Research Article

The effect of hepatitis C virus treatment on ovarian reserve

Mahmoud F. Midan[1], Samia M. Eid[1], Mahmoud A. El-Latif[2]*

[1]Faculty of Medicine Al-Azhar University, Damietta, Egypt
[2]Mansoura International Hospital, Mansoura, Egypt

*Correspondence:
Dr. Mahmoud A. El-Latif,
E-mail: m010113044000@yahoo.com

ABSTRACT

Background: The hepatitis C treatment effect on ovarian reserve of the treated women and so on their reproductive potential attracting the attention and becoming an issue of concern. In this study, we examine the possible action of interferon and ribavirin regimen on the ovarian reserve through assessing the change of AMH level pre-and post-treatment.
Methods: This study is a prospective longitudinal one, includes fifty women in childbearing period with chronic HCV infection fulfilling the criteria of attending the Egyptian national program for HCV treatment and has been referred for antiviral therapy with PEG IFN-α2a or PEG IFN-α2b, plus oral ribavirin for a total of 48 weeks. All patients were tested to AMH level at the beginning of the treatment program (the mean AMH level was 1ngml-3ngml) and retested at the end of treatment program. In addition, they examined by vaginal ultrasound to measure ovarian volume.
Results: At the end of the treatment program 28% of the studied cases remain within pre treatment level of AMH & in 32% of the studied cases the level of AMH decreased where's in 40% of the studied cases the level of AMH increased.
Conclusions: It is likely that interferon and ribavirin affect ovarian reserve of treated patients as a change occurs in the level of AMH in 72% of them.

Keywords: PEG IFN, Ribavirin, Ovarian reserve, AMH

INTRODUCTION

Egypt has the highest hepatitis C prevalence of the world where's 12.2% of women have hepatitis C virus antibodies, as well as 17.4% of men have tested positive, which makes a total of 14.7% of the 15-49 years old population having hepatitis C.[1]

The hepatitis C virus, is highly infectious, kills an estimated 40 000 Egyptians a year and at least one in ten of the population aged 15 to 59 years old is infected. People at all levels in Egypt today recognize hepatitis C as a major challenge.[2]

At birth, the female ovary has a fixed number of oocytes presented for ovulation during her reproductive years. Ovarian reserve is an estimate of the primordial follicle pool in the ovaries. It is a parameter for calculating reproductive potential and the remaining reproductive life span of a woman.[3]

Anti mullerian hormone is a glycoprotein secreted by granulosa cells in preantral and small antral follicles and appears in serum in detectable and stable amounts throughout the menstrual cycle. Anti mullerian hormone, measurement allows an assessment of ovarian reserve with several advantages over other biochemical and biophysical markers.[4]

Women represent nearly fifty percent of the Egyptian total population, the majority of them in childbearing period. Numerous of infected women have enrolled in program of hepatitis C treatment provided by the national committee for control of viral hepatitis, which provides a regimen of interferon and ribavirin as a treatment.

Thanking about if there is an effect of the hepatitis C treatment on ovarian reserve of the treated women and so on their reproductive potential attracting our attention and becoming a subject of concern. To the best of our knowledge there is no study about the effect of HCV treatment on ovarian reserve.

The aim of this study was to assess the effect of hepatitis C virus treatment program provided by the national committee for control of viral hepatitis on the ovarian reserve and so the future fertility of the woman.

METHODS

Study design

This study is a prospective longitudinal one, includes fifty women in childbearing period with chronic HCV infection previously fulfill the criteria of attending the Egyptian national program for HCV treatment and have been referred for antiviral therapy with PEG interferon alfa in combination with ribavirin at Mansoura center for treatment of hepatitis C. The study was performed between December 2012 and December 2014. The national committee for control of viral hepatitis investigated the patients before attending the treatment program and we reviewed the results from their medical records.

The inclusion criteria

Women aged between 20-40 years at the beginning of the HCV treatment program, their menstrual patterns were regular and they had been using non-hormonal contraception. They had no other chronic medical diseases and they do not use drugs except for the liver condition. The level of AMH at the beginning of the study was within limits of; 1.0 ng/ml - 3.0 ng/ml.

The exclusion criteria

Women with possible factors that may affect their ovarian reserve such as; ovarian abnormalities (single ovary, ovarian surgery, polycystic ovary syndrome), Hysterectomy, uterine artery embolization, women with pelvic infections, previous exposure to chemotherapy or radiotherapy, and women with cigarette smoking habit.

Treatment program

Each patient was treated with PEG interferon alpha-2a (Pegferon\ Hoffmann-La Roche) 180µg/week or PEG interferon alpha-2b (Peg-Intron\ Schering-Plough) 1.5 µg/kg/week subcutaneously around the umbilicus, plus oral ribavirin 800 mg/day (Minapharm). The treatment program was continued for a total of 48 weeks for the responder patients. The fifty selected women in our study were complete the 48 week course of HCV treatment.

Ultrasonic assessment of ovarian volume

Patients were examined twice; at a random day during the menstrual cycle with initiation and ending of the therapy. Transvaginal ultrasound examination was performed using ultrasonic machine (Chison 600M\China) fitted with 6 MHz transvaginal transducer.

The ovarian volume was calculated by measuring each ovary in three planes and using the formula for the volume of an ellipsoid (Volume = length x height x width x 0.5233). The ovarian volume was estimated for each ovary and the mean volume of both ovaries was calculated and used in analysis. Interpretation of the results were done guided by the normative model of ovarian volume throughout life performed by Kelsey et al 2013.[5]

Table 1: Normative model of ovarian volume in millimeters for ages from 20- 40 years.[5]

Age	3SD below	2SD below	1SD below	Mean	1SD above	2SD above	3SD above
20	37	48	61	77	96	120	150
22	37	47	60	76	95	119	147
24	35	45	57	72	90	112	140
26	32	41	53	67	84	105	131
28	30	39	49	63	79	99	124
30	28	37	47	60	76	95	119
32	28	36	46	59	75	94	117
34	27	36	46	59	74	93	116
36	27	36	46	58	74	92	115
38	26	35	45	57	72	90	113
40	25	33	42	54	68	86	107

Quantification of serum AMH level

The AMH levels of the patients were tested at the beginning of the treatment program and the fifty selected women with AMH range indicative of optimal fertility (1ng\dl-3ng\dl) retested at the end of their treatment program (after 48 weeks).

The AMH concentrations were quantitatively determined using the commercially available AMH Gen II enzyme linked immunosorbent assay kit for the quantitative measurement of AMH in human serum and lithium heparin plasma (AMH Gen II assay; Beckman Coulter, Inc., Webster, Texas). These assays were used according to the manufacturer's protocols.

Interpretation of the results

Interpretation of the results were done guided by AMH interpretation guidelines from fertility literature.[6]

Table 2: AMH blood level and ovarian fertility potential.[6]

AMH blood level	Ovarian fertility potential
Over 3.0 ng/ml	High (often PCOS)
1.0 - 3.0 ng/ml	Normal (optimal fertility)
0.7 - 0.9 ng/ml	Low normal (satisfactory fertility)
0.3 - 0.6 ng/ml	Low (low fertility)
Less than 0.3 ng/ml	Very Low (very low /undetectable)

Classification of the studied cases according to the results

We categorize the studied cases according to level of AMH post- treatment into three categories:

- Cases with increased AMH level: Over 3.0 ng/ml
- Cases with unchanged AMH level: 1ng/ml - 3ng/ml
- Cases with decreased AMH level: blow 1ng/ml

Statistical analysis

Statistical analysis was performed using a commercially available software package (SPSS, Inc., Chicago, IL, USA).

The distribution of AMH & ovarian volume pre and post treatment were assessed using Kolmogorov-Smirnov test.

Difference between the level of AMH & ovarian volume pre and post treatment were tested using Wilcoxon signed rank test.

Analysis across categories was by Kruskal–Wallis test. The Mann-Whitney test was used to test differences

All patient characteristics were described in numbers and percentage. Results are expressed as mean±SD, minimum & maximum

Spearman correlation coefficients were used to assess the relationships between the parameters (Values between 0 and ±0.3 indicate a weak±linear relationship. Values between ±0.3 and ±0.7 indicate a moderate ±linear relationship. Values between ±0.7 and ±1.0 indicate a strong ±linear relationship).[7] P-value ≤0.05 was considered statistically significant.

RESULTS

Table 3: Description of age, parity & body mass index (BMI) of the studied cases.

	Minimum	Maximum	Mean	Median	SD
Age	20	40	32.48	-	5.7080
Parity	0	5	-	2	-
BMI	22	30	28.12	-	2.1631

Table 4: Classification of the studied cases according to the change in the level of AMH post treatment.

	Decreased AMH	Unchanged AMH	Increased AMH
Number	16	14	20
percentage	32 %	28 %	40 %
Minimum	0.16	1.20	3.20
Maximum	0.70	2.30	14
Mean	0.3913	1.88	7.56
SD	0.2436	0.4555	3.3049

Table 5: Comparison between cases as regard basic characteristics.

Basic characteristics	Mean rank			P value
	Decreased AMH	Unchanged AMH	Increased AMH	
Age	38.00	19.79	19.50	0.000
Parity	26.00	24.71	25.65	0.906
BMI	28.13	35.36	16.50	0.000
ALT	28.25	34.50	17.00	0.000
AST	28.25	34.50	17.00	0.000
Alfa fetoprotein	24.50	24.50	27.00	0.216
Alkaline phosphates	24.50	24.50	27.00	0.216
TSH	25.63	22.50	27.50	0.216
HCV RNA	30.00	25.50	21.90	0.179
HBsAg	22.50	26.07	27.50	0.083
Anti-schistosomal antibodies	33.38	25.79	19.00	0.003
Liver biopsy	29.25	29.79	19.50	0.006

Table 6: Difference between the classified cases as regard significant variables.

	AMH		Z	P.
	Decreased	Unchanged	-3.199	0.001
Age	Decreased	Increased	-3.985	0.000
	Unchanged	Increased	-0.141	0.888
	Decreased	Unchanged	-1.725	0.084
BMI	Decreased	Increased	-2.607	0.009
	Unchanged	Increased	-3.674	0.000
	Decreased	Unchanged	-1.976	0.048
ALT, AST	Decreased	Increased	-2.646	0.008
	Unchanged	Increased	-4.021	0.001
Anti-schistosomal antibodies	Decreased	Unchanged	-1.844	0.065
	Decreased	Increased	-3.402	0.001
	Unchanged	Increased	-1.559	0.119
	Decreased	Unchanged	0.000	1.000
Liver biopsy	Decreased	Increased	-2.947	0.003
	Unchanged	Increased	-3.165	0.002

Table 7: Correlation between AMH level post-treatment and significant variables pre-treatment.

	Variable	Correlation coefficient (C.c.)	P value
	Age	- 0.537	0.000
	BMI	- 0.332	0.018
AMH post-treatment	ALT, AST	- 0.491	0.000
	Anti-schistosomal antibodies	- 0.437	0.002
	Liver biopsy results	- 0.386	0.006
	AMH pre treatment	± 0.313	0.027

Table 8: Comparison between decreased level AMH cases and increased level AMH cases.

Variables	Decreased AMH	Increased AMH
Age	36.8	29.9
BMI	28.5	26.9
ALT-AST ALT AST	75% elevated 75.3 80.6	30% elevated 51.8 42.9
Anti-schist. antibodies	87.5% elevated	30% elevated
Liver biopsy	62.5% A1F1 25.0% A2F2	100% A1F1 0.00% A2F2

Thiers is no significant difference between the 3 categories as regard HCV RNA level, HBsAg presence, elevation of Alpha fetoprotein, elevation of alkaline phosphates &elevation of TSH.

DISCUSSION

In this study, the mean age of the studied cases was 32.48 years old, a result that is comparable to that reported by El-Zanaty and Associates, as they mentioned that Over 80% of HCV infections in the Egyptian population are among individuals aged 30 years and above, and increases with age (reaching >25% among persons aged >50 years).[1]

In this study AMH values pre-treatment follow the normal distribution curve (P = 0.063) and so baseline AMH levels in hepatitis C patients similar to age-matched fertile women of the same age, while the values at the end of the treatment program not follow the normal distribution curve (P = 0.000), meaning that some sort of change occurred.

The mean serum AMH concentration in patients before therapy was 1.93 ng/ml; after therapy, the level was 3.67ng/ml. This difference was statistically significant (P = 0.036).

The ovarian function was intact prior to initiation of HCV therapy. Sifer, et al and Devaux, et al mentioned that active chronic HCV infection does not affect ovarian follicle development despite the detection of HCV RNA in the follicular fluid of 89% of HCV PCR positive females, irrespective of the degree of viremia.[8,9]

In our study, all the patients start the treatment program with the same range of AMH (1ng\ml-3ng\ml). At the end of the program AMH, values in 28% of cases remain in the pre-treatment range i.e. unchanged (1ng\ml - 3ng\ml) while 40% had a level more than 3 ng\ml (increased) and 32% had a level blow 1 ng\ml (decreased). All of them receive the same drugs (interferon and ribavirin), for the same period (48 weeks).

The data concerning the impact of interferon and ribavirin on AMH values are conflicting. We suggested that AMH concentrations influenced by HCV treatment.

In the light of the study results, we suppose that HCV treatment affect ovarian reserve of the patients, classifying the studied cases according to AMH level into three categories (decreased level - unchanged level – increased level). We explain this difference through comparing the baseline data of the three categories.

In this study, the mean age of patients with decreased AMH post treatment was 36.87, while the mean age of patients with increased AMH was 29.9, which means that diminished ovarian reserve may be due to aging process. There's inverted moderate correlation between the level of AMH levels at the end of the treatment and the patient Age (C. c. = 53.7%) (P = 0.0).

Faddy et al, mentioned that the rate of follicle depletion might increase after the age of 35 years.[10] In addition, Menken et al, maintained that prevalence of infertility increases significantly after the age of 35 years; about 99% of patients expected to be infertile with 45 years of age.[11]

Elevated levels of liver enzymes in general signify some form of liver damage. In this study there is significant difference between the three categories of patients as regard elevation of ALT & AST (P = 0.0). Liver enzymes elevated more in patients with decreased AMH than in patients with unchanged AMH and patients with increased AMH.

At hand is inverted moderate correlation (Correlation coefficient = 49.1%) between the ALT, AST elevation and the level of AMH post- treatment (P = 0.6). ALT, AST elevated in 75% of cases with decreased AMH (mean ALT: 75.31, mean AST: 80.62) and elevated only in 30% of cases with increased AMH level post treatment (mean ALT: 51.80, mean AST: 42.95).

Prada et al results based on 867 patients strongly suggest that almost all patients with elevated ALT levels have damaged their liver at least in the past and may potentially progress.[12] David E mentioned that patients with cirrhosis often have normal or only slightly elevated serum AST and ALT levels.[13] Thus, AST and ALT lack some sensitivity in detecting chronic liver injury and of course, AST and ALT levels tend to be higher in cirrhotic patients with continuing inflammation or necrosis than in those without continuing liver injury.

In this study There's significant difference between patients with decreased AMH and patients with increased AMH as regard presence of anti schistosomal antibodies (P = 0.001), the antibodies present in 87.5% of patients with decreased AMH while it present only in 30% of patients with increased AMH .

Abdel-Rahman et al retrospective analytic study in 2013 stated that positive schistosomal serology has no effect on fibrosis staging but is significantly associated with failure of response to HCV treatment despite anti schistosomal therapy.[14]

Liver biopsy provides information about the extent and distribution of inflammation and allows grading and staging of the disease (the amount of fibrosis). Furthermore, the liver biopsy allows some assessment of the rate of disease progression whenever the date of onset of infection is known.[15]

In this study the grade of inflammation activity and stage of hepatic fibrosis is more in patients with decreased AMH than patients with increased AMH (P = 0.03). All patients with increased AMH had mild activity (A1) and portal fibrosis without septa (F1), as 62.5% of patients with decreased AMH had mild activity and portal fibrosis without septa and 25.0% of them had moderate activity (A2) and portal fibrosis with few septa (F2). Theirs inverted moderate correlation (Correlation coefficient = 38.6%) between the Liver biopsy results and the level of AMH at the end of the treatment (P = 0.06).

Perrillo RP mentioned that the presence of diffuse fibrosis or cirrhosis correlates with a lower likelihood of response to antiviral therapy, and the finding of severe necroinflamatory and fibrotic changes is helpful in determining the relative importance of beginning treatment early rather than deferring therapy.[15]

There's no significant difference between the patients categories as regard HCV RNA level, supposing that viral load not affecting woman fertility. WHO, stated that the response to therapy is influenced by duration of therapy, dosage, infecting viral load and disease stage.[16] The strain of the infecting virus may also affect the clinical response.

In this study there is no significant difference between ovarian volume pre and post-treatment (P = 0.566). It has been suggested that there are no major changes in ovarian volume during reproductive years until the premenopausal period.[17,18] in women older than 40 years, there is a dramatic drop in ovarian volume, which is not related to parity.[19]

Bowen et al mentioned that there are significant correlations between reduced ovarian measures, increased age, and elevated serum FSH.[20]

There's no reported previous studies about effect of interferon and / or ribavirin on the ovarian reserve or on the AMH level but there is studies about the effect of many of the chemotherapeutic regimens used in the treatment of cancers on ovarian function.

Lee et al mentioned that many of the chemotherapeutic regimens used in the treatment of common cancers are

gonadotoxic and increase the risk of premature ovarian failure in young women.[21]

Bo Yu, et al aiming to assess levels of AMH, E2, FSH and menstrual status, in young women with breast cancer undergoing chemotherapy maintained that Chemotherapy decreases ovarian reserve rapidly and dramatically (median AMH after 6 weeks 0.08 ng/ml, after 12 weeks 0.05 ng/ml), compared to (median AMH 0.86 ng/ml) before the start of chemotherapy.[22] At 36 and 52 weeks after the start of chemotherapy, AMH levels remained suppressed (0.05 ng/ml at week 36 & 0.07 ng/ml at week 52). Although the secretory function of the ovary may recover to some extent and menses may return following completion of chemotherapy treatment, ovarian reserve remains persistently affected. Neither baseline nor change in AMH predicts return of menstrual function, suggesting that ovarian reserve and endocrine function may be affected differently or may recover differently from chemotherapy.

Howell and Shalet, Meirow and Nugent; maintained that chemotherapy and radiotherapy regimens differ widely in their effect on fertility.[23,24] Highly gonadotoxic chemotherapy regimens include alkylating agents and procarbazine. Wallace, et al maintained that radiotherapy carries a high risk where there is a direct or scatter dose to the gonad.[25]

UK Royal Colleges in the year 2007 recommends that all patients who require anti-cancer treatment should informed about potential gonadotoxic side effects at the time of diagnosis and before potentially gonadotoxic treatment.[26]

Chemotherapy disrupts ovarian function by depleting the primordial follicle pool in a drug- and dose-dependent manner.[27] Analyses of ovarian function following cancer therapy have mostly described the prevalence of ovarian failure following treatment, although follicular depletion may occur despite maintenance of regular menstrual cycles.[28,29]

In a small study of 3 patients, Oktay, et al demonstrated that with each cycle of chemotherapy the AMH levels fall significantly, also dropping to undetectable by 6-weeks in women who have disruption of their menses. They, however, found less of a decline in a woman who maintained her periods through treatment.[30]

Chemotherapy drugs act by a range of mechanisms but with particular cytotoxicity to dividing cells. Oocytes and somatic cells will have different vulnerabilities to cytotoxic agents, in developing follicles; the oocytes are non-dividing, although rapidly growing, while the somatic cells of such follicles have a high degree of proliferation.[31]

Chemotherapy treatment may induce premature ovarian failure by directly killing primordial follicles, reduction

of the primordial pool can arise indirectly, via the loss of activated, growing follicles.[31,32]

At any time, there are follicles within the ovary at various stages of maturation. It is possible that specific stages are more susceptible to chemotherapy-induced damage than others are. Oktem et al found that ovarian biopsies from patients treated with chemotherapy had significantly lower primordial follicle counts than untreated controls.[33]

A study by Yucebilgin et al also found that primordial follicle counts decreased following the administration of paclitaxel or cisplatin to rats in vivo.[34] More mature follicle classes are also vulnerable to damage by chemotherapeutic agents. Pre-antral follicles have been shown to be susceptible to chemotherapy with deterioration in follicle quality following treatment both in vitro and in vivo.[35,36]

The rapid falls in serum concentrations of inhibin B and AMH during chemotherapy also indicate loss of follicles at the pre-antral and early antral stages.[37-38] The loss of the production of inhibitory substances by growing follicles could lead to accelerated depletion of the primordial reserve, with more primordial follicles undergoing growth initiation to replace damaged growing follicles.[31]

Chemotherapeutic drugs may act at the level of the nucleus to cause DNA damage or interfere with DNA transcription and replication. They can also act on the mitochondria to induce the release of cytochrome c into the cytoplasm. These pathways all interconnect and lead to cell death, often through the caspase family of proteins (cysteine-aspartic proteases-a family of cysteine proteases that play essential roles in apoptosis, necrosis, and inflammation) which are associated with apoptosis.[31]

Ribavirin is a nucleoside analogues act as antimetabolites incorporated into growing DNA strands and affect mitochondrial DNA.[40] Mean peak plasma concentrations increased with severity of hepatic impairment.[41] Ribavirin has a long half-life (12 days after multiple doses) and may persist in nonplasma compartments for as long as 6 months.[42-44]

There is no FDA guidance on the use of Ribavirin in women of reproductive potentials.[45] In a fertility study in rats, ribavirin showed a marginal reduction in sperm counts with no effect on fertility.[46] In animal studies, ribavirin produced changes in sperm at subclinical doses possibly accumulate in sperm to induce defects.[47]

Likewise, ribavirin may accumulate in oocyte lead to cell death and so reduction in primordial follicles pool and decrease in the rate of recruitment. In addition, it can affect the growing follicles granulosa cells through incorporation into its growing DNA strands inhibiting its activity.

The previous possibilities may explain the reduction in the AMH level (damage to the proliferating ovarian granulosa cells and/or to the oocytes, subsequently, follicular depletion and reduction in the ovarian reserve.)

Interferons are cytokines, have different biologic effects; they display antiviral activity, influence cellular metabolism and differentiation, and possess antitumor activity.[48,49]

The effects of interferon-α on women fertility not systematically investigated. Its use in men offered for cases of idiopathic infertility and prophylaxis of sterility after mumps orchitis.[50,51]

Initial recruitment, started due to stimulatory factors (intra ovarian and/or other unknown factors) or may be due to a release from inhibitory stimuli that maintain the resting follicles in stasis.[52]

Many years of research have demonstrated that control of primordial follicle activation requires complex bidirectional signaling between the oocyte and the surrounding somatic cells, involving specific cytokines and growth factors.[53,54]

Likewise, interferon as cytokines may improve folliculogenesis through primordial follicle activation. It acts as stimulatory factors increase the rate of recruitment. This possibility may explain the increase in the AMH level.

Ernstoff, et al, Gisslinger et al reported that interferon administration resulted in stimulation of adrenocorticotropic hormone (ACTH) release.[55,56] ACTH stimulates the synthesis of cortisol, glucocorticoids, mineralocorticoids and Dehydroepiandrosterone (DHEA).[57]

Dehydroepiandrosterone (DHEA) has been reported to improve oocyte/embryo yields and oocyte/embryo quality in women with diminished ovarian reserve. AMH concentrations significantly improved after DHEA supplementation over time which is more pronounced in younger women. Women under age 38 years demonstrated higher AMH concentrations and improved AMH concentrations more than older females.[58]

This mechanism may occur in the same manner in our cases interferon stimulate ACTH release which stimulates the synthesis of DHEA explain the increase in the AMH level especially in younger women.

In attempt to explain the difference in response of the patients ovaries to the treatment we suppose that ribavirin and interferon act oppositely; ribavirin (chemotherapy) has inhibitory effect and interferon (cytokines - stimulate ACTH) has stimulatory effect. The end result of these tow effects depend on the patients baseline characters.

Suspecting the change of AMH depends on:

- Primordial follicles pool volume (the younger the age the more the resting pool).[10]
- Continuing liver injury (elevated ALT&AST) may associate with failure of response to HCV treatment.[12]
- Inflammation activity and portal fibrosis (the less the grade the increase likelihood of response to antiviral therapy).[18]
- Anti Schistosomal, antibodies presence (may associated with failure of response to HCV treatment).[14]

The patients with increased level of AMH (more than 3ng\ml) after treatment (40%) characterized by young age (mean±29.9years) so resting primordial follicles pool still high and many follicles (50-60/available at any given time) are recruited to the active follicle pool, also the number of primordial follicles falls steadily. liver enzymes elevated in 30% of cases (mean ALT: 51.8000, mean AST: 42.9500) supposing that 70% of them without continuing liver injury. Anti schistosomal antibodies present only in 30% of patients (may associated with failure of response to HCV treatment).

All of them had mild inflammation activity and portal fibrosis without septa; so increase likelihood of response to antiviral therapy. We suggest that in these patients interferon and ribavirin act better. Interferon increase the rate of recruitment ,and increase rate of granulosa cells proliferation, while ribavirin increase the rate of follicular atresia but the number of primordial follicles pool still many so the end result is more secretion of AMH (increased level) post treatment.

The patients with decreased level of AMH (less than 1ng\ml) after treatment (32%) characterized by old age (mean±36.9 years) so resting primordial follicles pool become low and fewer follicles are recruited to the active follicle pool, also the rate of loss of primordial follicles accelerates (increase after the age of 35 years).[10]

Liver enzymes elevated in 75% of cases (mean ALT: 75.3125, mean AST: 80.6250) supposing that the majority of them with continuing liver injury. Anti schistosomal antibodies present in 87.5% of patients (may associated with failure of response to HCV treatment).

62.5% patients with mild inflammation activity (A1) and portal fibrosis without septa (F1), and 25.0% A2F2 and so less chance of response to antiviral therapy. In these patients interferon and ribavirin action is less. ribavirin increase the rate of follicular atresia and this rate increase after the age of 35 years and accelerates about twofold at age of 37.5±1.2 years so the number of primordial follicles pool depleted progressively towards exhaustion and also may accumulate in growing follicles affecting their function, while Interferon is less effective so the rate

of recruitment decreased so the end result is less secretion of AMH (decreased level) post treatment.[10]

CONCLUSIONS

Interferon and ribavirin may affect the AMH level either increase or decrease according to patient basic characteristics and so the patient ovarian reserve and future fertility may be affected after administration of hepatitis C treatment.

Recommendation

1. HCV-infected women who prepared to enroll in hepatitis C treatment program should be counseled about their future fertility.
2. Women in 2nd & 3rd decade of life advised to start HCV treatment as early as possible as their fertility not changed a lot and their reserve may be even better after treatment.
3. Discussion of fertility preservation techniques with patient of low reserve before starting hepatitis C treatment.
4. Based on limited available information about the effect of interferon on ovarian function further studies is in need (larger sample, prolonged follow up, specific categories as women with low AMH level).

REFERENCES

1. El-Zanaty F, Way A. Egypt demographic and health survey. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International. 2008. Available at: http://www.measuredhs.com/ pubs/pdf/ FR220/FR220.pdf
2. World Health Organization. Egypt steps up efforts against hepatitis C: World Hepatitis Day. 2014. Available at: http://www.who.int/features/ 2014/ egypt-campaign-hepatitisc/ en/ index.html.
3. Scheffer G, Broekmans F. Antral follicle counts by transvaginal ultrasonography is related to age in women with proven natural fertility. Fertil Steril. 1999;72(5):845-51.
4. William L. Clinical utility of measurement of anti-mullerian hormone in reproductive endocrinology. J Clin Endocr Metab. 2010;95(12):5144-54.
5. Kelsey TW, Dodwell SK, Wilkinson AG, Greve T, Andersen CY, Anderson RA, et al. Ovarian Volume throughout Life: A Validated Normative Model. PloS One. 2013;8(9):e71465.
6. Sherbahn, Catenacci, Asemota. Advanced Fertility Center of Chicago; Anti-Mullerian Hormone Testing of Ovarian Reserve. 2011. Available at: http://www.advancedfertility.com/amh-fertility-test.htm
7. Ratne B. The correlation coefficient: Its values range between +1/−1, or do they? Journal of Targeting, Measurement and Analysis for Marketing. 2009;17:139-42.
8. Sifer C, Benifla JL, Branger M, Devaux A, Brun-Vezinet F, Madelenat P. Effects of hepatitis C virus on the apoptosis percentage of granulomas cells in vivo in women undergoing IVF: preliminary results. Hum Reprod. 2002;17:1773-76.
9. Devaux A, Soula V, Sifer C, Branger M, Naouti M, Porcher R. In P Brun, Vezinet F, Feldmann G. Hepatitis C virus detection follicular fluid and culture media from HCV + women, and viral risk during IVF procedures. Hum Reprod. 2003;18:2342-9.
10. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles. Hum Reprod. 1992;10:1342-6.
11. Rosen MP, Johnstone EB, Gillham SJ, Modan AE, Lipshutz AK, Reijo-Pera R. Is antral follicle count a genetic trait? Menopause. 2010;17:109-13.
12. Pradat P, Alberti A, Poynard T, Esteban JI, Weiland O, Marcellin P. Predictive Value of ALT Levels for Histologic Findings in Chronic Hepatitis C: A European Collaborative Study. Hepatology. 2002;36:973-7.
13. Fujisawa M, Fujioka H, Tatsumi N, Inaba Y, Okada H, Arakawa S. Levels of interferon alpha and gamma inseminal plasma of normozoospermic, oligozoospermic, a azoospermic men. Archives of Andrology. 1998;40:211-4.
14. Abdel-Rahman M, El-Sayed M, El Raziky M, Elsharkawy A, El-Akel W, Ghoneim H, et al. Co-infection with hepatitis C virus and schistosomiasis: Fibrosis and treatment response. World J Gastroenterol. 2013;19(17):2691-6.
15. Perrillo RP. The role of liver biopsy in hepatitis C. Hepatology. 1997;3(1):57S-61S.
16. World Health Organization. Global Alert and Response: Hepatitis C prevention and treatment. 2002. Available at: http://www.who.int/ csr/disease/ hepatitis/ whocdscsrlyo2003/en/ index5.html
17. Kwee J, Elting ME, Schats R, McDonnell J, Lambalk CB. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reproductive Biology and Endocrinology. 2007;5(9).
18. Hendriks DJ, Kwee J, Mol BW, Te Velde ER, Broekmans FJ. Ultrasonography as a tool for the prediction of outcome in IVF patients: A comparative meta-analysis of ovarian volume and antral follicle count. Fertil Sterile. 2007;87:764-75.
19. Higgins RV, van Nagell JR, Woods CH, Thompson EA, Kryscio RJ. Interobserver variation in ovarian measurements using trans vaginal sonography. Gynecol Oncol. 1990;39:69-71.
20. Bowen S, Norian J, Santoro N, Pal L. Simple tools for assessment of ovarian reserve (OR): individual

ovarian dimensions are reliable predictors of OR. Fertil Sterile. 2007;88(2):390-5.

21. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24:2917-31.

22. Bo Yu, Douglas N, Ferin MJ, Gary S. Changes in markers of ovarian reserve and endocrine function in young women with breast cancer undergoing adjuvant chemotherapy Cancer. 2010;116(9):2099-2105.

23. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinology and Metabolism Clinics of North America. 1998;27:927-43.

24. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7:535-43.

25. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18:117-21.

26. Royal College of Physicians, The Royal College of Radiologists, Royal College of Obstetricians and Gynecologists. The effects of cancer treatment on reproductive functions: Guidance on management. Report of a Working Party. London: RCP. 2007. Available at: https://www.rcplondon.ac.uk/ sites/ default/files/ documents/ effects-of-cancer-treatment-reproductive-functions.pdf

27. Sonmezer M, Oktay K. Fertility preservation in female patients Hum Reprod Update. 2004;10(3):251-66.

28. Bath LE, Wallace WH, Shaw MP, Fitzpatrick C, Anderson RA. Depletion of ovarian reserve in young women after treatment for cancer in childhood: detection by anti-Mullerian hormone, inhibin B and ovarian ultrasound. Hum Reprod. 2003;18(11):2368-74.

29. Larsen EC, Muller J, Schmiegelow K, Rechnitzer C, Andersen AN. Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. Clinical Endocrinology and Metabolism. 2003;88(11):5307-14.

30. Oktay K, Oktem O, Reh A, Vahdat L. Measuring the impact of chemotherapy on fertility in women with breast cancer. J Clin Oncol. 2006;24(24):4044-6.

31. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Update. 2012;18(5):525-35.

32. Meirow D, Biedermann H, Anderson RA, Wallace WH. Toxicity of chemotherapy and radiation on female reproduction. Clin Obstet Gynecol. 2010;53:727-39.

33. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer. 2007;110:2222-9.

34. Yucebilgin MS, Terek MC, Ozsaran A, Akercan F, Zekioglu O, Isik E, et al. Effect of chemotherapy on primordial follicular reserve of rat: An animal model

of premature ovarian failure and infertility. Aust Nz J Obstet Gyn. 2004;44:6-9.

35. Raz A, Fisch B, Okon E, Feldberg D, Nitke S, Raanani H, et al. Possible direct cytotoxicity effects of cyclophosphamide on cultured human follicles: an electron microscopy study J Assist Reprod Gen. 2002;19:500-6.

36. Abir R, Ben-Haroush A, Felz C, Okon E, Raanani H, Orvieto R, et al. Selection of patients before and after anticancer treatment for ovarian cryopreservation. Hum Reprod. 2008;23:869-77.

37. Anderson RA, Themmen APN, Al-Qahtani A, Groome NP, Cameron DA. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Hum Reprod. 2006;21:2583-92.

38. Decanter C, Morschhauser F, Pigny P, Lefebvre C, Gallo C, Dewailly D. Anti-Müllerian hormone follow-up in young women treated by chemotherapy for lymphoma: preliminary results. Reprod Biomed Online. 2010;20:280-5.

39. Rosendahl M, Andersen CY, La Cour FN, Juul A, Lossl K, Andersen AN. Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age. Fertil Steril. 2010;94:156-66.

40. Drug Bank. Version 4.2. created on June 13, 2005 / Updated on October 08, 2013. Available at: http://www.drugbank.ca/drugs/DB00811

41. Sharp M. Dohme Corporation. Rebetol (ribavirin) capsules and oral solution prescribing information. 2013 November Available at: https://www.merck.com/product/ usa/pi_circulars/ r/rebetol/ rebetol_pi.pdf

42. Lass A, Silye R, Abrams DC, Krausz T, Hovatta O, Margara R. Follicular density in ovarian biopsy of infertile women: A novel method to assess ovarian reserve. Hum Reprod. 1997;12:1028-31.

43. Seifer D, Mac Laughlin T, Penzias S, Behrman R, Asmundson L, Donahoe K, et al. Gonadotropin-releasing hormone agonist-induced differences in granulosa cell cycle kinetics are associated with alterations in follicular fluid müllerian-inhibiting substance and androgen content. JCEM. 1993;76:711-4.

44. Cook CL, Siow Y, Taylor S, Fallat M. Serum Müllerian inhibiting substance levels during normal menstrual cycles. Fertil Steril. 2000;73:859-61.

45. Drugs com. FDA prescribing information, side effects and uses Ribavirin. Revised: 08/2014 Available at: http://www.drugs.com/ pro/ ribavirin.html

46. Manns MP, McHutchison JG, Gordon SC. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C:a randomized trial. Lancet. 2001;358:958-65.

47. Mishkin D, Deschênes M. Conception soon after discontinuing interferon/ribavirin therapy: a

successful outcome. Am J Gastroentero. 2001;96(7):2285-86.

48. World of Microbiology and Immunology; 2003. Encyclopedia.com. 2015. Available at: http://www.encyclopedia.com/doc/1G2-3409800319.html

49. Jonasch E, Haluska FG. Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. Oncologist. 2001;6(1):34-55.

50. Yamamoto M, Miyake K. Successful use of interferon for male infertility. Lancet. 1994;344(8922):614.

51. Erpenbach KH. Systemic treatment with interferon-alpha 2B: an effective method to prevent sterility after bilateral mumps orchitis. Urology. 1991;146:54-6.

52. Erickson G. Follicle Growth and Development. Global library of women's medicine. 2008;ISSN:1756-2228. Available at: http://www.glowm.com/section_view/heading/Follic le%20Growth%20and%20Development/item/288.

53. Skinner MK. Regulation of primordial follicle assembly and development. Hum Reprod Update. 2005;11:461-71.

54. Hutt KJ, Mc Laughlin EA, Holland MK. KIT/KIT ligand in mammalian oogenesis and folliculogenesis: roles in rabbit and murine ovarian follicle activation and oocyte growth. Biol Reprod. 2006;75:421-33.

55. Ernstoff MS, Fusi S, Kirkwood JM. Parameters of interferon action: I. Immunological effects of whole cell leukocyte interferon (IFN-alpha) in phase I-II trials. J Biol Resp Modif. 1983;2:528-39.

56. Gisslinger H, Svoboda T, Clodi M, Gilly B, Ludwig H, Havelec L. Interferon-alpha stimulates the hypothalamic-pituitary-adrenal axis in vivo and in vitro. Neuroendocrinology. 1993;57:489-95.

57. Society for Endocrinology. Corticotrophin-releasing hormone. J Endocrinol. 2012;175(1):89-97.

58. Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve after dehydroepiandrosterone supplementation. Reprod Biomed Online. 2010;21(3):360-5. Available at: http://www.fertilitynutraceuticals.com/article/improv ement-in-diminished-ovarian-reserve.