Research Article

Impact of Chronic HCV Infection on Male Fertility

Amr M. Abdel Hamid*, Tarek K. FathElbab[*], Ehab M. Galal[*], Hanaa K. FathElbab, Magdy Fouad**, Hosny K. Salem***, Mahmoud R. Mohamed**** and Waleed Mahmoud*****

* Department of Urology, El-Minia Faculty of Medicine,
** Department of Tropical medicine, El-Minia Faculty of Medicine,
*** Department of Urology, Faculty of Medicine, Cairo University,
**** Department of Internal medicine, El-Minia Faculty of Medicine,
***** Department of Clinical pathology, El-Minia Faculty of Medicine,

Abstract

Introduction and Objectives: Hepatitis C virus (HCV) infection is gaining attention as a global health problem especially in Egypt which has the highest prevalence. Treatment with peg interferon ٢ alfa and ribavirin (PEGIFN/RBV) is the standard of care for chronic hepatitis C. There is some evidence that sperm quality may be impaired in HCV patients. Furthermore limited data is available on the effect of PEGIFN/RBV treatment on human sperm quality and male fertility in HCV patients. Aim: To study the impact of chronic HCV infection and standard antiviral treatment on male fertility. **Materials and Methods:** Thirty male patients proved pathologically as chronic hepatitis C complaining of infertility (with mean age ٣٥+٣.٦ years) and ١٥ healthy volunteers were studied. In all subjects semen samples were analyzed, and estimation of hormonal levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), free testosterone, prolactin and dehydroepiandrosteron sulphate (DHEAS) before treatment and after ١٢, ٢٤and ٤٨ weeks of treatment. **Results:** The sperm concentration, motility and morphology were significantly decreased in HCV patients compared to that of controls (P ٠.٠٠١), (P ٠.٠٣) and (P ٠.٠٤) respectively. An impairment of spermic morphology occurred, while other seminal parameters did not change significantly during antiviral treatment. Hormonal pattern of patients did not significantly change after treatment except free testosterone, prolactin and DHEAS in INF responders. Advanced liver fibrosis and high viral load were risk factors for male infertility.
Conclusion: HCV infection has a negative impact on male fertility through impaired reproductive hormones, quantitative and qualitative alterations of spermatogenesis.

Key Words: Hepatitis C, infection**,** male fertility

Introduction

Hepatitis C virus (HCV) is considered one of the major causes of chronic liver disease worldwide. More than ٢٠٠ million peoples are infected with HCV which is a RNA virus belonging to the family Flaviviridae. [١] High prevalence of hepatitis C infection was recorded in Africa and the Middle East, especially in Egypt[٢]. It was reported that viral infections contribute to male infertility by direct toxic effects on the male reproductive cells and/or indirect effect by causing a local inflammatory or immune-logical reaction[٣-٧]. The effect of HCV infection on male fertility is still being debated; hormonal abnormalities were accused in chronic HCV infection however the actual physiological mechanisms respon-sible for these hormonal abnormalities are still poorly understood[٥]. The presence of HCV-RNA in semen particularly in men with very poor seminal parameters and fertility was evaluated in few studies and the results about its presence in seminal fluid are controversial [٦-١٠]. Ribavirin and pegylated interferon is currently recognized as the slandered treatment for chronic HCV resulting in sustained virological response

(SVR) rate ٤٠-٥٠٪ in genotype٤ [١١]. Various adverse effects have been reported due to combination treatment such as semen alterations and sperm DNA fragmentation [١٢, ١٣]. Limited data is available on the effect of PEGIFN/RBV treatment on human sperm quality and male fertility as some reports have linked HCV infection to altered gametogenesis found in HCV patients.

The aim of this study is to evaluate the impact of chronic HCV infection and its standard antiviral medications on male fertility.

Materials and methods

This study was done in the period between January ٢٠١١ and January ٢٠١٣. Included in this study, infertile men with histologically confirmed chronic hepatitis by liver biopsy (according to Metavir score) with history of infertility and who fulfilled the criteria for treatment of HCV in the form of ١٨٠ mg SC peginterferon ٢α per week and daily oral ribavirin ١٠٠٠mg-١٤٠٠ according to body weight.

Excluded from the study; patients with history of any known endocrine abnormalities, patients with other causes of infertility as varicocele or history of cryptorchidism, patients with a history of any genital surgery, epididymo-orchitis, drug abuse, tobacco use, venereal disease or concomitant medical problems known to be associated with diminished fertility and lastly the use of antidepressants (especially with dopaminergic effects) due to their known interference with serum hormone levels.

Accordingly, ٣٠ patients fulfilled the inclusion criteria of our study in addition to ١٥ healthy volunteer (control group).

The end treatment response was assessed by undetectable qualitative PCR at the end of the treatment (RT-PCR Amplicor HCV monitor test version ٢.٠ Roche Diagnostics) while non responder defined by the presence of viremia less than ٢ log reduction at ٣m,٦m or at the end of the treatment (١٢m).

All patients and controls were subjected to preliminary evaluation, including full medical history, physical examination and laboratory investigations, in the form of semen analysis and hormonal profile to measure the baseline resting levels of free testosterone, LH, FSH, prolactin and DHEAS. Two early-morning blood samples at ٢٠-min interval were obtained from each subject to be analyzed using commercial radioimmunoassay. For these parameters, follow up was done at ٣, ٦ and ١٢ m of treatment.

Semen analysis: Semen sample was provided from each subject after ٢-٣ days abstinence period. All semen samples were analyzed by the same operator according to WHO (١٩٩٩) guidelines. When semen values differed by ≤٢٠%, a second test should be done. When there was a difference in semen values by >٢٠٪, a third analysis was performed. Therefore each sample was analyzed at least twice. Sperm concentration, percentage motility, percentage forward progression and the percentage of normal morphology were noted. Sperm morphology was assessed based on Kruger strict criteria. WHO values included a sperm concentration of ≥٢٠ × ١٠٦ spermatozoa /mL, motility of ≥٥٠٪ with forward progression and > ١٤٪ normal forms were considered normal.

Sperm **DNA integrity (DNA Fragmentation Index):** Sperm Chromatin Structure Assay (SCSA) was used to determine sperm DNA integrity. This assay is used to measure the susceptibility of DNA in sperm cells to acid induced denaturation using the metachromatic properties of special dye known as Acridine Orange (AO). After acid application, sperms are labeled with AO that attaches only to the denaturated DNA**.** By using flowcytometry, sperms either appear orange (broken) or green (intact). The metachromatic shift of AO from green to orange is quantified by flowcytometry to determine the extent of DNA fragmentation, giving an index known as DNA fragmentation index (DFI). Normal fertile men have DFI less than ١٥٪. Men with poor fertility

potential have DFI greater than ٣٠٪. Men with DFI Between ١٦٪ and ٢٩٪ have good to fair fertility potential that becomes poorer as it gets nearer to ٢٧٪.[١٤]

The study was approved by the ethics committee of our center and all patients and volunteers provided a written consent before enrollment in the study

Statistical analysis

Data entry and analysis were done with IBM compatible computer using SPSS soft ware version ١٣ (SPSS, Chicago, IL, USA). Results were expressed as mean± standard deviation (SD), median or number %. Comparisons were done using unpaired t-test and Mann-Whitney test. Logistic regression analysis was performed to determine the risk factors. P value of<٠.٠٥ was considered significant.

Results

The mean age of patients was ٣٥±٣.٦ years while that of controls was ٣٣±٢.٩ with no significant difference (P > ٠.٠٥). All semen variables showed significant differences between patients and control groups. Both mean sperm concentration and mean motility were significantly lower in patient group than control group (P<٠.٠٠١ and ٠.٠٣ respectively). Moreover, the normal morphological level was significantly less in group ١ than in group ٢ (P < ٠.٠٥).

Data on sperm DNA integrity were available in ٢٠ patients. At baseline, ٥ of ٢٠ patients (٢٥٪) had a DFI >٣٠٪. The median DFI increased very markedly during treatment (from ٢٠.٥٪ before to ٦٥.٢٪ at ٢٤ weeks of treatment) and remained elevated thereafter.

The mean basal levels for FSH, LH, free testosterone and DHEAS were significantly lower in group ١ than group ٢ (P < ٠.٠٠٢, ٠.٠٠٠١, ٠.٠٠٠١ and ٠.٠٠٠١ respectively), While the mean basal levels of prolactin did not differ significantly between the two groups (*P* > ٠.٠٥) (Table١).

Table (١) Seminal and hormonal parameters in patient and control groups (mean±SD)

	Patient group No=٣٠ Mean +SD	Control group No=١٥ Mean+SD	P
Sperm concentration (million/mL)	٣٣±٤.٦	٣٩.٦±١٨.٨	٠.٠٠١
Normal Motility%	٥٨.١±٥.١	٦٢.٤±٧.٥	٠.٠٣
Normal forms%	٢٨.٩±٨.٢	٣٥.٢±٦.٩	٠.٠٤
DFI%(Median)	٢٠.٥	١٦.٥	٠.٠٣
FSH ٢- ١٨ mU / mL	٣.١±١.٤	٤.٤±١.٢	٠.٠١
LH ١.٥-٧ mU / mL	٢.١±٠.٧	٤.٠٨±١.٣	٠.٠٠٠١
Free testosterone (١٦–٤١ pg/mL)	١٩.٢±٧.٣	٣٠.٣±٥.٣	٠.٠٠٠١
Prolactin (٧–١٨ ng/mL)	١٠.٧±٣.٢	٩.٤±٢.٥	٠.٥
DHEA(١٢٠-٥٢٠µg\L)	١٢٠±٥٦	٤٨٥±٢٠١	٠.٠٠٠١

DFI: DNA fragmentation index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, DHEAS: dehydroepiandrosteron sulphate.

Out of thirty patients, twenty were considered responders and completed the study up to one year, while ١٠ men were considered non responders after ٣ and ٦ months of treatment. There were no significant differences between results obtained after ٣m and ٦m of treatment and that recorded before treatment in all semen parameters except spermatic morphology and DFI which increased significantly from ٢٠.٥% to ٦٥.٢٪ (P١< ٠.٠٠١& P٢<٠.٠٠١ respectively). While from the studied hormonal parameters, free testosterone and DHEAS showed statically significant difference (Table ٢).

Table (٢): Follow up of the seminal and hormonal parameters of patients group

	٠ Week No=٣٠	١٢week NO=٢٤	P١	٢٤ week N٠=٢٠	P٢
Sperm concentration	٢٥.٦±٤.٦	٢٤.٥±٣.٦	٠.٨	٢٥.١± ٢.٦	٠.٧
Motility	٢٦.٢±+٥.١	٢٣.١ ±٢.٩	٠.١١	٢٤.٥ ±٣.٩	٠.٦
Normal forms	٢٨.٩±٨.٢	٢٠.٣ ±٢.٣	٠.٠٣	٢١.٣± ٣.٦	٠.٠٤
DFI%(median)	٢٠.٥٪	٤٠.٥٪	٠.٠٠١	٦٥.٢٪	٠.٠٠١
No=٢٠					
FSH	٣.١±١.٤	٣.٥±١.٣	٠.٦	٣.٧±١.٣	٠.١٨
LH	٢.١±٠.٧	٢.٤±١.٤	٠.٥	٣.٢ ±٢.٣	٠.٤٤
Free testosterone	١٩.٢±٧.٣	٢٢.١± ٨.٣	٠.٠٤	٢٢.٣± ٢.٨	٠.٠٤
Prolactin	١٠.٧±٣.٢	٩.٧ ± ٣.١	٠.١١	٨.٥± ٢.٨	٠.١١
DHEAS	١٢٠±٥٦	٢٠٠±٨٧	٠.٠٤	٣٥٠±١٢٠	٠.٠٢

DFI: DNA fragmentation index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, DHEAS: dehydroepiandrosteron sulphate.

The responders showed an overall better hormonal pattern than non-responders while DFI was still higher up to ١٢m of antiviral treatment (Table ٣).

When logistic regression analysis was done, it showed that high viral load and advanced liver fibrosis were associated with male infertility (Table ٤).

Table (٣): Seminal and hormonal parameters of responder and non-responder patients

	Non responder N٠=١٠	Responder N٠=٢٠	P value
Sperm concentration	٢٣.٥± ٣.٥	٢٦.١± ٤.٩	٠.٤
Motility%	٢٧.١± ٣.٩	٢٨.٣± ٣.٩	٠.١١
Normal forms %	٢٠.٢± ٥.٥	٢١.٣± ٦.٤	٠.٥
DFI % (median)	٦٥٪	٦٠٪	٠.١
No=٢٠	No=٦	No=١٤	
FSH	٥.١± ١.٥	٤.٧ ±١.٩	٠.٤
LH	٣.٥± ١.٦	٣.١± ٢.١	٠.٣
Free testosterone	١٩.١ ±٧.٥	٢٤.٢± ٣.٥	٠.٠٤
prolactin	٩.٥ ±٣.٥	٧.٢ ±٢.٨	٠.٠٣
DHEAS	١٥٠±٥٩	٤٥٠±٢٠١	٠.٠١

DFI: DNA fragmentation index, FSH: follicle-stimulating hormone, LH: luteinizing hormone, DHEAS: dehydroepiandrosteron sulphate.

Table (٤): Logistic regression analysis of the factors associated with male infertility

	OR	٩٥٪CI	P
Age	١.٦٥	٠.٨٤—٣.٢٣	٠.٢٤
ALT	١.٧٦	٠.٩—٣.٣٥	٠.٢٧
Viral load	٣.٤	١.٢—٥.٤	٠.٠٠٢
Liver fibrosis	٣.٦	١.٤— ٥.٦	٠.٠٠١
Response to treatment	١.١٤	٠. ٦— ٢.٩	٠.٧٣

OD: Odds Ratio, CI: confidence interval.

Discussion

Male factor infertility is the singular cause of infertility in nearly ٢٠٪ of infertile couples, while both male and female factors are responsible for ٣٠% to ٤٠% of infertility[٣]. Thus, male factor infertility is found in approximately ٥٠٪ of all infertile couples.

Patients with chronic hepatitis C had impaired reproductive capacity by two mechanisms; a hepatopituitary testicular (HPT) axis dysfunction, and elevated proportions of sperm with extra missing chromosomes or sperm DNA damage [٥].

HCV infection has been linked to many extra-hepatic manifestations, but not all associations have been definitely demonstrated. One of these associations is male germinal cells dysfunction[١٢].

At present, few reports are available on the correlation between altered semen parameters and HCV chronic infection. Our study showed that chronic HCV infection could affect the semen parameters (sperm concentration, motility, and morphology), sperm DFI in addition to the reproductive hormonal levels. Similar results were reported by Durazzo et al.,[٤] and Lorusso et al.,[١٣] who found that chronic infections like HCV infection had significantly impaired sperm quality (specially the progressive motility and morphology) compared with that of controls as a consequence of altered gametogenesis.

On the contrary, Garrido et al., observed comparable seminal parameters between HCV-infected and control men[١٥].

It has been suggested that the core protein of HCV may stimulate the production of reactive oxygen species (ROS) causing mitochondrial damage and/or injury to the genomic integrity in male germinal cells, thus altering spermatogenesis. Also ROS damage the sperm membrane which in turn reduces the sperm's motility and ability to fuse with the oocyte and this was documented by Vignera et al.,[١٦] who found that HCV patients had significantly higher basal and stimulated ROS levels in semen compared to control subjects. Also they showed that sperm DNA damage was suspected in chronic HCV patients. As sperm DNA integrity plays an important role in sperm function and fertilizing capacity, chronic HCV patients could have a poor reproductive outcome and possibly birth defects[١٦].

This suggestion will explain our results regarding increased sperm DFI in patients group than control group.

As regard the hormonal levels, the present study showed a significant statistical difference between the patients and control group except for prolactin and this in agreement with Safarinjad et al.,[٥] and Hofny et al.,[١٧]

The actual physiological mechanisms responsible for these hormonal abnormalities are still poorly understood. It seems that patients with chronic hepatitis C had impaired reproductive capacity for fertility and this can be the result of a HPT axis dysfunction. Low serum FSH, LH and testosterone values indicate hypothalamo-pituitary disease, i.e. hypogonadotrophic hypogonadism[١٧].

During antiviral treatment and follow up of our patients, seminal parameters showed altered spermatic morphology and impairment in sperm DFI, in agreement with Donna et al.,[١٨] who reported semen abnormalities in chronic HCV patients, with further impairment during antiviral therapy.[١٨].

While we noticed early rise in serum free testosterone level, moreover, hormonal pattern after one year was generally better in responders than non responders and this in accordance with Durazo et al., [٤].

Although Azab et al.,[١٩] suggested that after combined treatment with patients achieved HCV negative PCR, they noticed no improvements in semen parameters or hormonal levels. This controversy may be explained by their small sample size (١٢ patients).

On searching for hepatic risk parameters for male infertility, we found that high viral load and advanced liver fibrosis were important two risk factors according to multivariate analysis and this in accordance with Guechet et al.,[٢٠] who stated that screening for both fertility impairment and androgen deficiency associated with hepatitis C infection, should target men with a proofed higher degree of fibrosis. In men with advanced hepatic disease, the alteration of serum sex hormones level are reversible and might be attributed to the liver disease. Guéchot et al.,[٢٠] reported that, ٦ months after successful hepatic transplantation, serum testosterone, dihydrotestosterone, LH and FSH levels increased while serum prolactin, oestrogen, androstenedione and sex hormones binding globulin (SHBG) levels decreased. However, serum-free testosterone and LH concentrations showed no change during interferon therapy[٢٠].

The combined therapy for HCV could cause more impairment in spermatic morphology. On the contrary, its antiviral property could improve the process of spermatogenesis as a consequence of increased free testosterone levels in responders after twelve months of therapy. Furthermore, at the end of treatment, the responders experienced an overall better hormonal profile than non-responders. Hence both HCV infection and its antiviral therapy could affect spermatogenesis.

Conclusion

HCV infection has a negative impact on fertility potential of men through impairment of reproductive hormones, alterations of spermatogenesis and DNA abnormalities. Both high viremia and advanced liver fibrosis are risk factors for male infertility. Successful treatment with antiviral therapy has possible beneficial effect and will improve the hormonal pattern.

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