product from the 5' non-coding region, followed by restriction fragment length polymorphism (RFLP) analysis of the ampli­con. The restriction enzymes ScrF1, HinF1, Mva1 and BstU1 were used to digest the amplicon, and were visualised under UV light after ethidium bromide staining. The band patterns produced by these enzymes can distinguish HCV types 1a, 1b, 2a, 2b, 3a, 3b, 4, 5, and 6 (18).

The frequencies of HCV genotypes in the patient group were compared to those from previous studies of HCV-infected Italian patients (19–22) (Table 1).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Present study</th>
<th>Previous Italian studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lichen planus</td>
<td>CAMMAROTA et al.* (22)</td>
</tr>
<tr>
<td>1a</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>1b</td>
<td>51%</td>
<td>63.2%</td>
</tr>
<tr>
<td>2a</td>
<td>27%</td>
<td>12%</td>
</tr>
</tbody>
</table>

*Patients with community-acquired infection
b Chronic hepatitis C patients
c Acute and chronic hepatitis C patients
d All genotyped as type 1
* All genotyped as type 2

Table 1. Frequency of distribution of the HCV genotypes 1a, 1b and 2a in 33 Italian patients with lichen planus

Table 2. Characteristics of patients with HCV genotypes 1b and 2a

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HCV 1b</th>
<th>HCV 2a</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>6/11</td>
<td>3/6</td>
<td>ns*</td>
</tr>
<tr>
<td>Mean age</td>
<td>60</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Erosive vs non-erosive form of lichen planus</td>
<td>7/10</td>
<td>2/7</td>
<td>ns*</td>
</tr>
<tr>
<td>Chieti vs Turin</td>
<td>5/12</td>
<td>6/3</td>
<td>ns*</td>
</tr>
</tbody>
</table>

* Non-significant by chi-squared test.
* Non-significant by t-test for equality of means.

Table 2. Characteristics of patients with HCV genotypes 1b and 2a

Discussion

Hepatitis C virus is a positive stranded RNA virus, first identified in 1989 (23). It is now recognised as the main agent of the previously termed parenteral non-A, non-B viral hepatitis and is one of the major causes of chronic hepatic disease worldwide. The HCV genome comprises approximately 9500 nucleotides and, owing to the frequent errors and lack of repair mechanisms in the course of replication, it is extremely variable and is present as quasispecies in a single infected individual. Nucleotide sequencing of many isolates collected worldwide has permitted the development of different classification systems (24). In 1994 a common classification was proposed, dividing the known viruses into main “types”, numbered in order of discovery with Arabic numerals; the more closely related groups observed within some types are divided into “subtypes”, which are identified by a lower-case letter, again in order of discovery (25).

The different HCV genotypes have a heterogeneous distribution worldwide. Subtype 1b seems to be the most common variant in Europe, USA and Japan, although its prevalence may vary considerably; genotype 2 is less common in Europe than in Japan and China; type 3 is the most frequent HCV
type in Thailand, Singapore and some parts of India; type 4 is found more commonly in Egypt, Middle East and Central Africa; and types 5 and 6 are the most frequent in South Africa and Hong Kong, respectively (26). The HCV genotype may also reflect the route of infection; in Europe, genotype 3a is prevalent in young people with a history of intravenous drug use or tattooing (27), while genotype 1b is the most common variant in the elderly group, in those who have received blood transfusions, or in people whose source of infection is unknown (28).

From a clinical point of view, the best documented aspect of HCV genetic diversity is that response to interferon alpha is significantly influenced by genotypes (29); patients infected by the variant 1b tend to be worse responders (30) and to relapse more frequently (31). There may be an association between genotypes and the level of HCV viruria (32–34), and with the increased risk for the development of hepatic fibrosis, cirrhosis and hepatocellular carcinoma (35–37). Furthermore, genotypes may influence the range of serologic reactivity to the viral antigens (38, 39), transmissibility (40) and the development of cryoglobulinaemia (41), although some studies have failed to confirm such findings (42, 43).

The HCV genotype distribution found in the present study was very similar to that reported in a recent multicentre study of 495 Italian patients with chronic hepatitis C, showing 57% of subjects to be infected with HCV-1 and 31% with HCV-2, and to those of other studies of Italian HCV-seropositive patients reporting genotype 1b prevalence varying from 54 to 63% (Table 1). There was no statistically significant difference in the distribution of the two major genotypes according to the area of residence of the present group of patients (Table 2). These observations confirm the findings of two previous studies that investigated a group of Japanese oral LP patients (44) and a small group of French patients affected by the cutaneous form of the disease (45), which were unable to detect a significant association between LP and any particular HCV genotype.

We observed that patients with symptomatic erosive oral LP tended to be infected by the subtype 1b; this variant is possibly associated with more severe liver disease and appears less responsive to interferon therapy than other genotypes (29, 39). Thus, it could also be responsible for the development of a more severe form of oral LP, though a much larger cohort of patients with HCV-related LP is necessary to determine with more certainty whether subtype 1b is correlated with erosive symptomatic oral LP.

It can be concluded that while Italian patients with oral LP may be frequently infected with HCV, there is no evidence from this small study on referred patients to suggest that HCV genotypic difference influences this association.

References


Antibodies to epithelial components in oral lichen planus (OLP) associated with hepatitis C virus (HCV) infection


Oral lichen planus (OLP) is a common chronic inflammatory disorder sometimes associated with hepatitis C virus (HCV) infection. An increased prevalence of autoimmune markers has been reported in patients with HCV infection. The aim of the present study was to determine, by conventional indirect immunofluorescence, the nature and frequency of circulating antibodies to epithelial antigens in the sera of HCV-positive patients who also have OLP. The study comprised four groups: 14 patients with OLP and HCV infection, 14 HCV-seronegative patients with OLP, 21 HCV-seropositive patients without OLP and 18 healthy controls. We found a significant association between the concomitance of OLP and HCV infection and the presence of such antibodies. It is concluded that some patients with HCV-associated OLP may have circulating antibodies to epithelial antigens, although their precise aetiopathological role in the development of this disease in HCV infection remains unknown.

The mechanisms involved in the initiation and progression of lichen planus (LP) are not yet fully known, but in certain circumstances LP has been found to be associated with hepatic disease. For example, a number of studies suggest that there is a close relationship between hepatitis C virus (HCV) infection and LP in certain groups of patients (1–6), and we have recently found that 12% of a group of patients with HCV-related chronic hepatic disease were also affected by mucocutaneous LP (unpublished). Moreover, 25% of a group of Italian patients with LP (7) and 60% of a similar Japanese group (8) were found to be HCV-seropositive. Despite the apparent association between HCV and LP, however, the precise mechanism underlying the relationship between the development of LP and HCV infection remains unclear. It is unlikely that HCV-related LP represents solely a drug-induced reaction to the administration of interferon-alpha (IFN-α) (9–13), since LP has been shown to arise in HCV-infected patients who have not been treated with this drug and LP in HCV infection sometimes resolves with IFN-α therapy (14). These observations suggest that other mechanisms may be involved in the aetiopathogenesis of LP in HCV patients.

Cell-mediated immunity is considered to play a major part in LP pathogenesis (15) and while the role of humoral immunity may not be of primary importance, patients with oral lichenoid lesions have been reported to have circulating antibodies to nuclear (16) and basal membrane zone (17) antigens of stratified epithelial substrates. In addition, an oral mucosal disease, clinically similar to LP but characterized by epithelial deposits of anti-nuclear antibodies, has been described in a small group of patients of unknown HCV status (18). Moreover, patients with HCV infection frequently have autoimmune disease (19) and circulating tissue-specific and non-specific autoantibodies (20). In the present study, in order to assess the possible role of humoral immunity in the development of oral lichen planus (OLP) in HCV infection, we have examined the sera of HCV-positive patients with and without OLP for the presence of circulating antibodies to epithelial antigens.

Material and methods

Study groups

Four groups of patients were included in this study:

1. Patients with HCV infection and OLP (HCV+ve/OLP+ve): 14 Caucasian patients known to be HCV-infected, as determined by second generation HCV ELISA and 4-antigen RIBA assays. Six had liver biopsies showing histological features of chronic active hepatitis and had been treated with different regimes of IFN-α. Previous blood transfusions and previous injecting drug use were identified as HCV risk activities in 3 and 1 of these patients, respectively.

Key words: antibody; epithelium; fluorescent antibody technique, indirect; hepatitis C; lichen planus, oral

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All of these 14 had clinical and histological features of non-erosive OLP.

2. Patients without HCV infection but affected by OLP (HCV-ve/OLP+ve): 14 anti-HCV seronegative Caucasian individuals with clinical and histological features of non-erosive OLP.

3. Patients with HCV infection but without OLP (HCV+ve/OLP-ve): 21 HCV-seropositive Caucasian patients who had no mucocutaneous disease. These sera were taken at the time of the diagnosis and thus none of these patients had received IFN-α therapy.

4. Patients without HCV infection OLP (HCV-ve/OLP-ve): 18 healthy Caucasians who were HCV-seronegative and had no mucocutaneous disease.

All of the above clinical and histological diagnoses of OLP were made on the basis of World Health Organization criteria (21).

None of the above subjects had received any drug therapy (other than IFN-α, as noted in the text) likely to induce lichenoid reactions (22).

Indirect immunofluorescence

Circulating antibodies against epithelial antigens in the sera were detected by an indirect immunofluorescence technique, using pre-fixed sections of monkey oesophagus (BioDiagnostics Ltd., England) as substrate. Sections were washed with phosphate-buffered saline (PBS) and incubated with 20% normal goat serum (NGS) (Dako Ltd., England) in PBS for 30 min to block non-specific binding. All incubations were carried out at room temperature in a humidified chamber. Sera were diluted as indicated in PBS containing 20% NGS and incubated on the sections for 1 h, and then washed with PBS 3 times for 15 min each. Serum antibodies reactive with mucosal antigens were detected using FITC-conjugated goat anti-human IgG (Fc-specific) (Sigma Ltd.) diluted 1:60 in PBS containing 20% NGS. After 45 min of incubation, the sections were washed 3 times with PBS and mounted with FITC Mounting Medium (BioDiagnostics Ltd.). Fluorescence was visualized using a Zeiss Axioskop microscope. Sera were scored as positive for each particular staining pattern when they produced fluorescence at a dilution of 1:100 or more. End-point titres were determined for each of the positive sera. Negative controls were sections incubated with sera from healthy individuals (HCV-ve/ LP-ve group) and sections incubated with NGS/PBS only.

Statistical analysis

Statistical analysis was carried out with the Fisher two-tailed exact test, employing the Nanostat (Alpha Bridge Ltd 1992) computer analysis program. The results were also checked manually. Three tests were carried out comparing the following groups: HCV+ve/ OLP+ve vs HCV-ve/OLP+ve; HCV+ve/OLP+ve vs HCV+ve/OLP-ve; HCV+ve/OLP+ve vs HCV-ve/OLP-ve.

Results

As shown in Table 1, antibodies that recognised antigens expressed in stratified squamous epithelium were detected in the serum of a total of 8 of the 14 patients in the HCV+ve/OLP+ve group. However, the pattern of staining was not uniform for all sera, 3 different fluorescence staining profiles being readily detected. Figure 1 shows the reactivity against epithelial cell nuclei (pattern I). This punctate staining was observed with the sera of 6 of the patients, having titres from 1:100 to more than 1:1000. The sera of 2 of these patients also exhibited staining pattern II, a marked pericellular or membrane-associated reactivity against epithelial cells in the suprabasal region (Fig. 1). One other serum of this group gave only the latter intense surface staining, with no anti-nuclear reactivity (not shown). A third pattern was also produced by the serum of another HCV-positive/OLP-positive patient. As shown in Fig. 2, fluorescence staining pattern III was characterised by punctate granules distributed throughout the cytoplasm of all epithelial cells, frequently being excluded from the nuclear region.

Despite the marked differences in specific staining profiles produced by the sera of these patients, none of the other 53 sera samples examined was found to give a positive reaction using the same standard mucosal assay specimen (monkey oesophagus). The results in Table 1 show that none of the 14 patients with OLP only, and none of the 21 with HCV only, produced any detectable immunofluorescence when tested at a dilution of 1:100 or greater. The comcomitance between OLP and HCV infection and the presence of circulating antibodies to epithelial antigens was found to be statistically significant when compared with the other two patient groups (HCV-ve/OLP+ve and HCV+ve/OLP-ve) or with the normal controls (HCV-ve/OLP-ve group) (P<0.01). Because of the lack of com-

Table 1. Immunofluorescence staining patterns of human sera

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Number of positive sera (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV OLP</td>
<td>I</td>
</tr>
<tr>
<td>+ +</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>- +</td>
<td>0</td>
</tr>
<tr>
<td>+ -</td>
<td>0</td>
</tr>
<tr>
<td>- -</td>
<td>0</td>
</tr>
</tbody>
</table>

* Staining patterns were anti-nuclear (I), anti-membrane (II) and anti-cytoplasmic (III), as described in the text.

b Patterns I and II were produced together by the sera of 2 patients.
Discussion

HCV infection has been associated with a number of immunologically-mediated disorders including mixed cryoglobulinaemia, membranoproliferative glomerulonephritis, autoimmune hepatitis, autoimmune thyroiditis and Sjögren’s syndrome (23). Moreover, tissue-specific and non-specific autoantibodies are frequently present in the sera of some groups of patients with chronic HCV infection (20). Some groups of patients affected by HCV infection also have a high incidence of LP, and an increased prevalence of HCV infection has been reported in certain Italian LP patients compared with the normal local population (8). These observations suggest that the two disorders are closely linked, although the cellular and molecular features responsible for this association are still not understood.

In the present study we found that patients with HCV who develop OLP have relatively high titres of circulating antibodies directed against antigens expressed in oral mucosa. Notably, this type of immune reactivity was not detected in the sera of patients who have HCV but not OLP; nor was there anti-epithelial activity in the sera of OLP patients without HCV. While the aetio-pathogenic role of different antibodies directed against epithelial antigens in systemic autoimmune pathologies such as pemphigus and pemphigoid is well documented (15), the presence of anti-epithelial immunoglobulins in some LP patients and in drug-induced lichenoid reactions (DLR) has not yet been explained. In one of the previous studies describing circulating antibodies in LP (16), no data were presented about the HCV status of the two patients described, most probably because serodiagnostic tests for HCV were not readily available at that time. It is notable, however, that one of the patients was affected by chronic hepatitis and the other was seropositive for antibodies against hepatitis B virus surface antigens. In addition, although antibodies producing a pattern similar to the type II described in the present study have previously been associated with DLR (17) and IFN-α therapy (24), we have not been able to confirm the relationship between IFN-α therapy and the appearance of similar antibodies since none of the patients in the three control groups had received this drug. The type II staining pattern was, however, found in one of the HCV+ve/OLP+ve patients who did not receive IFN-α therapy, showing that the presence of this type of circulating antibody is not necessarily linked to IFN-α therapy. Whether these abnormal humoral responses we have described are directly elicited by viral presence in the epithelium of HCV-infected patients or reflect the expression of cross-reactivity between HCV determinants and mucosal epitopes is not yet known. However, despite our finding that there is a highly diverse immunological response between different patient groups, there is nevertheless an apparent similarity in the clinical outcome. This suggests that a number of multiple factors, including certain drugs, may act by a similar molecular mechanism to elicit the development of LP and LP-like lesions.

Acknowledgements - The authors are grateful to Dr. John Bulmaas for assistance with the statistical analysis and to Alun Kirby for his helpful suggestions about immunofluorescence. This work was supported in part by a scholarship awarded by the University of Milan to G. L.

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Development of Squamous Cell Carcinoma in Hepatitis C Virus-associated Lichen Planus

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The hepatitis C virus may occasionally be associated with oral lichen planus. The present report details the features of a patient with hepatitis C virus infection and oral lichen planus who developed a squamous cell carcinoma of the tongue. © 1997 Elsevier Science Ltd. All rights reserved.

Key words: hepatitis C virus, lichen planus, oral squamous cell carcinoma

INTRODUCTION

Recently, an association between hepatitis C virus (HCV) infection and lichen planus (LP) has been suggested [1]. Lichen planus may simultaneously develop upon acquisition of HCV [2–7] and there can be coincident resolution of cutaneous and oral LP with interferon alpha therapy of chronic HCV-associated hepatic disease [8]. Five to twelve per cent of patients with HCV-related chronic hepatic disease may have LP [9, 10], while up to 26% of a group of Italian patients with LP were HCV seropositive and up to 65% of Italian patients with LP and chronic hepatic disease were HCV-seropositive [11]. Twenty-three per cent of 40 American LP patients were positive for HCV by second-generation ELISA [12], but none of 50 English patients with oral LP and only 2 of 52 French patients without hepatic disease had HCV antibodies [13, 14].

There is some evidence to suggest that LP may have a small but clinically significant premalignant potential [15], particularly if the LP lesions are long-standing and possibly erosive and/or atrophic.

However, there is no information concerning the premalignant potential of HCV-associated oral LP we now report the features of a patient with longstanding HCV-associated oral LP who developed a squamous cell carcinoma of the oral cavity.

CASE STUDY

A 61 year old Indian male was referred to the Department of Oral Medicine of the Eastman Dental Institute London, U.K. for investigation and treatment of generalised oral mucosal soreness that had been present for 3 years. The patient had previously been under the care of oral surgeons who had undertaken two biopsies of lesional tissue and had established the cause of the oral discomfort to be erosive lichen planus. Previous management of the erosive lichen planus had included hydrocortisone hemisuccinate mouth rinses, triamcinolone acetonide in carboxymethylcellulose paste, betamethasone mouth rinse, benzydamine hydrochloride and chlorhexidine gluconate mouth rinses and sprays, systemic prednisolone and topical cyclosporin suspension. Each agent had provided only transient relief of his oral symptoms.

The patient was otherwise well and a review of systems revealed no notable abnormalities, aside from mild hesitancy of micturition due to known prostatism. The patient had had non-insulin dependent diabetes mellitus for 12 years, was mildly hypertensive and had had hepatitis C virus-associated chronic active hepatitis for at least 1 year. The source of the HCV infection was not known.

The patient’s current medication comprised glibenclamide and bendrofluazide. He had no known allergies. The patient was heterosexual, married with five adult children and had been resident in the U.K. for the previous 20 years. He did not smoke tobacco, drink alcohol and denied any tobacco–betel type habits or injecting drug use.

Extra-oral examination revealed no cervical lymphadenopathy or orofacial stigmata of chronic hepatic disease. Intra-orally there was extensive erosive lichen planus affecting the buccal mucosa, ventral and dorsal aspects of the tongue, the labial and palatal/lingual free- and attached-gingivae, and the upper and lower labial mucosa. There was a 1.5 cm diameter raised, speckled mass on the posterior aspect
with oral cancer had previously had oral LP, and it is unclear if the co-occurrence of HCV with oral malignancy is of any aetiological significance, especially as HCV RNA has not been found in any of the squamous cell carcinoma tissue of HCV-infected patients [19].

The risk of oral malignancy and premalignancy in longstanding LP is small [15]. Nevertheless, the present report has highlighted the possibility of HCV-associated LP being a risk factor for the development of oral squamous cell carcinoma.

**DISCUSSION**

Lichen planus (LP) may occur in up to 12% of patients with hepatitis C virus infection [9, 10]. The precise cause of this association remains unclear, but it need not be due to concomitant drug therapy [1]. To date there have been no reports of oral squamous cell carcinoma developing in patients with HCV-associated LP.

The present report indicates that squamous cell carcinoma can occur in patients with HCV-infection and oral lichen planus. However, this must be interpreted cautiously. It is not known how long the patient had had HCV-related hepatic disease, indeed his mode of acquisition of the virus is quite unclear, particularly as he denied any risk activities for HCV transmission [16]. Nevertheless, as HCV-associated hepatic disease is generally a long-term problem giving rise to morbidity rather than mortality, it is possible that the patient may have had HCV infection for many years [17]. It is possible that the oral LP may have been associated with glibenclamide therapy [18] but the patient had only been receiving this therapy for the past 2 years and thus after the development of oral LP.

An association between HCV-infection and oral squamous cell carcinoma has been recently suggested as 24% of a group of Japanese patients with oral squamous cell carcinoma were HCV seropositive, 17% having HCV RNA in serum. It is not known if any of these HCV-infected persons of the anterior two-thirds of the left side of the dorsum of the tongue (Fig 1). No trigeminal or hypoglossal nerve abnormalities were detected. The likely clinical diagnosis was a squamous cell carcinoma in association with pre-existing lichen planus. Haematological investigations prior to biopsy revealed mild thrombocytopenia, elevated serum alkaline transaminase, normal prothrombin and activated partial thromboplastin times and hepatitis C virus seropositivity.

Biopsy from the tongue confirmed the lesion to be a moderately well-differentiated squamous cell carcinoma. The patient has now undergone laser-excision of the squamous cell carcinoma and is under regular clinical review.

**Fig. 1.** Squamous cell carcinoma with associated lichen planus of the dorsum of the tongue.
LETTER TO THE EDITOR

Lack of association between Hepatitis C virus and Sjogren’s Syndrome

The precise aetiology of Sjogren’s Syndrome (SS) remains unknown. A number of viruses give rise to a similar clinical picture, including Epstein–Barr virus (Mariette et al, 1991; Maitland and Scully, 1994), human immunodeficiency virus (Couderc et al, 1987), and human T-lymphotrophic virus 1 and other retroviruses (Green et al, 1989). An association with hepatitis C virus (HCV) and SS has also been proposed, as up to 77% of selected groups of French and Italian patients with HCV-related hepatitis or cirrhosis have salivary glands with histological features characteristic of SS (Haddad et al, 1992; Pawlotsky et al, 1994; Pirisi et al, 1994) or xerophthalmia in the absence of sialadenitis (Guisset et al, 1993). Further, French patients with SS have a higher prevalence of HCV antibodies than the general population (De Bandt et al, 1991; Mariette et al, 1993). However, in one study, none of 26 Italian (Aceti et al, 1992) and, in another, possibly only one of 48 US patients with SS (King et al, 1994) had evidence of HCV seropositivity. In a further study, less than 3% of another group of French patients were HCV-infected (Wattiaux et al, 1995).

These conflicting data may be due to the inclusion of patients with hypergammaglobulinaemia (Vitali et al, 1992). We have examined the HCV serostatus of a group of British patients with SS in which hypergammaglobulinaemia was excluded.

The study group comprised 18 patients (17 female, median age 54 years, range 45–75 years) with clinical, labial gland histopathological, and serological features of SS (Vitali et al, 1993) in the absence of cryoglobulinaemia. Eleven patients had primary SS while seven had secondary disease. Serum samples were tested for the presence of IgG antibodies to HCV using two second-generation enzyme-linked immunosorbent assays (Ortho Diagnostic System, Raritan, CA, USA; Murex Diagnostics, Dartford, UK). Additional confirmatory assays were not required.

None of the eighteen patients with primary or secondary SS had serum IgG antibodies to HCV.

Thus, while some cohorts of European patients with HCV-infection may have clinical and histological evidence of Sjogren’s-like salivary gland disease, there is no evidence that British patients with well-defined SS have HCV-infection. It is of course possible that variation in HCV genotype distribution, and perhaps clinical expression, may explain some of the conflicting data between Europe and the US and UK, but until such a study is undertaken, this will remain unknown.

References


Occupational Risk of Hepatitis C Virus Infection to Dental Health Care Staff: An Update

Giovanni Lodi and Stephen R. Porter

This article highlights current knowledge of the epidemiology and transmission of hepatitis C virus and reviews current information on the risk of transmission of the virus during dental health care.

Hepatitis C virus (HCV) is an RNA virus comprising six types and more than 40 different subtypes. Infection with HCV results in chronic carriage of the virus in 70 to 80% of patients. In 20% of these patients hepatic cirrhosis develops in about 15 years, and 10% of this group may develop hepatocellular carcinoma.

In view of the potential for transmission via parenteral routes (including needlestick injuries) during dental treatment and the possible clinical consequences of infection, dental health care workers are becoming concerned as to the potential risk of HCV acquisition and transmission during routine dental care.

**EPIDEMIOLOGICAL DATA**

First-generation anti-HCV antibody tests indicated that up to 2% of blood donors in developed countries had antibodies to HCV. Later studies, using more specific serological assays (second-generation enzyme-linked immunosorbent assay—ELISA—or recombinant immunoblot assay—RIBA) suggest these figures to be over-estimates. After the first year of screening with second-generation assays the prevalence of HCV infection in British blood donors was revised to 1 in 1400 (0.071%). The prevalence in Scotland is similar (0.088%), and less than 1% of all Japanese blood donations were found to be infected with HCV.

**PATIENTS AT RISK OF HCV INFECTION**

Injecting drug users are the main patient population in developed countries at risk of infection with HCV, needle sharing being responsible for acquisition of the virus in up to 83% of cases. Recipients of blood and blood products—sufferers of haemophilia, thalassaemia or leukaemia—and people undergoing haemodialysis, plasmapheresis, organ transplantation and intravenous immunoglobulin injection have also been at potential risk. More recently tattooing and male ear-piercing have been suggested as risk activities. Residents of areas with high HCV prevalence can be at risk of HCV infection and hence are possible carriers.

**ROUTES OF INFECTION**

HCV has been isolated from most body fluids, including semen, vaginal secretions and saliva. Although transmission via vaginal intercourse can occur, this is not a common route of transmission: neither is sex between men. Vertical spread of HCV, from mother to newborn child (either in utero or during birth) is also uncommon and more likely when the mother is also infected with HIV. There have been reports of vertical transmission skipping generations. For further reading see the reviews by Van der Poel et al. and Dusheiko et al.

**RISKS TO HEALTH CARE WORKERS**

Since the first report of a surgeon becoming infected with HCV following a needlestick injury, several further episodes of nosocomial transmission have been documented. Most of these incidents were caused by injury from sharp objects (such as needles) although exposure by other routes, such as conjunctival contact with HCV-infected blood, has been reported.

Prevalence studies indicate that up to 4.1% of healthcare workers may be HCV seropositive (Table 1). Retrospective studies of infection in healthcare workers known to have been accidentally exposed to contaminated body fluids suggest that less than 3% of exposures are likely to lead to HCV acquisition, although two reports give the risk as 6 to 10% (Table 2). It is noteworthy that all but one of the injuries reported was a needlestick injury.

In the absence of effective therapy or any passive or active vaccination there is a significant risk that HCV-infected healthcare workers will develop chronic hepatic disease.

**SALIVARY TRANSMISSION OF HCV**

Transmission of parenteral non-A, non-B hepatitis and HCV through saliva has been demonstrated experimentally, although there is little epidemiological data.
OCCUPATIONAL HAZARDS

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Country</th>
<th>Number of subjects</th>
<th>Tests</th>
<th>Prevalence in study group (%)</th>
<th>Prevalence in control group (%)</th>
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<td>Belgium</td>
<td>2031</td>
<td>Third-generation ELISA and third-generation RIBA</td>
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<td>3073</td>
<td>Second-generation ELISA and second-generation RIBA</td>
<td>2.2</td>
<td>-</td>
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</table>

Second generation ELISA tests C100-3, C33-c antibodies.
Second-generation RIBA tests C100-3, C33-c, C22-3, 5-1-1 antibodies.

Table 1. Recent prevalence studies of HCV infection among health care workers (1994 onward).

Several studies indicate that there is little risk of HCV transmission during dental procedures (Table 3). In 1990 a study found that 1% of a group of American dental health care workers were HCV seropositive; auxiliary dental personnel being at higher risk for infection (1.4%) than dental surgeons (0.3%). In a second study, eight of 456 (1.75%) dental healthcare workers in the New York City metropolitan area were HCV seropositive compared with one of 723 controls.

Table 2. Recent retrospective analyses of healthcare workers occupationally exposed to HCV (1994 onward).

to suggest that saliva is a major mode of transmission. There are very few reports of transmissions via bite injuries. The whole saliva of some (but not all) HCV-infected patients contains HCV RNA and levels of the virus in saliva may correlate with viraemia and hepatic function.
surgeons were at higher risk than other dentists. In a third study none of 94 dental surgeons in Wales were found to be infected even though 68% of the group had sustained inoculation injuries. Another study found that three of 461 (0.65%) dentists from Taiwan were infected, the seroprevalence rate being similar to that of local blood donors (0.95%) and pregnant women (0.63%). None of the 90 dentists providing dental care at the San Francisco General Hospital were seropositive for HCV.

More recently the low risk of HCV carriage amongst dental health care staff was confirmed by the finding of 2% of 343 US oral surgeons and 0.7% of 305 dentists being HCV seropositive.

To date no dental health care worker has knowingly transmitted HCV to a patient during dental treatment. Nevertheless nosocomial transmission of HCV is possible as a consequence of sharps injuries during surgery.

**CONCLUSIONS**

Infection with HCV can cause profound liver problems, and all dental health care staff should take appropriate steps to minimize transmission of the virus during dental procedures, either from patient to dentist or from dentist to patient. Few dental staff have become infected occupationally with HCV, and maintenance of effective cross-infection control should minimize transmission during dental treatment.

**Table 3. HCV seroprevalence in groups of dental healthcare workers.**

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Country</th>
<th>Number of subjects</th>
<th>Tests</th>
<th>Prevalence in study groups (%)</th>
<th>Prevalence in control group (%)</th>
</tr>
</thead>
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<td>22</td>
<td>USA</td>
<td>960 (333 dentists + 627 auxiliaries)</td>
<td>First-generation ELISA and first generation RIBA</td>
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<td>456</td>
<td>First-generation ELISA and first-generation RIBA</td>
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<td>Second-generation ELISA</td>
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<td>0.3*</td>
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<td>Second-generation ELISA</td>
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<tr>
<td>10</td>
<td>USA</td>
<td>90</td>
<td>Second-generation ELISA and second-generation RIBA</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>27</td>
<td>USA</td>
<td>343 oral surgeons 305 general dentists</td>
<td>Second-generation ELISA and second-generation RIBA</td>
<td>2.0</td>
<td>—</td>
</tr>
</tbody>
</table>

First-generation ELISA tests C100-3 antibody. Second-generation ELISA tests C100-3, C33-c, C22-3 antibodies. First-generation RIBA tests C100-3, 5-1-1 antibodies.

* Local blood donors.

**References**

15. Mitsui T, Iwano K, Masuko K et al.
Book Review


Two new authors have been drafted in to update and revise this title, both of whom have a wealth of experience in clinical paediatric dentistry. Professor Curzon is a consultant in paediatric dentistry and John Roberts runs a very successful private practice limited to children. The information, opinions and advice in the book is based on the authors' practical clinical experience, and is usually reinforced with selected scientific evidence; unfortunately many of the references are now rather dated.

The book consists of four sections which cover the principles of restorative care for children, diagnosis and patient management, restoration of teeth and pulp treatment. It is further subdivided into 20 chapters. As expected the book covers all aspects of the restoration of the cavitated dentition in children including isolating using rubber dam, simple restorations, preformed stainless steel crowns on posterior teeth and composite strip crowns on primary incisor teeth, ending with five chapters on pulp therapy.

For the dentist who wants to undertake high quality restorative treatment of the primary dentition this book gives much valuable information and advice. The authors tell the reader that by using rubber dam routinely for all the restorative treatment, the time in the dental chair is reduced by up to one-third, the restorations are of a higher quality and therefore require less frequent replacement. In addition, by using preformed crowns for anything other than an occlusal or minimal two surface fillings, the long term prognosis of restorations can be virtually guaranteed.

The book is illustrated with black and white photographs and pictures of radiographs. Looking back at my second edition many of the illustrations have been retained. However it was disappointing to see that the quality and sharpness was noticeably worse in the new edition, especially the illustrations of radiographs which lack clarity. Bearing in mind the weight of clinical material passing through the authors' clinics I would like to have seen new illustrations rather than ones which are obviously over 20 years old.

As a clinician who restores primary teeth, carries out pulp treatment and places preformed crowns and strip crowns, there is much I agree with in this book, and I would recommend it to all dentists who want to do this type of work. However, I find that not all my child patients are candidates for this type of restorative work. When reading the book I had the impression that every child with a carious primary tooth has to have it restored. Indeed, the authors come out with a very strong statement: "In our opinion the failure to restore decayed teeth with cavitation is negligent on the part of the dentist and borders on malpractice. In our experience the simultaneous restoration of the teeth encourages acceptance and implementation of the preventive programme".

I was interested to read in the section on dental materials that the authors come out strongly in favour of the use of amalgam: "Its time-proven qualities make it the material of choice for restoring all posterior cavities (unless a crown is indicated) and for anterior teeth where aesthetic appearance is of secondary importance". Glass-ionomer is considered to be a temporary filling material to be used if a tooth is within 6-12 months of exfoliation.

Regarding preformed crowns on primary molars these should be seen as more than just a way of restoring teeth, but preventive restorations. If there is evidence of developing carious lesions on the buccal or lingual of primary teeth this would be an indication of a preformed crown, as these surfaces would be covered and hence further caries would be prevented. I find this philosophy to be rather mechanistic with an over-reliance on operative therapy. Personally I want to motivate the family so that they will all carry out good personal dental health behaviour. Once these are demonstrated, the teeth can then be restored to the highest standard.

This book is an essential buy for any dentist or postgraduate trainee who wants to carry out restorative dentistry of the highest quality for their child patients, or who will be taking any further examinations where paediatric dentistry is likely to be included. Undergraduate dental students will find it useful additional reading following on after their recognised undergraduate textbooks, which should have given them the basics of practical treatment planning for their child patients.

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Prevalence of HCV infection in health care workers of a UK dental hospital

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Objective To determine the prevalence of hepatitis C virus (HCV) antibodies in a group of dental health care workers (DHCW).

Design Retrospective cross sectional study.

Setting A UK dental hospital.

Subjects and methods The sera of 167 unselected DHCW were tested for the presence of IgG antibodies to HCV using two, third-generation enzyme-linked immunosorbent assays (ELISA). HCV viremia was determined by reverse transcription polymerase chain reaction (RT-PCR) analysis.

Results Two (1.2%) of the serum samples were found to be anti-HCV positive; one was also viremic. The two antibody-positive subjects were a qualified dental nurse and a student dental nurse, both females, without any known risk factor for HCV acquisition. No dentist was HCV seropositive.

Conclusions Since the prevalence of HCV infection in the UK general population varies between 0.08% and 0.55%, these results suggest that DHCW, and auxiliary staff in particularly, may have a slightly increased risk of HCV infection.

Hepatitis C virus (HCV) is an RNA virus comprising at least 6 types and more than 40 different subtypes. HCV infection is serious in that it results in chronic carriage in 70–80% of patients, with 20% developing hepatic cirrhosis in about 5 years — 10% of whom may develop hepatocellular carcinoma.1 Injecting drug users are the main at-risk patient population in developed countries, needle sharing being responsible for HCV acquisition in up to 83% of such individuals.1 Recipients of blood and blood products have also been at risk of HCV acquisition.1 Tattooing and male ear piercing1,2 have been implicated as risk activities. Persons living in areas of high HCV prevalence can also be at greater risk of HCV infection and hence carriage.6 Sexual transmission is possible, although rare, via either heterosexual or male homosexual routes.7,8 Vertical spread of HCV, from mother to new-born child (either in utero or perinatally), is also uncommon and appears to be more likely when the mother is co-infected with HIV.9

Currently the prevalence in the general population in the UK varies between 0.08%10 and 0.55%.11 Prevalence studies have indicated that up to 4.1% of groups of health care workers in different geographic regions may be HCV seropositive.12 Retrospective studies of HCV infection in medical staff known to have been accidentally exposed to contaminated body fluids suggest that less than 3% of occupational exposures are likely to lead to HCV acquisition,13 although there are reports of a 6%14 and 10% risk.15 To date there have been few published reports16–21 of the frequency of HCV infection in dental health care workers (DHCW), only one18...
considered UK DHCW — a small group in Wales. Some of these studies employed early-generation serodiagnostic tests for HCV, and most suggest that there is little risk of HCV transmission as a result of occupational injury during routine dental care. However, the incidence of HCV is rising, and one study did suggest a risk to dentists. The present retrospective investigation was thus carried out using currently available HCV-specific serological assays, to determine the frequency of HCV seropositivity in a large cohort of DHCW working in London, UK.

Materials and methods

The study group comprised 167 apparently healthy DHCW who had worked in a UK dental hospital between 1992 and 1995. Details of the occupational status, gender and age of the DHCW are provided in Table I. As part of a yearly occupational health assessment, each DHCW underwent venepuncture for hepatitis B virus serological assessment. Sera thus derived, that had been stored at -20°C, were used for this study. At the time of venepuncture staff gave written consent for their blood to be used for additional research investigations; local ethical approval was obtained prior to commencement of the study.

Serum IgG antibodies to HCV were tested in January 1996 using two third-generation enzyme-linked immunosorbent assays (ELISA) (Ortho Diagnostic Systems, Emmeryville, California; Sanofi Diagnostic Pasteur, Marnes la Coquette, France). The presence of HCV viremia was determined by reverse transcription polymerase chain reaction (RT-PCR) analysis (Roche Diagnostic Systems, Branchbury, New Jersey).

Results

Two of the 167 (1.2%) DHCW had detectable serum IgG antibodies to hepatitis C virus — a qualified dental nurse and a student dental nurse who had just commenced dental duties. The student nurse was also HCV viremic. The mode of acquisition of the HCV by these two asymptomatic DHCW was not known. No other DHCW had serological evidence of HCV infection (Table II).

Discussion

Since the first report of a surgeon becoming infected with HCV following an injury with a contaminated needlestick, several further episodes of nosocomial transmission of HCV have been documented, the majority following sharps accidents. To date there have been six reported studies of the frequency of HCV seropositivity in DHCW (Table III). In 1990, 1% of a group of US DHCW were found to be HCV seropositive; auxiliary dental personnel were at a higher risk for HCV infection (1.4%) than were dental surgeons. Eight of 456 (1.75%) dentists from New York City metropolitan area were anti-HCV seropositive compared with one out of 723 controls, and oral surgeons were at a higher risk than practitioners of other specialities.

None of 94 dental surgeons in Wales were found to be HCV-infected, despite 68% of the group having sustained inoculation injuries.

Only three of 481 (0.65%) dentists from Taiwan were found to be HCV-infected, despite 68% of the group having sustained inoculation injuries. Furthermore, none of 90 dentists providing dental care to patients at the San Francisco General Hospital were found to be HCV-infected.
In Brief

Key messages:

- Acquisition of hepatitis C virus is a potential occupational hazard for dental health care workers.
- Although low, the prevalence of hepatitis C virus infection among dental health care workers may be higher than that of blood donors.
- Auxiliary dental health care workers may be more liable to hepatitis C virus infection than dentists, but the reasons for this are not clear.

Hospital were seropositive for anti-HCV antibodies. Recently a US study confirmed the low risk of HCV carriage among dental health care staff by finding that 2% of 343 oral surgeons and 0.7% of 305 dentists were HCV seropositive.

The present results, showing evidence of HCV infection in 2 out of 167 DHCW (1.2%), together with those of the previous studies (Table III), suggest a slightly increased risk of HCV infection in DHCW, especially in auxiliary staff. However these data must be interpreted with caution. The sources of HCV infection of the present two HCV-infected DHCW are not known. One of the two HCV-infected DHCW had just commenced clinical duties and although she had no relevant problems in her social or medical histories it is most unlikely that she became infected as a consequence of nosocomial routes. The other dental nurse had been undertaking clinical duties for the past 5 years and it is likely that she could have sustained a sharps injury but there was no record of such an accident. At the time of the study both the nurses had left the hospital.

Even if it is assumed that only one of the present DHCW may have become infected by occupational routes, the present data suggests a higher HCV seroprevalence than that reported by Zuckerman and co-authors (0.28%) in a study of non-dental hospital health care workers in the same metropolitan area. This difference might reflect the higher number of surgical procedures performed by DHCW in comparison with most of the other health care workers, as well as the high incidence of sharps injuries occurring among dental staff.

In conclusion, the present results would suggest that dental auxiliary staff may be at slightly increased risk of HCV infection, but it is unclear if this reflects an increased occupational risk, or HCV acquisition via other routes. There is little evidence that infected health care workers can transmit HCV to patients during surgical treatment. Nevertheless an HCV-infected cardiac surgeon did transmit HCV to 5 of 222 patients in a 6-year period in Spain, possibly as a result of percutaneous injuries to the surgeon during wire closure of the sternum. Thus as similar sharps injuries are not uncommon during dental treatments, the current data highlights the need for all DHCW to maintain high standards of cross-infection control measures to avoid nosocomial transmission of HCV.

References

Hepatitis C virus (HCV) - an occupational risk to dentists?

Stephen R Porter* MD, PhD, FDS RCS(Edin), FDS RCS(Eng),
Giovanni Lodi** BDS

Hepatitis B virus has long been regarded as an occupational hazard in dentistry, however, as the availability of effective vaccination has reduced the risk of HBV infection, so dental staff have become aware of the potential risk of transmission of hepatitis C virus during dental treatment. This article outlines current relevant data suggesting that nosocomial transmission of HCV in dentistry is unlikely.

Hepatitis C virus (HCV) is an RNA virus comprising 6 types and more than 40 different sub-types. HCV infection results in chronic carriage in 30-80% of patients, 20% develop cirrhosis in about 5 years, 10% of whom may develop hepatocellular carcinoma. Treatment typically includes the use of interferon alpha, however, this does not usually produce complete clearance of HCV and has a variety of side-effects. Currently there is no passive or active immunisation for HCV infection.

Hepatitis C virus - transmission

Hepatitis C virus is predominantly transmitted by parental routes; for example sharing infected needles or via blood and blood products and during haemodialysis and plasmapheresis, although this is rare. Transmission via sexual routes or in utero is uncommon.

The persons likely to be infected with HCV are generally injecting drug users, patients who repeatedly received blood products prior to routine HCV testing of all blood donations, or who received other forms of specialised hospital care.

Hepatitis C virus is present in most body fluids of HCV-infected persons, including saliva. Salivary levels of HCV are generally low, and may correlate with hepatic function. Salivary transmission of HCV has not been documented in vivo, although transmission between experimental animals has been observed, and there is at least one report of HCV transmission via a bite injury. Nevertheless, as HCV is generally confined to distinct patient groups, saliva must be an uncommon vehicle for the transmission of HCV.

The risk to health care workers

Retrospective studies indicate that up to 4.1% of health care workers (HCW) may be HCV-antibody positive (seropositive). However, some initial investigations used assays that tended to over-estimate HCV seropositivity. In the UK 0.28% of a large cohort of health care workers (HCW) were recently found to be HCV-seropositive.

Studies of dental health care workers (DHCW) also reflect the relatively low nosocomial transmission of HCV. In 1990, 1% of a group of DHCWs in the USA were found to be HCV sero-positive, HCV infection being higher in dental auxiliaries (1.4%) than dental surgeons (0.3%). However, no control group was included in this study.

Another study reported 8 out of 456 (1.75%) DHCWs from the New York metropolitan area to be HCV-infected compared with 1 of 723 control persons; oral surgeons were possibly at higher risk of HCV infection than other dentists. Never-theless, none of 90 dentists working a large hospital in San Francisco were HCV seropositive.

In the UK, none of 90 dental surgeons were found to be HCV-infected, despite 68% of the study group having sustained sharps injuries in dental practice. Furthermore, only 3 of 481 (0.65%) dentists from Taiwan were found to be HCV-infected, the seroprevalence rate being similar to that of...
Hepatitis C virus – an occupational risk to dentists?

It is evident from the above figures that transmission of HCV during dental treatment is rare. Nevertheless, DHCWs must continue to maintain high standards of cross-infection control to minimise the possible spread of HCV during dental treatment. The recent identification of other hepatitis viruses such as hepatitis G virus (HGV) highlights the need for the dental profession to regularly review cross-infection control policies.

Conclusion

In summary, dental health care staff are unlikely to acquire HCV through occupational routes. Nevertheless, in view of the significant medical problems associated with HCV acquisition and the lack of an effective long-term therapy or vaccination programme, DHCWs must continue to maintain high standards of cross-infection control to minimise the possible spread of HCV during dental treatment.

Precautionary action

It is evident from the above figures that transmission of HCV during dental treatment is rare. Nevertheless, DHCWs must continue to maintain high standards of cross-infection control as dental patients may be HCV-infected. It is important that DHCWs understand that transmission of HCV can occur via unrecognised routes in medical units, and know the consequences of HCV infection, including the possible lack of sustained response to therapy, the risk of re-infection or emergence of new HCV strains within the same patient, and the lack of effective immunisation. The BDA clinical guidelines on cross-infection control in dentistry presently seem appropriate.

The current General Dental Council guidelines indicate that DHCWs known to carry HCV should not perform expensive prone procedures. The advisory group on hepatitis and the UK advisory panel for health care workers infected with blood-borne viruses have advised that all HCV-infected health care workers do likewise.

References

1. Iwarson S. The natural course of chronic hepatitis C. FEMS Microbiology Rev 1994; 14: 201-204.

Erratum

We regret that in the latest issue of Dental Business (Vol 1, No 3), it was stated in a pullquote that dental records need to be made available within 24 hours, although the author's article clearly stated the time period to be 14 days.

This editorial error may have caused confusion, for which we apologise. Please note that dentists are required under the National Health Service (General Dental Services) Regulations 1992 to make their records available to either a dental officer, the DPB or the FHSA upon request and within 14 days.