Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection?

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Abstract

AIM: To evaluate the possible influences of HCV infection and relative antiviral treatment on seminal parameters and reproductive hormonal serum levels.

METHODS: Ten male patients with HCV-related chronic hepatitis and 16 healthy male volunteers were studied. In all subjects seminal parameters (nemaspermic concentration, progressive motility, morphology) and hormonal levels were determined. Seminal parameters and inhibin B, follicle-stimulating hormone, luteinizing hormone, total and free testosterone, estradiol, prolactine in patients were measured after six and twelve months of antiviral combined (interferon + ribavirin) treatment.

RESULTS: Patients before treatment showed a significantly lower nemaspermic motility and morphology as well as lower inhibin B and free testosterone levels than controls. Inhibin B levels in cases were improved six and 12 mo after treatment in five responders (161.9 ± 52.8 pg/mL versus 101.7 ± 47.0 pg/mL and 143.4 ± 46.1 pg/mL versus 95.4 ± 55.6 pg/mL, respectively). Hormonal pattern of patients did not significantly change after treatment, with the exception of estradiol levels with an initial reduction and an overall subsequent increment (19.7 ± 6.4 pg/mL versus 13.6 ± 5.0 pg/mL versus 17.3 ± 5.7 pg/mL). However in 1-year responders a significant increment of free testosterone (14.2 ± 2.54 pg/mL versus 17.1 ± 2.58 pg/mL) occurred. An impairment of nemaspermic morphology occurred, while other seminal parameters did not change significantly during antiviral treatment.

CONCLUSION: Patients with HCV infection show worse spermatic parameters than controls, suggesting a possible negative influence of virus on spermatogenesis, with further mild impairment during antiviral treatment. However therapy could improve the spermatic function, as suggested by the increased inhibin B levels and improved hormonal pattern in responders. Further studies are needed to confirm these preliminary intriguing results.

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INTRODUCTION

Hepatitis C virus (HCV) chronic infection is widespread in the world with about 150-180 millions of carriers[1]. Chronic HCV-related hepatitis is a frequent condition with a significant clinical impact due to the rare spontaneous virus disappearance in carriers. Virus transmission occurs predominantly *via* parenteral routes, but is often unclear. Sexual and maternal transmissions are rare (about 5%)[2]. Combined administration of peghilated α-interferon (PEG-IFN) plus ribavirin is the treatment of choice for HCV infection (from 45% to 80% of virus eradication)[3].

HCV is involved in many extrahepatic conditions and at least one of these manifestations is present in 74% of cases (the term "HCV disease" has been used to underline its systemic aspects)[4,5]. The association between extrahepatic diseases and HCV infection has not been definitely proven. Moreover, no organ-specific antibodies (ANA, SMA, anti-LKM) are present in 20%-40% of cases with uncertain role, which might be the results of lymphocyte B stimulation by HCV or the HCV-induced anti-LKM1 recognition of specific antigens[6,7].

Mixed cryoglobulinemia is the most frequent extrahepatic HCV manifestation (43%-90% of cases)[8,9]. Other associated diseases include crioglobulinaemic nephropaty and glomerulonephritis[10,11], thyroid diseases (during or after interferon treatment)[12,13], autoimmune gastritis and

less frequently Sjögren or Sjögren-like syndromes[14] idiopathic pulmonary fibrosis[15], porphyria cutanea tarda[16], lichen planus[17], type 2 diabetes mellitus[18]. Moreover interferon frequently seems to precipitate latent autoimmune gastritis, particularly in females, which is often associated with antithyroid antibodies[19].

Very few studies have been performed to evaluate the presence of HCV-RNA in seminal fluid and spermatozoa, particularly in candidates for assisted reproduction techniques, whose seminal parameters and fertility are very poor.

The presence of the virus in the cervico-vaginal secretions has been already demonstrated, while data about its presence in semen are controversial[2]. Indeed polymerase chain reaction (PCR) inhibitors in seminal fluid, especially *Taq* polimerase inhibitors, could interfere with results obtained by this methodology. Levy *et al*[20] suggested that HCV-RNA could be found in males' semen when *Taq* inhibitors have been suppressed by diluitions, thus explaining the previous contradictory results. The authors demonstrated that 30% of the studied males show semen abnormalities (two oligozoospermias and ten oligoasthenoteratozoospermias), confirming the presence of HCV-RNA in the seminal fluid before antiviral treatment for high serum viral load carriers[21]. On the contrary, Debono and co-workers[22] showed that HCV-RNA could not be detected in semen (seminal fluid and spermatozoa) either by PCR or by branched DNA or *in situ* hybridisation[22]. Moreover, these studies have confirmed the low prevalence of HCV sexual transmission.

The aim of this study was to evaluate the seminal parameters (volume, pH, nemaspermic concentration, motility and morphology) and reproductive hormone serum levels including follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactine (PRL), 17-β-estradiol (17-βE), total and free testosterone, inhibin B, in subjects with chronic HCV-related hepatitis before and after six and twelve months of standard antiviral combined therapy (peg-interferon plus ribavirin), in order to verify the possible influences of the virus and/or antiviral treatment on seminal and hormonal variables.

MATERIALS AND METHODS

Patients

Ten consecutive male patients (age range 33-39 years) affected by HCV-related chronic hepatitis, followed at our department, with viral replication (17 240-5 176 100 copies/mL, branched DNA vers.3.0) and increased aminotransferase levels (1.4-4 X) were enrolled in March-April 2003. They showed normal hepatic function (values of bilirubin, albumin and coagulative parameters within ranges) and no ultrasonographic features of portal hypertension. Viral genotypes were 1 (60%), 2 (20%), and 3 (20%). Causes of viral transmission were unknown in all patients. Two subjects (monitored as blood donors) were infected in the last few months, the mean duration of infection was 11.0 ± 2.7 years. Nobody showed either history of cryptorchidism, varicocele or other conditions at risk for infertility, or other causes of liver disease (HBV, alcohol, autoimmunity, hemochromatosis, *etc*) and previous treatment for hepatitis C.

Sixteen male healthy volunteers aged 18-52 years, without history or features of liver disease, were enrolled in the same period and served as the control group.

Table 1 Characteristics of controls and patients before antiviral treatment (mean ± SD)

Parameters	Cases	Controls
Number	10	16
Age (yr)	36.3 ± 2.4	29.7 ± 7.3
Sperm concentration (n/mL)	85.5 ± 63.5	103.6 ± 54.1
Normal motility (%)	45.7 ± 8.8	60.4 ± 6.9
Normal morphology (%)	28.1 ± 9.6	42.0 ± 6.7
Inhibin B (pg/mL)	101.7 ± 47.0	192.8 ± 78.6
Free testosterone (pg/mL)	15.2 ± 2.2	21.4 ± 3.1
Total testosterone (μg/L)	6.72 ± 2.1	6.7 ± 1.3
Prolactine (μg/L)	8.19 ± 5.1	4.6 ± 1.9
LH (mU/mL)	4.02 ± 2.2	2.5 ± 0.7
FSH (mU/mL)	4.81 ± 1.8	3.6 ± 1.4
17-βE (pg/mL)	19.7 ± 6.4	14.9 ± 2.9

A trained blinded operator investigated chemical-physical characteristics of seminal fluid (volume, pH, sperm concentration, motility and morphology) in patients and controls, according to the WHO criteria and recommendations (1992/1999)[23]. The following hormones were measured in HCV patients and controls: FSH, LH, PRL, estradiol, total and free testosterone, inhibin B. FSH, LH and PRL were determined by immunoradiometric assay (IRMA) (FSH: normal values [nv] 1.2-10 mU/mL; intra- and inter-assay coefficients of variation [CV] 7.5%-8.3%; sensitivity 0.18 mU/mL; LH: nv 1.5-7 mU/mL, CV 5.8%-13.8%, sensitivity 0.20 mU/mL; PRL: nv 2-12 ng/mL, CV 2.7%-8.9%, sensitivity 0.5 ng/mL). 17-βE and total/free testosterone levels were determined by radioimmunoassay (RIA) (17-βE: nv 10-40 pg/mL, CV 10.5%-9.1%, sensitivity 1.4 pg/mL; total testosterone: nv 3-10 ng/mL, CV 8.7%-13.7%, sensitivity 0.05 ng/mL; free testosterone: nv 16-41 pg/mL between 20-50 years of age and 9-31 pg/mL > 50 years of age, CV 10.6%-9.9%, sensitivity 0.15 pg/mL). Serum inhibin B was measured by enzyme linked immunosorbent assay (ELISA) (nv 97-330 pg/mL, CV <7%, sensitivity < 15 pg/mL).

The same seminal parameters and hormone levels were determined in cases at 6 and 12 mo after starting antiviral combined treatment with 1.5 μg/kg peg-interferon α2b a week plus ribavirin 800-1200 mg a day (according to weight). The duration of treatment was twelve months.

Statistical analyses were performed using Student's *t*-test and when appropriate, using matched pair t-test. Age-adjustments were performed by a multiple regression model.

RESULTS

The sperm concentration was not significantly different between controls and cases (before antiviral therapy), while nemaspermic motility and morphology were lower in the latter (after age adjustment: $P = 0.0002$ and $P = 0.0003$ respectively). The two patients with more recent HCV infection presented the worse seminal parameters: progressive motility < 40%, normal morphology < 20%. Inhibin B and free testosterone were significantly lower in patients than in controls (after age-adjustment: $P = 0.004$ and $P < 0.001$ respectively) (Table 1).

After six months of combined antiviral treatment, sperm concentration and motility did not significantly

Table 2 Seminal and hormonal parameters in responders and non responders before, during and after treatment (mean ± SD)

	Month 0	Month 6	Month 12	Month 0	Month 6	Month 12
		Non responders			Responders	
Sperm concentration (n/mL)	84.2 ± 56.6	69.0 ± 35.5	66.6 ± 36.1	86.9 ± 76.6	35.2 ± 25.8	76.8 ± 75.3
Normal motility (%)	45.2 ± 7.0	51.4 ± 13.9	46.0 ± 7.4	46.2 ± 10.7	37.0 ± 17.8	43.8 ± 24.3
Normal morphology (%)	34.8 ± 7.6	24.6 ± 11.3	26.0 ± 6.8	21.4 ± 6.0	14.4 ± 9.2	21.6 ± 12.8
Inhibin B (pg/mL)	98.0 ± 49.2	166.4 ± 30.3[b]	95.4 ± 55.6	105.3 ± 50.1[a]	157.4 ± 72.7	143.4 ± 46.1
Free Testosterone (pg/mL)	16.2 ± 1.6	16.4 ± 2.0	16.9 ± 3.4	14.2 ± 2.5	14.6 ± 2.8	17.1 ± 2.6[c]
Total testosterone (μg/L)	7.4 ± 2.0	7.9 ± 0.9[d]	6.6 ± 1.0	6.0 ± 2.0	7.3 ± 2.1	6.9 ± 1.0
PRL (μg/L)	7.0 ± 3.8	7.9 ± 1.8	8.9 ± 3.3	9.4 ± 6.3	7.4 ± 3.9	6.5 ± 2.8
LH (mU/mL)	4.2 ± 2.1	3.6 ± 1.8	3.0 ± 1.2	3.8 ± 2.6	4.5 ± 2.5	3.6 ± 0.7
FSH (mU/mL)	4.8 ± 1.7	4.9 ± 1.0	5.7 ± 1.6	4.8 ± 2.1	5.2 ± 0.8	5.1 ± 0.9
17-βE (pg/mL)	19.4 ± 5.3[e]	14.6 ± 5.1	20.2 ± 6.8	20.0 ± 7.9	12.7 ± 5.2	14.5 ± 2.8

[a]$P = 0.039$ (0 *vs* 12 mo); [b]$P < 0.01$ (6 *vs* 12 mo); [c]$P < 0.05$ (0 *vs* 12 mo); [d]$P < 0.05$ (6 *vs* 12 mo); [e]$P < 0.01$ (0 *vs* 6 mo).

change in HCV patients, while further alterations in morphology appeared ($P = 0.01$). At the same time inhibin B levels significantly increased ($P = 0.004$) (Table 2).

Viraemias at the sixth month were negative in seven patients and there were not any significant differences in seminal and hormonal parameters between the former and the three patients with persistent viraemia. Two responders had virological breakthrough at 12 mo and a total of five patients (50%) showed a full response to combined antiviral therapy.

At 12 mo sperm concentration and motility did not significantly change with respect to basal values and 6 mo parameters, and nemaspermic morphology improved slightly in responders. Inhibin B levels increased after six months of therapy both in responders and in non responders. However after twelve months inhibin B values significantly decreased only in non responders (Table 2). Hormone levels were within normal range in all the patients, even if estradiol values significantly decreased at six months with respect to basal values in non responders, but at twelve months its values were raised in non responders and reduced only in responders (Table 2).

A significant increment in free testosterone levels from baseline to the 1 year evaluation occurred (respectively 14.2 ± 2.54 pg/mL versus 17.1 ± 2.58 pg/mL, $P = 0.04$). Furthermore 1-year hormonal pattern was generally better in responders than the corresponding basal levels, even if the values did not reach the statistical significance probably owing to the small number of subjects.

DISCUSSION

Some reports have linked HCV infection to many extrahepatic manifestations, but not all associations have been definitely demonstrated[4]. These data suggest that HCV chronic infection could alter seminal parameters, particularly reduce the percentage of spermatozoa with normal progressive motility and morphology. Patients showed significantly lower levels of free testosterone and inhibin B, a glycoproteic hormone produced by Sertoli cells which is considered a sensible marker of good spermatogenesis[24].

This reduction could be one of the consequences of the altered gametogenesis found in HCV patients.

The combined antiviral therapy could cause a further alteration in spermatic morphology. On the other hand, its antiviral activity could improve spermatogenesis, as demonstrated by the increased inhibin B levels after six and twelve months of therapy in responders. Moreover, after one year of therapy, the responders showed an overall better hormonal pattern than non-responders. Therefore both HCV chronic infection and related antiviral treatment could act on spermatogenesis.

HCV sexual transmission is quite rare and not all authors have identified viral RNA in sperm[20-22]. Previous studies were performed in the context of assisted reproductive techniques, in order to investigate the risk of infection transmission during performing such techniques. The possible interference of HCV with spermatogenesis has not been previously assessed, thus any comparisons with literature are quite difficult.

It has been shown that HCV can stimulate production of reactive oxygen species (ROS) through expression of core protein with resulting *in vitro* and *in vivo* mitochondrial injury, which might explain its hepatic damage, at least in part[25]. Furthermore, oxidative stress could injure genomic integrity in male germinal cells and cause infertility[26]. Thus, oxidative stress induced by HCV-RNA presence in seminal fluid, could directly or indirectly impair spermatogenesis.

These preliminary data show the negative influence of HCV infection on spermatogenesis and the possible beneficial effect of antiviral therapy. A further follow-up of this cohort is therefore necessary. In the present study, some patients did not eliminate HCV after treated for six months, but interferon could act on HCV sufficiently to eliminate adverse seminal effects. This has been demonstrated for HCV-related membranoproliferative glomerulonephritis[11]. In interferon responders, proteinuria decreased from 6.1 g to 1.3 g, while in non-responders a lower decrement was observed.

This is the first report about hormonal and seminal changes in patients with HCV-related chronic hepatitis

during antiviral treatment. Further studies are necessary to confirm these associations and to determine the exact action on seminal and hormonal pattern in order to establish if semen cryoconservation is useful before the therapy in these patients.

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