

Hepatitis C virus infection and assisted reproduction

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BACKGROUND: In assisted reproduction, hepatitis C virus (HCV) transmission may pose a risk for the baby, technicians, and gametes or embryos from non-contaminated parents. This study aimed at determining the prevalence and risk factors for HCV infection in a group of infertile couples. **METHODS:** HCV infection was investigated in 409 patients attending the infertility clinic at Hospital de Clínicas de Porto Alegre, Brazil, between 1997 and 1998. Serum was screened for anti-HCV using ELISA and for hepatitis B surface antigen (HBsAg) using an enzyme-linked fluorescent assay (ELFA). HCV infection and semen viraemia was also investigated using HCV RNA detection. **RESULTS:** The overall prevalence of anti-HCV was 3.2% (8/248) among women and 3.7% (6/161) among men. All subjects were negative for hepatitis B virus (HBV) and human immunodeficiency virus (HIV). From the 14 HCV-positive patients, two were lost, and serum was collected from the remaining 12 patients for assessment of HCV RNA, resulting in five HCV-positive cases (one woman and four men). Only one of the HCV-positive men had viraemia levels >500 000 RNA copies/ml. There was a significant risk associated with being HCV-positive in women with HCV-positive male partners ($P < 0.001$). In male patients, the correlation between use of intravenous drugs and HCV-positivity was also significant ($P < 0.001$). **CONCLUSIONS:** Since the risk for vertical and laboratory HCV infection is not well determined, and HCV prevalence is not negligible in this group, we recommend that infertile patients be screened before assisted reproductive techniques.

Key words: hepatopathy/infertility/in-vitro fertilization/non-A non-B hepatitis

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide (Choo *et al.*, 1989). At least 85% of HCV-infected patients develop persistent infection, while almost 70% develop chronic hepatopathy with increased levels of liver enzymes. Chronic HCV infection may lead to cirrhosis and hepatocellular carcinoma (Centres for Disease Control and Prevention, 1997; Moyer *et al.*, 1999). The prevalence of HCV infection in the general population is 1.8% in the USA (National Institute of Health, 1997; Alter *et al.*, 1999; EASL International Consensus Conference on Hepatitis C, 1999), 1.5% in Europe (Botta-Frilund, 1994; Zeuzem *et al.*, 1996; Pawlotsky, 1997), and 1.7% in Brazil (Fonseca *et al.*, 1998).

Epidemiological and experimental studies indicate that the main route of HCV transmission is parenteral, through transfusion of blood and plasma derivatives (Genesca *et al.*, 1991). Other routes of parenteral transmission include intravenous drug use, haemodialysis and organ transplantation (Ho, 1991). In addition, healthcare workers have developed non-A, non-B hepatitis after accidental needle-stick exposure, as well as in

the absence of apparent percutaneous exposure (Genesca *et al.*, 1991).

Non-parenteral HCV transmission is also frequent. At least 50% of patients with hepatitis C have had no parenteral exposure. Serological studies have also shown vertical HCV transmission (Stevens, 1994), and mother-to-child transmission of HCV in women co-infected with HCV, and human immunodeficiency virus (HIV) has been detected using PCR (Novati *et al.*, 1992). However, although viral RNA has been detected in saliva and semen, the role of sexual, perinatal, and other possible non-parenteral routes of hepatitis C transmission is still unclear (Tedder *et al.*, 1991; Kao *et al.*, 1996; McKee *et al.*, 1996; Alter *et al.*, 1999).

In assisted reproduction, HCV transmission may pose a risk for the baby, and also for technicians and gametes or embryos from non-contaminated parents (Levy *et al.*, 2000). The exact risk for HCV transmission in this population is, however, unknown; therefore, specific guidelines to prevent HCV infection in reproductive medicine have not yet been established. It is important to determine HCV prevalence among infertile couples who seek assisted reproduction so that specific health

policies can be devised and infertile patients with HCV infection can be counselled during ART cycles.

The aim of this study was to determine the prevalence of HCV infection in a population of infertile couples, and to search for risk factors associated with this condition.

Materials and methods

Design

We performed a cross-sectional study with 409 patients (248 women, 161 men) attending the infertility clinic at Hospital de Clínicas de Porto Alegre (HCPA), a teaching hospital in southern Brazil, between 1997 and 1998. The research protocol was approved by the Ethics Committee of the hospital.

Patients

All patients received counselling before and after being tested for hepatitis C. Those patients with positive results were submitted to gastroenterologic evaluation and management according to the National Institute of Health Consensus Statement on the Management of hepatitis C (National Institute of Health, 1997). Hepatitis B, HIV, and known risk factors associated with HCV transmission were also evaluated. Every patient was also tested for *Chlamydia* and *Treponema* infection, in addition to the investigation of possible causes for their infertility.

The couples answered a questionnaire concerning previous history of personal and familial hepatitis, sexual exposure, use of injectable drugs, and exposure to whole blood or plasma derivatives in several situations.

We included only infertile patients who agreed to participate (248 women and 161 men) and who, if HCV test positive, agreed to have their disease investigated and monitored. At the beginning of the study, all subjects involved gave their informed consent to participate and to have their blood samples tested. Only two women and one man refused to be included.

During the initial visit, all patients were submitted to a thorough examination. Previous medical history was evaluated and current use of any medication was recorded. Exclusion criteria were: age (>40 years) or having any acute health disorder. Blood contact was defined as any contact with blood (with or without protection) and surgery defined as any procedure (out-patient/in-patient) performed in a hospital.

Assays

The detection of antibodies to hepatitis C virus (anti-HCV) and to hepatitis B surface virus antigens (HBsAg) was included as part of routine infertility investigation. Sera from patients were screened for anti-HCV by ELISA (Ortho HCV 3.0; Ortho-Clinical Diagnostics, Neckargewünd, Germany), and for HBsAg by enzyme-linked fluorescent assay (ELFA) (Vidas HBsAg; bioMérieux, Marcy-l'Étoile, France). HIV was also investigated by ELISA (Vironostik[®] HIV Uni-Form II plus O; Organon, Boxtel, Holland).

The specificity of the anti-HCV test (ELISA) in a low-risk population is 99.96% (4 units per 10 000 donations); the intra-assay coefficient of variation (CV) is 9.9% ; the inter-assay CV range 1.2–14.8%.

In patients who tested positive for anti-HCV (ELISA), RNA was extracted as described (Chomcczynski and Sacchi, 1987). RT-PCR was carried out to detect hepatitis C virus RNA using primers drawn from the 5' non-coding region of HCV (NS-5'R) for amplification (Simmonds *et al.*, 1993). The technique's detection limit was 200 copies/ml. To confirm the results, a second amplification was carried

Table I. Clinical and demographic characteristics of patients

	Women (n = 248)	Men (n = 161)
Type of infertility		
Primary	154 (62%)	105 (65%)
Secondary	94 (38%)	56 (35%)
First sexual experience	18.5 ± 3.4 years ^a	17.25 ± 2.5 years ^a
Ethnicity		
Native Brazilian	6 (2.4%)	5 (3%)
Black	61 (24.6%)	44 (27%)
Latin (white)	181 (73%)	112 (70%)

^aData are mean ± SD.

out using internal primers (nested PCR). HCV-genotyping was performed by restriction fragment length polymorphism (RFLP) analysis of the nested PCR products using three restriction enzymes in two separate digestion systems. Digested products were submitted to electrophoresis, stained, and separated on polyacrylamide gels. Band patterns for the different HCV genotypes were derived from those described (McOmish *et al.*, 1994) and from the 5'NCR (non-coding region) sequences obtained from gene databases.

Statistics

Data were stored and analysed using the EPI-INFO 6.02 software (Atlanta, USA). Statistical analysis was carried out by estimation of relative risk (RR) and by the Fisher's exact test.

Results

The prevalence of anti-HCV positivity among women was 3.2% (8/248). The mean age of female patients was 31.6 ± 4.1 years (range 17–39). Regarding previous history of parenteral exposure, 7.9% of the women in the study had undergone transfusion, 1.9% had used injectable drugs, 59.5% had undergone surgery, 7.5% had had contact with blood and 3.7% reported history of hepatitis.

The clinical and demographic characteristics of studied patients are presented in Table I. *Treponema* infection was negative in all investigated cases. The prevalence of *Chlamydia* infection was 15% in HCV-negative patients and 17% in HCV-positive patients (*P* = not significant, Fisher's exact test). Considering other infertility factors: 103 patients (41.5%) presented with tubal occlusion; 43 (17.3%) with endometriosis; and 17 (7%) with ovulatory dysfunctions. In 23 (9.2%) the infertility was idiopathic, and in 62 cases (25%), male infertility was present.

The prevalence of anti-HCV positivity among men was 3.7% (6/161). The mean age of male patients was 33.9 ± 5.7 years (range 21–57); 7.0% had undergone transfusion, 6.1% had used injectable drugs in the past, 49% had undergone surgery, 3.5% reported contact with blood and 9.6% had developed hepatitis.

One HIV-positive man also presented with anti-HCV positivity. Two patients (one man and one woman) were HBsAg-positive; both were anti-HCV-negative.

From the 14 anti-HCV positive patients, two were lost to follow-up. Serum was collected from the remaining 12 patients (7 women and 5 men) for assessment of viraemia levels (HCV

Table II. Features of anti-HCV-positive subjects

Patient	Sex	Age	PCR	Genotype	Viral load ^a	Risk factor
1	F	28	-	-	-	HCV ^{+ve} partner
2	M	32	+	3	100 000	Injectable drugs
3	F	37	-	-	-	Surgery
4	F	29	-	-	-	HCV ^{+ve} partner
5	M	44	+	3	400 000	Injectable drugs, surgery, history of hepatitis
6	F	31	-	-	-	Not identifiable
7	M	33	-	-	-	Not identifiable
8	F	34	-	-	-	Transfusion, surgery hepatitis in family
9	M	26	+	1a	250 000	Injectable drugs
10	F	38	+	2	300 000	HCV ^{+ve} partner
11	M	36	+	3	200 000 000	Injectable drugs
12	F	35	-	-	-	Not identifiable

^aRNA copies/ml

F = female; M = male.

RNA). Among those patients, only one male patient presented high viraemia levels (20×10^6 RNA copies/ml). The patients' genotype and levels of viraemia are described in Table II.

We also evaluated the risk of sexual HCV transmission among infertile couples and the relationship between anti-HCV and injectable drug use. In women, an association was observed between the risk for presenting anti-HCV antibodies and having an anti-HCV positive male partner [RR = 16.09, 95% confidence interval (CI) = 1.48–262.0, $P = 0.0124$, Fisher's exact test]. In men, an additional association was found between use of intravenous drugs and anti-HCV positivity (RR = 23.24, 95% CI = 3.21–541.36; $P = 0.0013$, Fisher's exact test).

Discussion

Since the introduction of specific antibodies to HCV, several studies have been developed with the aim of improving the epidemiology and clinical course of this infection (Lynch-Salamon and Combs, 1992). In general, antibodies to HCV are found ~0.5–5% of the population, depending on the geographic region being studied (McKee *et al.*, 1996). In this study, in which couples were evaluated before assisted reproduction technology procedures, the prevalence of HCV was in agreement with the local prevalence and the data from the literature (Duffaut and Valla, 1997; Fonseca *et al.*, 1998).

We showed that the prevalence of HCV infection was lower in infertile patients than in the general population. In the presence of infection, semen viraemia was extremely low. Our data is in accordance with other reports (Levy *et al.*, 2000) in which a low viral load of HCV was detected in semen of infertile men using the same methodology employed by us (RNA detection).

HCV is found in the semen of HCV-positive patients in very low or of undetectable levels by the available methodology; however, these data must be interpreted with care, since semen is known for its ability to inhibit PCR (Debono *et al.*, 1996; Semprini *et al.*, 1998; Levy *et al.*, 2000). Moreover, the importance of HCV virus in semen can be related to virus

concentration, since viral infections might contribute to male infertility by causing an inflammatory/immunologic reaction or by a direct toxic effect (Keck *et al.*, 1998).

The risk for sexual HCV transmission is estimated at 5% (Dienstag, 1997) and in women, the risk for infection by HCV-positive partners is higher in relationships lasting more than 20 years. In these cases, transmission is more feasible if exposure is repeated and long-lasting (Caporaso *et al.*, 1996; Kao *et al.*, 1996). We also found a positive association between HCV-positivity in males and risk for HCV-positivity in their female partners. Some investigators have demonstrated that sexual transmission is more probable in cases with co-infection by HIV, suggesting an association with the elevated HCV charge produced by immune suppression (Eyster *et al.*, 1991; Soto *et al.*, 1994).

It is known that HCV transmission occurs essentially by the parenteral route, with a huge part of HCV-serum positive patients reporting a history of blood transfusion and use of drugs in the past (Diago *et al.*, 1996). In our population, we found a relative risk of 23.24 for HCV infection in male patients who were users of injectable drugs. It is also known that in 40–50% of hepatitis C cases the parenteral risk factor is not identified, suggesting the importance of other possible routes (Everhart *et al.*, 1990).

Vertical HCV transmission is less frequent, except in cases of mothers with high viraemia levels (HCV RNA $\pm 10^6$ copies/ml), including those co-infected with HIV (Lin *et al.*, 1994; Ohto *et al.*, 1994). In our study, none of the female patients had HCV RNA levels $>3 \times 10^6$ copies/ml. Although current evidence indicates that the risk for sexual and perinatal transmission is low, the prognosis of neonates born with HCV infection remains unknown. In addition, there are few data concerning prevalence of HCV within infertile populations, repercussion of HCV infection in reproductive health and risk for transmission by assisted reproductive procedures.

Another unsolved question concerns the variety of viral genotypes and their relation with the vertical or laboratory risk for transmission. Some types of HCV virus could be more aggressive and should be managed differently.

In conclusion, our results show that most HCV-positive patients in this population of infertile couples had undetectable or low levels of HCV RNA. We should consider the inclusion of serum typing for HCV in the investigation of the infertile couple, to allow the definition of management and risks associated with viraemic levels when one or both partners are infected with HCV.

These data may be useful for counselling and management of couples who seek assisted reproduction, and also for further studies analysing the risk for HCV transmission in infertile couples.

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