

Assessment of antisperm antibodies in patients with hepatitis C virus infection : A controlled study

Original Article

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ABSTRACT

Background: Antisperm antibody (ASA) is defined as an immunoglobulin with antibody activity against a sperm antigen that plays a role in fertility. It has been hypothesized that hepatitis C virus (HCV) infection of lymphocytes is the cause for the increased autoimmune phenomena and autoantibody production reported in association with HCV. The development of ASA has been previously reported in cases with human papilloma virus (HPV), where infertile males with HPV in semen showed higher ASA percentages compared with infertile males with no HPV in semen.

Aim: To investigate the influence of HCV infection on the development of ASAs in diagnosed HCV male patients.

Patients and Methods: The study included two groups: group one included 25 patients who were infected with HCV, and group two included 25 healthy controls. Semen analyses by CASA were performed and serum and semen samples were taken for all patients to detect ASA by enzyme-linked immunosorbent assay.

Results: In the patient group, 28% were positive for ASA in semen versus 4% in the control group with a statistically significant difference between the two groups ($P=0.049$). ASA levels in serum were positive in 36% of patients versus 12% in the control group with a statistically significant difference between the two groups ($P=0.047$). There were significant negative correlations between progressive motility, agglutination, and the level of patient's ASA in both serum ($r=-0.635$, $P<0.001$, $r=-0.749$, $P<0.001$, respectively) and semen ($r=-0.764$, $P=0.001$; $r=-1$, $P<0.001$, respectively).

Conclusion: The coincidence of ASA in males with hepatitis C infection diseases was significantly higher than healthy controls.

Key Words: Antisperm antibody, hepatitis C virus, infertility.

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INTRODUCTION

Since not every antibody that binds to the sperm surface influences sperm function, antisperm antibodies (ASA) are defined as immunoglobulins with antibody activity against a sperm antigen that plays a role in fertility^[1].

ASAs are found in the serum and seminal plasma and are bound directly to spermatozoa. ASAs frequently impair sperm motility^[2].

The association between viral infections and the development of ASAs has been previously reported in cases with human papilloma virus (HPV), where infertile male patients with HPV in semen showed higher ASA percentages than infertile males with no HPV in semen^[3].

The hepatitis C virus (HCV) is a viral infection that causes both hepatic and extrahepatic disease. HCV infection is a major and common public health issue. It has been proposed that HCV infection of lymphocytes is

the cause of the increased autoimmune phenomena and autoantibody production associated with HCV^[4]. HCV-RNA was found in the semen of HCV patients, albeit in low concentrations and not in all HCV-positive patients^[5]. It was found that HCV infection was associated with impaired sperm motility^[6].

The same effect was observed in cases with positive ASAs in semen^[7]. On the basis of preceding data, we can conclude that HCV may influence the development of ASAs and impair sperm motility. So we aimed to assess the effect of HCV infection on the development of seminal and serum ASAs and their effect on motility in HCV males.

PATIENTS AND METHODS

This was a case-control, descriptive study. The study was carried out on fertile men attending the hepatitis C center in Tropical Hospital and Andrology Outpatient Clinic Suez Canal University Hospital. The study included

two groups: group 1 (the patient group) included: 25 HCV-positive fertile males, with elevated liver enzymes of more than 6 months and positive PCR test for HCV. Group 2 (control group) included: 25 HCV-negative fertile males. After receiving the Institutional Review Board approval, the study was carried out in accordance with the Helsinki Declaration guidelines (1964). All participants provided written informed consent. Exclusion criteria included: males above 60 years old, patients who had azoospermia or severe oligozoospermia ($<5 \times 10^6$ sperm/ml), and patients who had clinical evidence of varicocele, history of testicular surgery testicular torsion, trauma, epididymo-orchitis and cryptorchidism. Males in the two groups were subjected to detailed history taking and clinical examination. Laboratory investigations for all participants included the following: for semen analysis the semen was collected by masturbation after 4–5 days of sexual abstinence and the collected semen was allowed to liquefy at 37°C. Analyses of samples were performed according to WHO 2010 guidelines^[8]. Seminal plasma IgG ASA and serum IgG ASA were measured by enzyme-linked immunosorbent assay (BIOSERV Kits, Germany). In brief, ASA levels in serum and semen were quantified using a BIOSERV Kits, Germany. The extinction was measured at a wavelength of 450nm with a microplate reader (Bio-Rad Laboratories, Hercules, California, USA).

The sample size was calculated using the following formula^[9]:

$$n = 2 \left[\frac{(Z_{\alpha/2} + Z_{\beta}) * \sigma}{\mu_1 - \mu_2} \right]^2$$

Where n=sample size, $Z_{\alpha/2}=1.96$ (the critical value that divides the central 95% of the Z distribution from the

5% in the tail.), $Z_{\beta}=0.84$ (the critical value that separates the lower 20% of the Z distribution from the upper 80%), σ =the estimate of the standard deviation of SperMar IgG test=13.67%, μ_1 =mean in the study group=8.83%, and μ_2 =mean in the control group=20.33%^[10].

So, the sample size will be 25 cases per group, giving a total sample size of 50 cases.

Statistical analysis

Data were fed into the computer and analyzed using IBM SPSS software package, version 20.0 (IBM Corp., Armonk, New York, USA). Qualitative data were described using number and percent. The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, SD, and median. Significance of the obtained results was judged at the 5% level. χ^2 test was used to study comparison and association between two qualitative variables. Student’s t test was used for comparison between two groups having quantitative variables with normal distribution (for parametric data).

RESULTS

This case–control study included two groups: the first group included 25 HCV-positive fertile males and the second group included 25 HCV-negative fertile males. Each patient underwent semen analysis, seminal IgGASA, and serum IgGASA done by enzyme-linked immunosorbent assay.

The mean age in HCV patients was 36 years and in control it was 34 years. The percentage of smokers in the patient and control groups was comparable (48.0 vs. 44%, respectively). All the participants were married. None of the controls suffered from any chronic illness; meanwhile, all of the 25 patients had HCV infection (Table 1, Fig.1).

Table 1: Demographic characteristics of the study groups

	Patient group	Control group
Age		
Mean \pm SD	36.04 \pm 8.55	34.96 \pm 7.32
Minimum–maximum	30.0–57.0	21.0–49.0
Smoking [n (%)]		
Yes	12(48)	11(44)
No	13(52)	14(56)
Marital status [n (%)]		
Married	25(100)	25(100)
Single	0	0
Chronic illness [n (%)]		
No	0	25(100)
Yes	25(100)	0

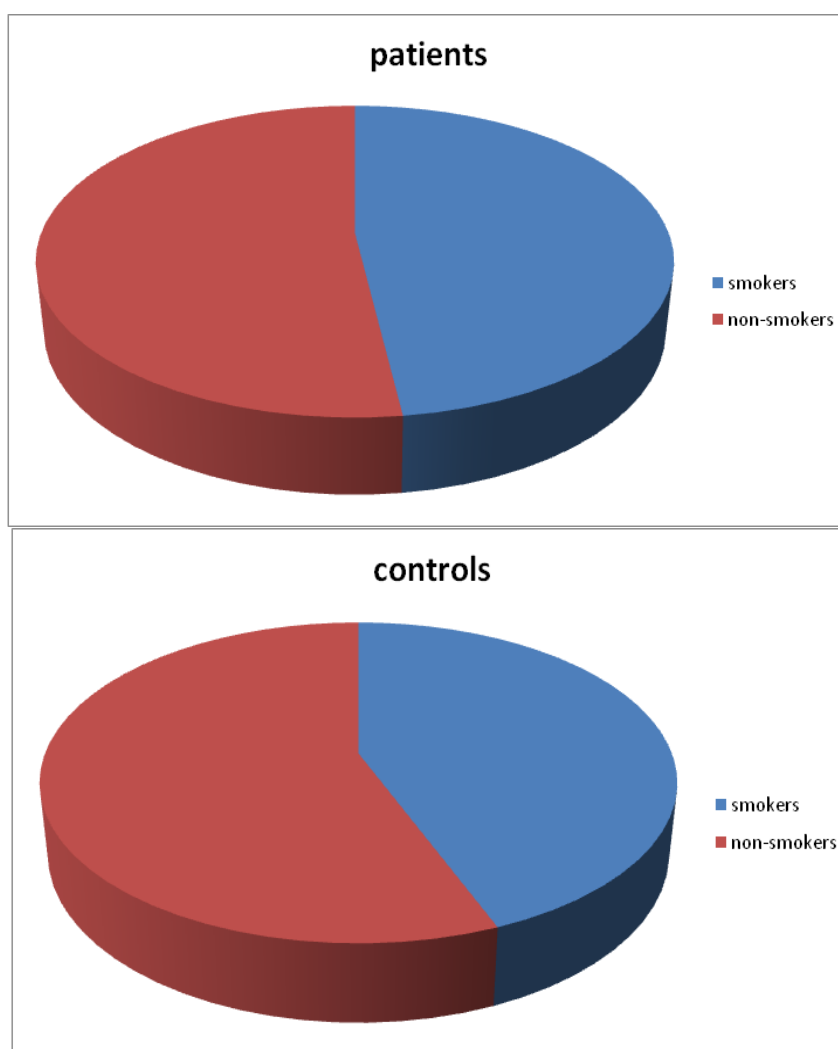


Fig. 1: Smoking in the study groups.

There was no significant difference in the mean of total sperm motility between the two groups ($P>0.05$). Meanwhile, progressive motility showed a statistically significant decline by about 8% in HCV patients compared with controls ($P=0.040$). Pyospermia was absent in the majority of HCV patients and controls, with no statistically difference between the two groups ($P>0.05$). Neither HCV patients nor controls had hemospermia (Table 2). There was a statistically significant increase

in sperm agglutination of patients by about 24% compared with controls ($P=0.049$) (Table 2). Nine (36.0%) patients with HCV had positive serum ASA against only three (12%) controls with a statistically significant difference between the two groups ($P=0.047$). A similar finding was in semen as seven (28%) HCV patients had positive semen ASA versus only one (4.0%) case in the control group with a statistically significance difference between the two groups ($P=0.047$) (Table 3).

Table 2: Semen analysis parameters of the study groups

	HCV (N=25)	Control (N=25)	<i>P</i>
Volume (ml)			
Minimum–maximum	1.50–8.0	1.50–9.0	0.906
Mean \pm SD	3.58 \pm 1.59	3.62 \pm 1.77	
Viscosity[n(%)]			

Normal	23 (92)	24(96)	1.00
Abnormal	2(8)	1(4)	
Concentration ($\times 10^6$)			
Minimum–maximum	10.0–79.0	20.0–180.0	
Mean \pm SD	51.12 \pm 22.01	87.96 \pm 27.91	<0.001*
Total motility			
Minimum–maximum	15.0–90.0	35.0–90.0	
Mean \pm SD	54.80 \pm 23.65	61.20 \pm 14.60	0.256
Progressive motility			
Minimum–maximum	10.0–65.0	28.0–70.0	
Mean \pm SD	39.96 \pm 17.07	48.52 \pm 10.71	0.040*
Abnormal forms			
Minimum–maximum	15.0–70.0	15.0–75.0	
Mean \pm SD	35.80 \pm 15.79	32.48 \pm 16.09	0.384
Pyospermia [n(%)]			
No	21(84)	22(88)	
Yes	4(16)	3(12)	1.00
Hemospermia[n(%)]			
No	25(100)	25(100)	
Yes	0	0	-
Agglutination[n(%)]			
Yes	7(28)	1(4)	
No	18(72)	24(96)	0.049*

HCV, hepatitis C virus.

*Statistically significant at *P* value less than or equal to 0.05

Table 3: Comparison between the two studied groups according to antisperm antibody

	HCV (N=25) [n (%)]	Control (N=25) [n (%)]	<i>P</i>
ASA in serum			
Positive	9 (36.0)	3 (12.0)	
Negative	16 (64.0)	22 (88.0)	0.047*
ASA in semen			
Positive	7 (28.0)	1 (4.0)	
Negative	18 (72.0)	24 (96.0)	0.049*

ASA, antisperm antibody; HCV, hepatitis C virus; *P*: *P* value for comparing between the studied groups.

*Statistically significant at *P* value less than or equal to 0.05.

In the patient group, there was a statistically significant relationship between agglutination and ASA in semen ($P \leq 0.001$) as all the seven cases, which showed agglutination in semen, were positive for ASA in semen as well all the 18 cases, which showed no agglutination, were negative for ASA in semen (Table 4).

There were statistically significant negative correlations between progressive motility, agglutination, and the level of patient's ASA in both serum ($r = -0.635$, $P < 0.001$; $r = -0.749$, $P < 0.001$, respectively) and semen ($r = -0.746$, $P = 0.001$; $r = -1$, $P < 0.001$, respectively) (Table 5).

Table 4: Comparison between agglutination and antisperm antibody in semen in the hepatitis C virus group (N=25)

	ASA in semen [n (%)]		
	+ve (N=7)	-ve (N=18)	
Agglutination			
No	0	18 (100.0)	
Yes	7 (100.0)	0	<0.001*

ASA, antisperm antibody; *P*: *P* value for comparing between the studied groups.

*Statistically significant at *P* value less than or equal to 0.05.

Table 5: Correlation between antisperm antibody in serum, antisperm antibody in semen, and different parameters in hepatitis C virus group (N=25)

	ASA in serum		ASA in semen	
	r_s	<i>P</i>	r_s	<i>P</i>
Progressive motility	-0.635*	0.001*	-0.746*	<0.001*
Agglutination	0.749*	<0.001*	-1.000*	<0.001*

ASA, antisperm antibody; r_s , Spearman coefficient.

*Statistically significant at *P* value less than or equal to 0.05.

DISCUSSION

This study assessed the ASA in serum and semen of men who were infected with HCV. The current study found that patients with HCV had statistically significantly higher semen and serum ASA than controls ($p=0.047$, respectively).

Bourlet *et al.*^[5] demonstrated that in HCV patients, the detection of HCV-RNA in the sperm was reported, albeit at low concentrations and not in all HCV-positive patients. That comes in agreement with Shiraishi *et al.*^[11] as they tested ASA in the sera of 70 males with systemic autoimmune diseases and 80 healthy controls and found that the incidence of ASA was significantly higher in the patient group, as five patients were with positive indirect-immunobead test; meanwhile, no positives existed in 80 healthy males. One of them had chronic hepatitis and possessed all three classes of IgA, IgG, and IgM.

Viral infections, including HCV infection, were thought to be a potential cause of autoimmune diseases. Therefore, Shiraishi *et al.*^[11] hypothesized that the state of chronic HCV infection may have induced ASA as an autoimmune disorder.

Hussein *et al.*^[10] conducted a study on 30 HCV-infected individuals and 30 healthy controls and found that HCV-

infected patients had higher IgG seminal levels than healthy controls. In addition, they detected a significant decrease in sperm motility in HCV patients compared with controls. In our study, though we did not find a significant difference in total sperm motility between the two groups ($P=0.256$), there was a significantly lower progressive motility in the patient group than controls ($P=0.04$).

This can be elucidated by the work of Zini *et al.*^[7], who reported that HCV could contribute to the impaired sperm motility seen in HCV patients through promoting the development of ASAs which interfere with sperm motility. In addition, Levy *et al.*^[12] hypothesized that HCV-RNA in the seminal plasma may interfere with sperm motility through passive adsorption into the cell membrane.

In the current study the presence of significant agglutination in patients group is congruent with the significant presence of ASA in this group ($p<0.001$). As well the negative correlation between progressive motility, agglutination and ASA in patients' both serum & semen supports the aforementioned data about the relationship between ASA and sperm motility.

In the current study, sperm concentration in the patient group was significantly lower than controls ($P<0.001$). This agrees with Durazzo *et al.*^[13], who reported a difference in sperm concentration between controls and HCV

patients, implying that HCV may have a negative impact on spermatogenesis. In another study, Hofer *et al.*^[14] also reported low sperm concentration in most or all of their chronic HCV patients compared with fertile controls.

Moretti *et al.*^[15], on the other hand, conducted a study on 34 HCV-infected patients and found that some patients had normal sperm concentration. This may be attributed to the evaluation of semen samples, according to the WHO 1999 guidelines^[16], whereas our study used the WHO 2010 guidelines^[8].

The small sample size and lack of randomization were significant limitations of this study. We, however, used a control group and an equation for sample size calculation to reinforce the study.

CONCLUSION

The incidence of ASA in males with hepatitis C infection diseases was significantly higher than in the healthy controls. These ASAs are directly related to and are negatively correlated with sperm progressive motility and agglutination.

CONFLICT OF INTEREST

There are no conflicts of interest.

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