CASE REPORT

Transmissions of hepatitis C virus during the ancillary procedures for assisted conception

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Since mother to child transmissions of hepatitis C virus (HCV) have been reported to be low, teams involved in assisted reproductive technologies have accepted HCV positive patients into their programmes. We report in the present paper two cases of undoubted patient to patient HCV transmission while patients were attending for assisted conception. In both cases, HCV genotyping and sequencing of the first hypervariable region of the HCV genome provided molecular evidence for nosocomial transmission. Investigations made to elucidate the route of contamination have shown that the most likely route of contamination is through healthcare workers. Such nosocomial HCV infection has been reported in other healthcare situations, mainly in dialysis units, and physical proximity was also suspected to be at the origin of the infection. We conclude that assisted reproduction teams must be very prudent when including such patients in their programmes.

Key words: hepatitis C/in-vitro fertilization/virus

Introduction

More and more patients infected by the hepatitis C virus (HCV) attempt IVF, raising the question of virus transmission. The risk of mother to child transmission of HCV has been well studied and appears to be low (MacDonald et al., 1996; Van der Poel and Ebeling, 1998), so that teams involved in assisted reproductive technologies have agreed to include such patients in their programmes (Fédération des BLEFCO, 1997). However, nosocomial transmissions have been reported despite no clear evidence of the route of contamination. A French study, including 6664 patients with chronic hepatitis C, revealed that 37% of contamination is due to blood transfusion and 15% to another nosocomial origin (Roudot Thoraval et al., 1997). However, viruses transmissions have been reported after percutaneous exposure to blood and blood-derived products in healthcare workers (Howard et al., 1997). These nosocomial transmissions have been proven by virus characterization, leading to strict recommendations in hygienic care and specially in the use of disinfectant products acting on HCV (Schvarcz et al., 1995; Howard et al., 1997; Ouzan, 1997; Van der Poel and Ebeling, 1998). In the present paper, we describe the contamination of two patients in our programme despite the strict observance of these rules.

Case report

The first case concerned a 37 year old anti-HCV negative patient, attempting intracytoplasmic sperm injection (ICSI) for male infertility. She had a twin pregnancy and presented with clinical acute hepatitis during the fourth month of gestation, with an increase in liver transaminases. Serum examination showed the presence of anti-HCV antibodies as well as HCV RNA. The pregnancy had a favourable evolution with a decrease in transaminase concentrations and resulted in the birth of two healthy boys. The search for HCV RNA was negative in the newborns and remained negative after 18 months of life. The patient remained HCV RNA positive 2 months after delivery. Since liver biopsy showed signs of severe hepatitis, the patient was treated by α-interferon, $3 \times 10^6$ units twice a week, for 11 months. The transaminases then returned to normal and HCV RNA remained undetectable after treatment interruption with a follow-up of 6 months.

The second case was a 32 year old anti-HCV negative woman having an IVF attempt with donor spermatozoa for non-obstructive azoospermia associated with tubal infertility. The HCV infection was clinically occult and the contamination was detected through a systematic search for anti-HCV antibodies and confirmed by the presence of HCV RNA in serum. Since she was not pregnant, the patient was immediately treated by α-interferon, $3 \times 10^6$ units twice a week for 6 months after which HCV RNA disappeared from her serum.

Since these two patients were negative for anti-HCV antibodies at the time of their attempts at assisted reproduction and did not present any risk factors (blood transfusion, endoscopy, piercing, etc.) we suspected a nosocomial transmission in the course of the various procedures carried out during assisted reproduction treatment. We investigated the patients’ records and the procedures used during these attempts. We found that each of them was treated concomitantly, but at different periods (one in May 1997, the other in September 1997), at the same time as a patient known to be HCV infected. In both cases the contaminated patient had follicular puncture immediately after the infected one. The HCV genotypes from the three
patients, characterized by the line probe assay (LIPA HCVII®; Innogenetics, Zwijndrecht, Belgium), were 1b. Two-strand direct sequencing was carried out on a nested polymerase chain reaction (PCR) product in the E2 gene encompassing the hypervariable region 1 of the HCV genome as previously described (Izopet et al., 1999). Phylogenetic analysis of E2 sequences from these 1b strains and a control panel consisting of 1b strains from the same geographical area plus 1b strains extracted from the EMBL (EMBL–European Bio-Informatics Institute) data bank indicated clustering of the two cases of de-novo infections with the already infected patient attending the IVF centre (Figure 1). We screened all other patients treated in the same periods (sharing same days of puncture, ultrasonographic examinations and blood sampling) for anti-HCV antibodies and none of them appeared to be infected. Moreover, all the staff of the assisted reproduction treatment centre (physicians, biologists, nurses, etc.) were tested and found to be negative. Follicular puncture was therefore not suspected as the cause of contamination. Moreover, the contaminated patients and the suspected contaminating one underwent follicular puncture in different rooms, with different probes and single use materials, the follicular fluids were treated in different laminar hoods and oocytes placed in different incubators. These findings led us to postulate that the contamination occurred outside the direct practice of IVF, and possibly through procedures practised by ancillary staff.

Discussion

Before concluding that the virus has been transmitted via health workers, we have carefully investigated each step of the assisted reproduction attempts shared by patients. Follicular growth was monitored by daily vaginal ultrasonography and serum oestradiol measurement. Ultrasonography is unlikely to be contaminating since probes were covered with a protective sheath and wiped after each patient with germicidal disposable cloths, which has been reported to be effective in the prevention of virus transmission (Milki and Fisch, 1998). Moreover, no other patient monitored during the same period has been infected.

Contamination during the follicle puncture itself seems also to be unlikely. Indeed, contaminating and contaminated patients were punctured in different rooms, with different probes and all other materials were disposable. This situation looks like the one reported in dialysis centres, where patients treated on different but adjacent machines were contaminated by the same viruses (Natov and Pereira, 1996; Pereira and Levey, 1997; Ouzan, 1997). Therefore a physical proximity during routine health care appears to be a risk of HCV transmission. This risk seems to be linked to percutaneous exposure during i.v. injection processes or to the use of multi-dose vials of drugs (MacDonald et al., 1996; Pereira and Levey, 1997; Couzigou, 1997). Cross-infection between patients has been reported in a care unit where nurses did not change their gloves but disinfected them with alcohol-based products, inactive on HCV viruses (Schvarcz et al., 1996; Van der Poel and Ebeling, 1998). The use of multi-dose vials of heparin has also been shown to be a route of contamination in dialysis patients (Pereira and Levey, 1997).

Laboratory contamination by hepatitis B has been reported due to the use of serum containing culture medium (Quint et al., 1994). This cannot be the case in our team since protein-
References


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free media without any supplementation are used for IVF (Parinaud *et al.*, 1998, 1999). Moreover, the laboratory instruments used for follicular fluid examination, IVF and embryo culture were different.

Contamination during embryo transfer is unlikely since it is not bloody, only disposable materials were used and no i.v. injection was done.

Patients with anti-HCV antibodies are now included in our programme only if HCV RNA is negative at the beginning of the IVF cycle. French law includes guidelines concerning assisted reproduction procedures, published in February 1999 (Ministère de l’Emploi et de la Solidarité, 1999). Assisted reproduction treatment in HCV positive patients (male or female partner) is possible only in the case of a clinical research protocol accepted by the governmental health ministry. However, these measures are mainly made to protect against partner to partner and mother to child contamination and not for nosocomial transmissions. Our experience leads us to recommend extreme prudence and to take all possible hygienic precautions when including such patients in an IVF programme. A way to minimize the risks could be to treat these patients before IVF in order to eradicate the virus.