Post interferon therapy decreases male fertility through gonadotoxic effect

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Abstract: Prevalence of hepatitis C virus (HCV) has been seen in more than 15% of Pakistani population. For the treatment of this infection, only two medicines, interferon, and ribavirin were approved in 1998. The concerned physicians evaluate side effects of these two antiviral drugs only during the treatment period. The long-term extra hepatic side effects are being neglected. This retrospective study was conducted with reference to induced infertility in HCV treated 40 male patients from the period 2008-2015. Possible effects of interferon therapy on fertility hormones and seminal parameters were assessed. Level of fertility hormones like serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and testosterone was measured. For seminal parameters, guidelines from World Health Organization (WHO) were followed. Among forty cases of HCV patients who received interferon, only 14 (35%) have children and 26 (65%) could not conceive (p = 0.0372). After HCV treatment, HCV positive patients showed a significant change in the level of FSH, LH (p<0.05). Especially, it decreased testosterone level (p=0.0096). Similarly, HCV treatment significantly decreased sperm count (p = 0.001) and motility (p = 0.0005).

Keywords: Gonadotoxicity, interferon, fertility hormones, seminal parameters, male fertility.

INTRODUCTION

Hepatitis viruses are of great concern in the modern scientific era. As a causative agent in hepatitis these are a major economic burden, illness, and death. In millions of people worldwide, this hepatitis leads to chronic liver disease and hepatocellular carcinoma (Lavanchy, 2009).

Global surveys show that among all hepatitis viruses the most virulent is hepatitis C virus. The ratio of Hepatitis C Virus reached up to 2.8% in 2005, which was 2.5% in 1990. The number of persons having anti-HCV was 122 million in 1990 with an increase to 185 million in 2005 (Mohd Hanafiah *et al*., 2013). In the patients infected with HCV, about 85% will develop chronic hepatitis C infection and are at great risk to progress into cirrhosis, hepatocellular carcinoma as well as extrahepatic manifestations (Chen *et al*., 2006).

The disease has become a major health problem in developing countries, including Pakistan, where HCV infection ranges from 4-8%. Healthcare workers are especially at great risk due to exposure to HCV. Potential exposure body areas are eyes, mucous membranes and needle stick injuries. In majority of Pakistani hospitals patients are being operated without prior hepatitis viral screening due to which hepatitis C carrier among medical staff is increasing. It was found 30% in one of the hospital in Abbottabad. Studies on small targeted groups, which include blood donating persons, doctors, paramedical staff, drug addicts and chronic liver disease patients indicate that the prevalence of hepatitis C is as high as 40% (Jiwani, 2011).

Until a few years ago, there were only two drugs (interferon and ribavirin) approved by the Food and Drug Administration (FDA) for hepatitis C treatment (Manns *et al*., 2001). Interferon is similar to a protein in our body and fights against infections. A modified form Pegylated-interferon which is a long-acting form is also available, and is administered in injectable form. Initially used alone, but now being used with ribavirin (Manns *et al*., 2001). Interferon has many significant side effects, and people with HCV were unable to take it or stopped therapy due to these side effects. In United States, recently approved stronger oral medications have largely replaced interferon. Interferon is recommended for hepatitis genotypes 1, 2 and 4; it is occasionally used against genotype 3. Interferon use is expected to decrease in other countries as all-oral treatment options become available (Poynard *et al*., 2003). Combined administration of pegylated α-interferon (PEG-IFN) plus ribavirin is the treatment of choice for HCV infection with 45% to 80% of virus eradication (Manns *et al*., 2001).

We hypothesize that interferon has gonadotoxic effects and is a cause of infertility. In this article, we analyzed the gonadotoxic effects of interferon therapy in hepatitis C

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virus-infected patients as measured by fertility hormones. We observed infertility in HCV positive patients who have received interferon therapy, by semen analysis.

MATERIALS AND METHODS

The research work was conducted at Department of Biochemistry, Government College University, Faisalabad and at Social Security Hospital Faisalabad, Pakistan. Gonadotoxic effects of interferon therapy were tested on 40 males (20-50 years old) suffering from hepatitis C virus (HCV). The patient group got treatment during the period 2008-2015. A control group of 40 normal married male individuals between the age group 20-50 year was also selected. Forty cases of HCV infected only male patients (prior to treatment) were also included. Social Security Hospital Faisalabad is a 300-bedded hospital providing free medical treatment to industrial workers of Faisalabad region. This hospital provides free HCV treatment as well. Following patients were selected from the record as mentioned below:

2008 Eleven
2009 One
2010 Six
2012 Nine
2013 Five
2014 Seven
2015 One
Total Forty Patients

Selection criteria
Male patients between the age group 20-50 years, having one or more children prior to the start of HCV antiviral therapy. The patients of HCV who were treated with antiviral therapy but having health problems were excluded from the study, azoospermia, varicocele, cryptorchidism, liver cirrhosis, diabetes, pancytopenia and renal failure were also excluded from the study (Azab *et al*., 2011).

Sample collection
Patients were invited at Social Security Hospital Faisalabad. Following procedure was adapted for sample collection:

I. Counseling
II. Written consent
III. History, which includes HCV infection, previous investigations if any available, start of therapy, a brand of interferon, duration of therapy (which was six months in all cases), children before and after therapy.
IV. Clinical examination to fulfill inclusion and exclusion criteria.

Sample collection was divided into two parts; Blood sample collection and semen sample collection.

Measurement of fertility hormones
Fertility hormones include Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, and Testosterone. Quantification of first three hormones was performed by sandwich immunoassay (De Gier *et al*., 2006). The level of serum testosterone was measured by fluorescence enzyme immunoassay technique (Ijiri *et al*., 2003).

Sample collection and delivery of semen sample
The samples were taken after a minimum of 48 hours and maximum 6 days of sexual inactivity. The name of the person, date and time of collection were recorded. The time interval between sample collection and test was 30 – 40 minutes. The samples were collected in the privacy of a room near laboratory into a clean, wide-mouthed plastic container.

During the primary microscopic investigation of the semen sample, estimated motility and count of spermatozoa was performed on a simple glass slide with coverslip. The presence of cells other than spermatozoa and of agglutination of spermatozoa was determined.

Total sperm count
The concentration of spermatozoa was determined by using the hemocytometer method. One liter of diluent was prepared for sperm count by using the following formula:

i) Sodium Carbonate 50 g
ii) 35 % formalin 10 ml
iii) Methylene blue 0.25 g
iv) Distilled Water 990 ml

A 1:20 dilution from each well-mixed semen sample was prepared by diluting 0.05 ml of liquefied semen with 0.95 ml of diluent. 10 *ul* of this diluted specimen was delivered on both chambers of the hematocytometer and after an interval of five minutes for the settlement of cells and diluents counting was performed. Binocular microscope "Zeiss" Germany was used for counting.

Active sperm motility
10 *ul* of semen volume was delivered onto a clean glass slide and covered with a 22 x 22 mm coverslip. This preparation was then examined under microscope at 400Xand room temperature was 25^{o}C. The microscopic field was scanned systematically. Motility of each spermatozoon encountered was graded a, b, c or d according to whether it shows:

(a) Rapid progressive motility.
(b) Slow or sluggish progressive motility
(c) Non-progressive motility.
(d) Immobility.

STATISTICAL ANALYSIS

GraphPad Prism 7.02 was used for all statistical analysis (Ali *et al*., 2016). In case of three groups, one way ANOVA was applied to datasets. For two data groups, unpaired t-test was used to calculate *p*-values. The outcomes were expressed as mean ± standard deviation. The differences between groups were tested by the student t-test, *p*-value was considered significant if it is <0.05 and non-significant if it is > 0.05.

RESULTS

Follicle stimulating hormone (FSH)

For the assessment of Follicle Stimulating Hormone (FSH) levels, three groups of people were selected. There were three groups, one with a healthy volunteer, second with HCV infected patients and the third group consisting of interferon-treated patients. The patients were treated for HCV from 2008-15.

'Healthy' individuals were not suffering from any illness. 'HCV infected' groups were carrier of HCV virus and have not started treatment. While 'treated' group was HCV positive and had received interferon therapy.

According to the statistical analysis, FSH level of healthy individual was same as HCV infected patients but significantly higher as compared to the patients who were treated with interferon. The mean for treated persons are 5.44 and for normal persons 7.98 (p = 0.0052). Both groups fall in the normal value of FSH as 2-11 *mmol/l* as shown in fig.1. Therefore, from our results, we can conclude that interferon therapy significantly decreases FSH level in the HCV infected patients who received interferon therapy.

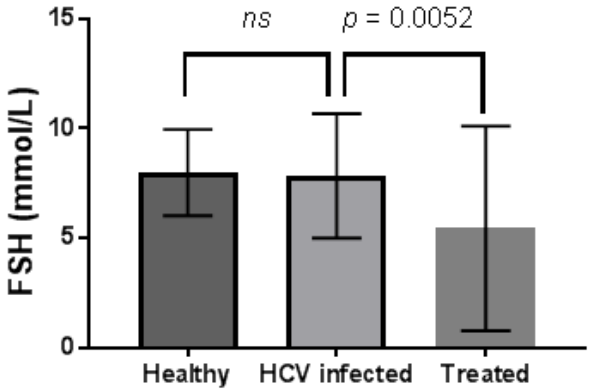

Fig. 1: Bar graph showing FSH level in healthy, HCV infected, and interferon treated individuals.

Luteinizing hormone (LH)

For the assessment of Luteinizing Hormone (LH) levels, three groups of people were selected. One group has 40 persons who were treated for HCV from 2008-15. Other group consists of volunteers with a healthy life. The third group consisted of untreated patients infected with HCV virus.

According to statistical analysis, the *p*-value for infected and treated patients appeared to be significant (p= 0.0055). While for healthy (9.59 ± 6.4) and HCV infected (prior to treatment) (10.46 ±7.3) individuals do not show a significant difference in LH levels. Both groups with HCV infected, fall in the normal value of LH as *3-25 mmol/l* (fig. 2). So from our results, we can conclude that there is some effect on LH levels because of HCV treatment. 'Healthy' individuals were not suffering from any illness. While 'treated' group was HCV positive and has received interferon therapy.

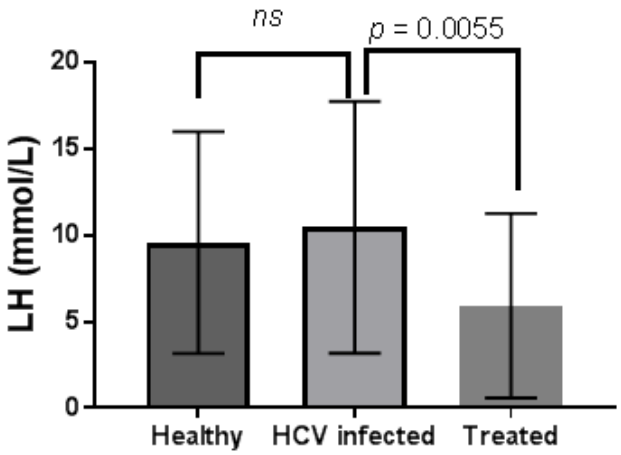

Fig. 2: Bar graph showing LH level in healthy, HCV infected, and interferon treated individuals

Testosterone

For the assessment of Testosterone Hormone levels, three groups of people were selected for this analysis. One group has 40 persons who were treated for HCV from 2008-15. Other group consists of normal persons with a healthy life. The third group consisted of untreated patients infected with HCV virus.

According to statistical analysis, the *p*-value (0.0096) is less than the level of confident 0.05 so the means of both groups are significantly different from each other. The mean for treated persons are 754.46 and for normal persons 1002.62. Both groups fall in the normal value of testosterone as 250 – 1100 *ng/dl* (fig. 3). As seen from our results, we conclude that there are effects of HCV treatment on testosterone of treated patients.

Sperm count

For the assessment of sperm counts, three groups of people were selected. One group has 40 persons who were treated for HCV from 2008-15. Other group consists of normal persons with a healthy life. The third group consisted of untreated patients infected with HCV virus.

Then the results of sperm count test were recorded as follow:

The statistical analysis shows that the means of all three groups were significantly different from each other. According to statistical analysis, the *p*-value (<0.001) is less than the level of confident 0.05 so the means of both groups are significantly different from each other. The mean for treated persons is 54.16 and for normal persons 75.15. Although both groups fall within the normal value of sperm count, 40 *million/ml* – 300 *million/ml* (fig. 4). But from our results, we can conclude that there are effects of HCV treatment on Sperm Count of patients as shown by *p*-value.

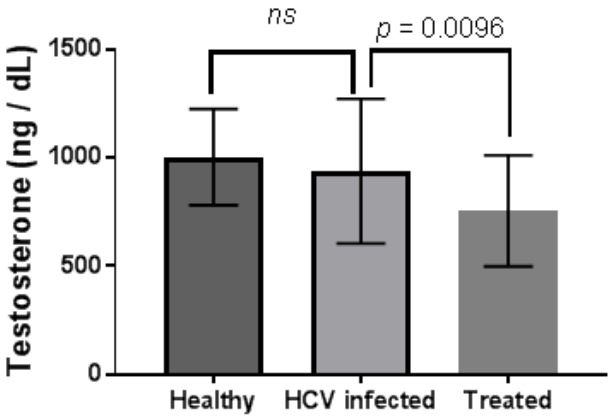

Fig. 3: Bar graph showing testosterone level in healthy, HCV infected, and interferon treated individuals

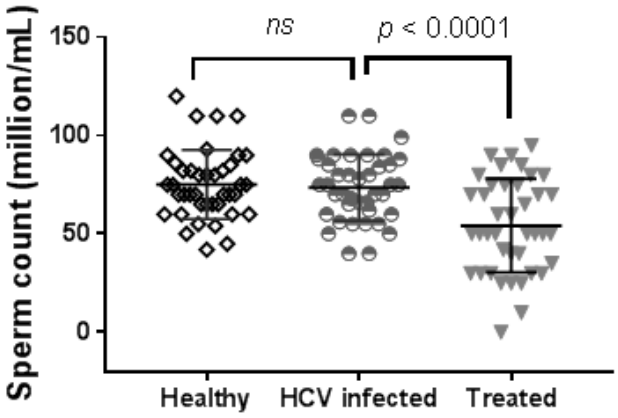

Fig. 4: Graph showing sperm count in healthy and interferon treated individuals

Sperm morphology

For the assessment sperm morphology, three groups of people were selected. One group has 40 persons who were treated for HCV from 2008-15. Other group consists of normal persons with a healthy life. The third group consisted of untreated patients infected with HCV virus as shown in fig. 5.

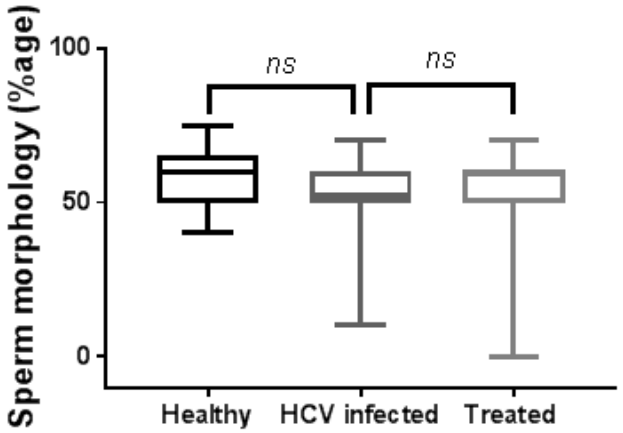

Fig. 5: Graph showing sperm morphology in healthy and interferon treated individuals

Sperm motility

For the assessment of sperm motility, three groups of people were selected.

According to statistical analysis, the *p*-value (0.0005) is less than the level of confident 0.05 so the means of three groups are significantly different from each other. The mean for treated persons are 41.75 and for normal persons 54.50 (fig. 6). So from our results, we can conclude that there is a significant effect of HCV treatment on sperm motility of patients.

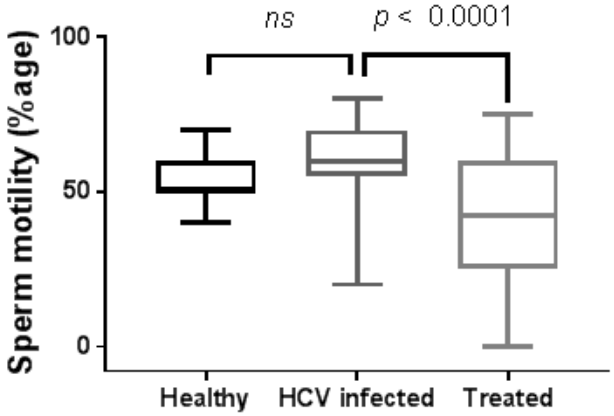

Fig. 6: Graph showing sperm motility in healthy and interferon treated individuals. The statistical analysis shows that the means of both groups were significantly different from each other.

Number of children

No of children were counted for treated patients in time span before and after treatment of HCV.

According to statistical analysis, the *p*-value (<0.0001) is less than the level of confident 0.05 so the means of both groups are significantly different from each other. There is a huge difference on the number of children, after

treatment the number decreases. Only 37.5% patients with interferon therapy have children after treatment. These children were born after at least 3 to 5 year of treatment. This shows adverse effects of HCV treatment on patients.

DISCUSSION

Hepatitis C virus chronic infection is one of the leading causes of morbidity and mortality. Great emphasis has been underway for the treatment of this life threatening disease. There has been a lot of improvement in HCV therapy over the last two decades. Sustained virological response (SVR) which was 30% initially has been achieved upto 50% now (Shiffman *et al*., 2002). Inspite of the fact that modified forms of interferon has shown good response rate than previous versions, side effects of the drug remained same. All approved interferon types showed similar side effects except frequency of adverse reactions, which vary with the brand (Fried, 2002). Among extra hepatic side effects our study focused on fertility problems in male after HCV therapy. Chronic HCV treatment could affect the semen contents in the form of spermatozoa count, sperm motility, and its survival in the fluid medium. The fertility hormones were not much affected.

Our results are in agreement with (Lorusso *et al*., 2010). They discover that the chronic hepatitis C infection significantly impaired sperm quality compared with that of controls. Other researchers found the treatment of HCV with antiviral drugs cause worsening of the semen parameters and advised the use of contraceptive methods during the period of treatment. They noted a significant difference in spermatozoa morphology, count, and mobility as our study indicates (Hofer *et al*., 2010).

Some other articles also found worsening influence of HCV infection on spermatogenesis, which augments our results and after treatment noted, no improvement in the sperm morphology and level of testosterone in the blood (Durazzo *et al*., 2006).

Hormonal patterns after one year were generally better in treated patients than freshly treated patients and this is in accordance with (Durazzo *et al*., 2006).

The main effect of all parameters is shown in the number of children difference before and after the treatment. The study shows that one year after treatment sperms were not strong enough to fertilize the egg. All the patients who have children after treatment took three years to revive their fertility as have shown by the study (Durazzo *et al*., 2006).

CONCLUSION

The study concluded HCV infection and their line of treatment has a bad effect on the male fertility and semen parameters, and some reproductive hormones are also affected. Further studies with large numbers of patients are necessary for more confirmation and to determine the exact action on the seminal and hormonal pattern. This will help in establishing the proper way to deal with infertile male patients infected with HCV infection. Physicians must monitor these long-term side effects of interferon.

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