Prevalence of Hepatitis virus C and Hepatitis virus B in patients who had ICSI at Azhar ART unit and its impact on the outcome?

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Abstract: Objective: To detect the prevalence of HBV and HCV in patients who had ICSI at Azhar ART unite and its impact on the outcome of the first ICSI-embryo transfer cycle. Study design: A retrospective cohort study.Setting: Azhar ART unites. **Material and methods:** 3764 patients had the first ICSI cycle to estimate the prevalence of HBV and HCV a among such patients, then we have chosen HCV seropositive patients (154 patients) and HBV seropositive patients (26 patients), in addition to 200 seronegative patients for HCV and HBV having the ICSI, embryo transfer cycle during the same time period used as control. Statistical analysis of data were done regarding, prevalence of HCV seropositive patients, HBV seropositive patient characteristics, pattern of ovarian stimulation and clinical pregnancy rate. Outcome measures: prevalence of HBV&HCV, duration of gonadotropin treatment, total dose of gonadotropin stimulation, number of growing follicles, oocytes retrieved, fertilization rate, number of grade 1 embryos, the number of embryos transferred, pregnancy rate (clinical pregnancy rate). **The results:** Among the 3764 patients, having the first ICSI cycle, the prevalence of HCV was 4.10 % (154 patients) while the prevalence of HBV was 0.7 % (26 patients). Regarding the duration of HMG stimulation, there were shorter duration of stimulation in an HCV seropositive group (11.32±2.06), and HBV seropositive group (10.7±3.91) if compared with control group (12.16±1.99). The highest clinical pregnancy rate was obtained in the control group 34 % versus 25% for HBV seropositive group and 26 % for HCV seropositive groups, but the differences were not statistically significant. **Conclusion:** The prevalence of HCV, HBV in our study much lower if compared with a national prevalence rate. Also we concluded that HCV, HBV did not affect the ICSI outcome, which support the worldwide trends of providing assisted reproductive surfaces for patients infected with HCV and HBV.

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Key Words: HCV, HBV, Prevalence, ICSI outcome.

1. Introduction

The world health organization has declared hepatitis C a global health problem, with approximately 3% of the world population (roughly 170- 200 million people) infected with HCV. Egypt contains the highest prevalence of hepatitis C in the world. The national prevalence of HCV antibody positivity has been estimated to be between 10- 13 % (1). Worldwide hepatitis B is most prevalent viral liver infection; more than 300 million People are carriers of the HBV. The prevalence of HBV in Egypt is 6.7 % among healthy populations (1, 2). Three quarters of individuals infected with HCV, HBV are in their reproductive years (3). So there is increasing demand for ART services in patients infected with HCV and HBV. In the ART , the infected patients with HCV or HBV arises concerns regarding the safety of ICSI procedures for patients and working staff, in addition to possible transmission to offspring, as well as impact of viral infection on the ICSI outcomes. The published articles in the literature regarding the impact of HCV and HBV on the ICSI outcome are scarce and conflicting, some researcher found significantly decreased in pregnancy outcomes in couples seropositive for HCV and HBV (4)while other investigators found significantly increased in pregnancy rate in ICSI patient infected with a virus (5).However the general trend in most of the world countries supports the view that these patients should be given the opportunity to have children (4, 5). The aim of this study is to detect the prevalence of HBV and HCV in patients who had ICSI at Azhar ART unite and its impact on the ICSI outcome.

2.Patients and methods

This is a retrospective cohort study on the first 3764 ICSI cycles performed at ART unite – Azhar university, in the period from January 2007 to June 2011 to estimate the prevalence of HBV seropositive and HCV seropositive among such patients, then we have chosen the HCV seropositive patients (number 150 patients) and HBV seropositive patients (number 26 patients) in addition to 200

patients seronegative for HCV and HBV having ICSI at the same time used as control. Screening for HBV & HCV is performed as part of the routine work-up of all patients undergoing ICSI at ART unit-Azhar University. Screening was done using a third generation test (enzyme linked immunosorbant assay). 4 patients with HCV seropositive and 2 patients with HBV seropositive were excluded from the program and sent for tropical consultation because they had abnormal liver enzyme levels. The husband had not received interferon treatment for at least 6 months before ovarian stimulation because it does exert a cumulative effect on the human sperms which could be extended for 6 months after initiation of therapy. Ovarian stimulation was performed by GnRH agonist long protocol, the patients received 0.1 mg/day subcutaneous triptorelin acetate or a single long acting Leuprolide acetate IM injection starting on the day 21 of the preceding cycle, after the complete down regulation which indicated by the E2 level less than 50 pg/ml, the controlled ovarian hyper stimulation affected with HMG. The daily HMG dose ranged between150 and 300 depending on the age of women and body mass index. The Ultrasound monitoring started on 6th day of stimulation and dose adjustment was done according the follicular development and serum E2 level. HCG 10000 IU was administrated when the leading follicles reached 20mm in diameter accompanied by ≥ 2 follicles more than 16 mm in diameter. Oocytes retrieval was undertaken 36 hrs after administration of hCG. Fertilization was achieved with ICSI in all couples. ET was performed on day 2 or3. Amaximum of three grade 1 embryos was transferred under ultrasound gaudiness using labotect catheter. Luteal support was achieved using 100 mg/day IM progesterone or progesterone suppositories 200 mg/12 hours which continued for 8 weeks if the pregnancy test was positive, Chemical pregnancy was diagnosed by serum B-HCG estimation 14 days after oocyte retrieval Clinical pregnancy was confirmed by the detection of a gestational sac by transvaginal Ultrasound examination 2 weeks after positive pregnancy test. Ongoing pregnancy was detected by demonstration of fetal heart rate by ultrasound examination.

2.1.Outcome measures

This included: prevalence of HCV & HBV, duration of gonadotropin treatment, total dose of gonadotropin stimulation, number of growing follicles, oocytes retrieved, fertilization rate, number of grade 1 embryos, the number of embryos transferred, pregnancy rate (clinical pregnancy rate)

2.2.Statistical methods

Data were statistically described in terms of range, mean± standard deviation, median, number of cases and percentage when appropriate. Comparison of numerical variables between the study groups was done using a Kruskal wallets test with pathos multiple 2 group comparisons. For comparing categorical data, chi square test was performed. Exact test was used instead when the expected frequency is less than 5. A P value less than 0.05 was considered statistically significant. All statistical calculation was done using computer program SPSS (Statistical package for social science; SPSS inc., Chicago, IL USA) version 15 for Microsoft windows

3. Results

The result shown that, the prevalence of HCV was 4.10 %, while the prevalence of HBV was 0.7 % as shown in figure1. Our patients had normal liver enzymes, the liver function tests were Normal with no significance difference among the studied groups as shown in table 1.

The number of HMG ampoules for HCV seropositive group was (33.80 ± 11.31) and for the HBV seropositive group was (33.93±13.14) and for the control group was (36.86 ± 9.88), the differences were not statistically significant.

The duration of HMG stimulation for HCV seropositive group was (11.32±2.06), and for HBV seropositive was (10.7±3.91) and for the control group was (12.16±1.99). There was significantly shorter duration of stimulation in an HCV seropositive group and the HBV seropositive group if compared with the control group. HCV were significantly shorter if compared with the control group.

The number of mature follicles in HCV seropositive patients was (13.38±6.95) and in HBV seropositive patients was 11.89± 6.89 and in the control group was 11.64± 64, but the differences were not statistically significant

The number of oocyte retrieved in HCV seropositive was 8.64±5.05 and in HBV seropositive patients was 6.56±3.39 and in control group was 8.02±4.21., but the differences was not statistically significant

The number of fertilized oocytes was 3.64± 2.74 in HCV seropositive group and 3.8±3.3 and 4.68±2.42 in the control group .The differences in the number of fertilized oocytes among the different group were not statistically significant

The number of grade 1 embryos was 2.8±1.81 in HCV group, and HBV group was 3.24±2.12and 3.04±1.41 in the control group, the differences was not statistically significant in all groups

The tab 3 presented the clinical and demographic data among the different groups, the age of women, the duration of infertility, the cause of infertility were comparable between the different groups. There were no significant difference among the different groups regarding the maternal age, day 2-3 FSH levels. Significant difference regarding the duration of infertility with 8.52 ±5. 17, 9.259± 5.59 and 5.116 ±3. 08 for HCV, HBV and control groups respectively

Also we found no significant difference between the 3 groups regarding the clinical pregnancy rate, the heights pregnancy rate was in the control group which was 34 %, 25 %for HBV group, 26 % of HCV group.

Table 1: Liver functions among the studied groups.

Liver functions	HCV	HBV	Control	*P* value
Bilirubin (mg/dl)	0.598±0.119	0.0560±0.152	0.546±0.108	1.6
Total protein(gm/dl)	7.39±0.40	7.5±0.36	7.40±0.34	1.07
Albumin(gm/dl)	4.14±0.28	4.46±0.34	4.37±0.28	0.067
ALT(U/L)	25.59±7.63	21±19	23.66±5.44	0.057
AST (U/L)	25.26±6.13	24.69±6.6	24.34±4.93	0.056

The *P* value is significant if less than 0.05

Table 2: The outcome measures among different groups.

Outcome	HCV	HBV	Control	*P* value
HMG dose(ampoules)	33.80 ± 11.31	33.93± 13.41	36.86± 9.88	0.169
Duration of stimulation	11.32±2.06	10.70 ±2.92	12.16±1.99	0.015
Total No of mature follicles	13.38 ± 6.59	11.89 ±6.89	11.64± 5.63	0.424
No of oocytes retraived	8.46 ± 5.05	6.056 ± 3.93	8.02± 4.21	0.299
No of oocytes fertilized	3.6 ± 2.74	3.8 ±3.31	4.8 ± 2.92	0.159
No of G1 embryos	2.8±1.16	2.08 ±1.15	3.04± 1.41	
No of transferred embryos	2.18 ± 1.16	2.08±1.15	2.7 ±0.79	0.0006
Clinical pregnancy	13 (26 %)	7 (25 %)	17 (34 %)	

The *P* value is significant if less than 0.05

Table 3: Demographic and clinical data among the different groups.

Clinical data	HCV	HBV	control	*P* value
Women age	30. 72±5.29	29.52±5.71	30.24±5.59	0.665
Infertility duration	8.52±5.17	9.259±5.59	5.116±3.08	0.04
D2-3 FSH(mIU/ml)	6.543±2.06	6.764±2.79	5.116±3.08	0.894
Unexplained infert	3(6 %)	1 (3.7)	5(10 %)	
Oligospermia	13(26 %)	9 (33.3 %)	10 (20 %)	0.413
Athenospermia	15(30 %)	11(40 %)	14(28%)	0.495
Azospermia	13(26 %)	7 (25.9 %)	9 (18 %)	0.579
Tubal factors	10 (20 %)	4 (14.8 %)	16 (32 %)	0.176
Endometriosis	2 (4 %)	0	0	0.209

The *P* value is significant if less than 0.05

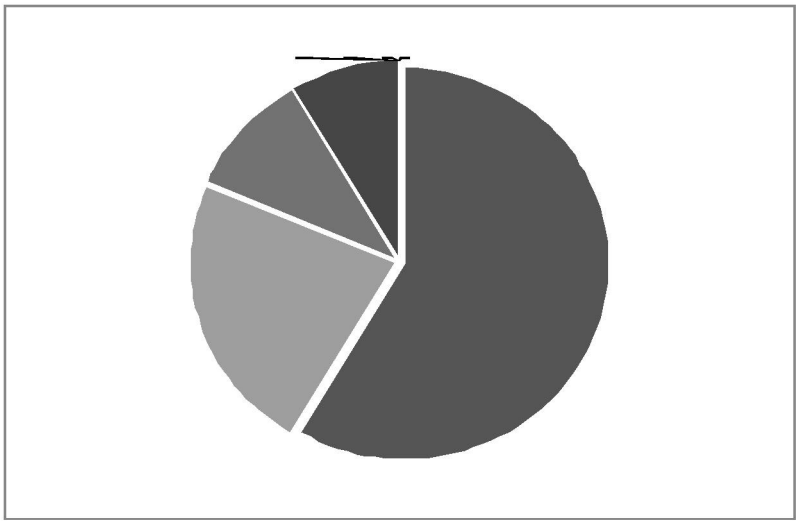

Figure1: The prevalence rate of HCV &HBV in 3764 ICSI patients

4. Discussion

The study was performed on 3746 first ICSI cycles carried out in the assisted reproductive unit-Azhar University between January 2007 and June 2011.The prevalence rate of HCV was 4.1 % while the prevalence rate of HBV was 0.7 %. The prevalence rate of HCV in our study appeared to be much lower than the national prevalence rate which varies from 10 to 13 %, the reasons for such difference is that our patients were female who are less affected by hepatitis virus than male, because many of them are working as a farmer with subsequently increased infectivity with schistosomiasis which had been an endemic disease in Egypt .In the 1960s, 1970s and early 1980s, mass campaigns were carried out to treat schistomiasis infection by tartar emetic injections. An association between HCV infection and a history of having received tartar emetic injection has been concluded (6-9).Based on this evidence, the studies suggest that inadequately sterilized needles and syringes used during the campaign were the probable causes for transmission of HCV in the region. HCV also affects males when they have their hair cut at barber shops, shaving by barbers was to be a potential source of transmission of HCV (10). A large proportion of addicts are among the males aged 25-49 yrs old (11, 12) the major risk factors are an injection drug user (12, 13). Another reason for the lower prevalence rate in our study is that ICSI expensive procedures done in the private sectors, not all infertile patients can afford for ICSI. So those who can afford for ICSI, are usually of better living standards and healthier than many of the population

In our study, there no significant difference regarding the cause of infertility among the studied groups, malefactors is the main cause of infertility, followed by tubal infertility, which may be due to increased prevalence of sexual transmitted diseases such as gonorrhea and Chlamydia causing pelvic inflammatory disease and tubal infertility

There is no significant difference among the studied groups regarding, the maternal age and basal hormone levels which predict a good ovarian response to stimulation and normal oocytes production in all groups, also in our study there is no significant differences in the liver enzyme levels among the studied groups, which denote the low risk of procedures on the patient health among the studied groups.

Also in our study, there are no significant differences among the three groups regarding HMG dose, the number of oocytes collected, number of Oocytes retraived, number of fertilized oocytes,. There is a significant difference regarding duration of HMG stimulation, number of grades A embryo transferred, there is slightly longer duration of stimulation in the control group in addition to the higher number of embryos transferred per cycle, this may be due to extra precaution during handling of embryos from hepatitis positive couples which may affect the embryos (14) .

Our study did not find significant differences in the clinical pregnancy rate among the studied groups, there was trend of a lower pregnancy rate of patients who are seropositive for HCV, HBV when compared with seronegative patients, but the number of patients is not enough to reach statistical significance, in future we need a prospective

randomized trial using a larger number of cases to address such issue

A recent study (15) revealed different results, this study was conducted on 40 women who proved to be positive for HCV, using PCR. Two control groups (both n= 40), who were negative for HCV by PCR were also included. The first control group was HCV seropositive and the second was HCV seronegative.

They reported that HCV infection in females undergoing ICSI has a negative impact on the outcome, and the impact is higher in PCR positive cases: they attributed that to hormonal disturbance associated with viral liver cirrhosis coinciding with active viral replication they have concluded that HCV infection in the females undergoing ICSI has a negative impact on the outcome, and this is higher with PCR positive. Our results did not agree with such study, as our patients had normal liver enzymes, (no significant differences regarding liver enzyme levels among the studied groups), furthermore, the previous study using a small number of cases (40 cases) 50 % of them had a poor response which had not been explained by the authors

The effect of HBV on the outcome in the ICSI treated cycle remains controversial, the pregnancy rate of HBV seropositive patients was significantly lower than that of control in one report (14) and they suggested that the extraprecausions on the handling of embryos from HBV seropositive may be the cause of the lower pregnancy rate. Another small study (16) showed there were no significant difference in the pregnancy rate and delivery rate in couples who was chronic carrier of HBV. A latest study (17) , demonstrate for the first time significantly higher PRs and implantation rates of IVF and embryo transfer cycles for couples with at least one partner being HBV seropositive. But the underlying mechanisms had not been elucidated by authors

There are several limitations of the present study. It was retrospective in nature, using a relatively small number of patients, PCR hasn't done for seropositive patients. So in future we need a prospective study using a huge number of patients to reach firm conclusions

We concluded that the prevalence of HCV, HBV in our study much lower if compared with a national prevalence rate. Also we concluded that HCV, HBV did not affect the ICSI outcome, so assisted reproductive surfaces can be provided safely to patients infected with HCV and HBV

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