Effect of Letrozole on Hyaluronic Acid Concentration of the Endometrial Wash

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ABSTRACT

Hyaluronic acid (HA) is an anionic, non-sulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. The study included 40 infertile females. A full history taking, complete general examination, complete gynecological examination, and infertility workup including basal hormonal analysis, and uterine cavity assessment by ultrasound and tubal patency evaluation by hysterosalpingogram was done in addition to husband's seminal fluid analysis. The control group did not receive any ovarian stimulation drugs, while the 2nd group was treated with letrozole + gonadotropin. The ovarian stimulation protocol was chosen for each patient according to her age, history, and hormonal assay. Evaluation of thickness and pattern of the endometrium, size, and the number of mature follicles was performed by transvaginal ultrasonography on the 2nd day of the menstrual cycle and cycle day 11-14 before hCG injection. The study showed no correlation between HA and LH at triggering day in women who received letrozole plus Gonadotropin but a positive correlation was documented between HA concentration and E₂ in addition to a positive correlation with progesterone, the number of follicles, and endometrial thickness at the day of triggering of ovulation. The study revealed that mean hyaluronic acid concentration was elevated significantly ($P < 0.05$) in pregnant women after stimulation protocol compared with nonpregnant (164.2 vs. 152.1 pg/ml). HA was significantly higher in women who undergo ovarian stimulation with letrozole which was a good protocol in the treatment of women with ovulatory problems.

Keywords: Hyaluronic acid; Female infertility; Letrozole; FSH; Gonadotropin.

1. Introduction

Infertility is a disease of the reproductive system, which is defined as the failure to achieve pregnancy after at least one year of regular unprotected sexual intercourse in women < 35 years who are not using contraception and after six months in women > 35 years or due to an impairment of a person's capacity to reproduce, either as an individual or with his/her partner, it affects about 10-15% of couples (*Bergqvist, et al* ^[1], *Campbell, S* ^[2]). According to the World Health Organization (WHO) definition, a couple is considered infertile if, after 2 years of regular sexual intercourse, without contraception, the woman has not become pregnant (*Chun, et al* ^[3]). Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan that has a significant role in establishing the cellular microenvironment conducive to the development of the proliferative process. The lack of hyaluronic acid synthase regulation genes causes abnormal production of HA and promotion of

abnormal biological processes such as metastasis and pregnancy loss (*Al-Hussaini and Shaaban* ^[4]). An increasing number of reports suggest the role of HA in female reproduction and interest in its application in assisted reproduction is rising. However, there are contrasting data about the effectiveness of adding HA to the embryo-transfer medium on improving pregnancy rates (*Oats, et al* ^[5]). Several studies evaluated the co-administration of Letrozole during ovarian stimulation in patients suffering from a diminished ovarian reserve, yielding conflicting results. In these studies, Letrozole was started either concomitantly with or before gonadotropins, and was given for a total of 5 stimulation days (*Elsedeek, et al* ^[6], *Ahmied, M.* ^[7]). Hypothetically, extending the duration of letrozole co-treatment may provide a more pronounced effect on intrafollicular androgens and circulating estrogen levels. Letrozole co-treatment during the entire stimulation course improves ovarian response in normal responders undergoing IVF-ET (*Hameed*

and Ahmeid ^[8]). The study aims to evaluate the use of letrozole in the stimulation of infertile women in relation to hyaluronic acid levels in endometrium.

2. Materials and Methods

This comparative prospective study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, from September 2019 to March 2020. The study included a total of 40 women who attended the infertility clinic of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University from September 2019 to March 2020. All couples subjected to full history taking, complete general examination, complete gynecological examination, and infertility workup including husband's seminal fluid analysis, basal hormonal analysis, and uterine cavity assessment by ultrasound and tubal patency evaluation by hysterosalpingogram. The females were classified into 2 groups each consists of 20 females. The control group did not receive

any ovarian stimulation drugs (natural cycle). In the 2nd group (studied group) ovulation induction programs were used, 20 were stimulated by letrozole 2.5 mg tablets from cycle 3rd day for 5 days two times daily in addition to gonadotropin ampule r-FSH 75 IU from day 4 of the cycle with dose adjusted according to patient's response. The ovarian stimulation protocol was chosen for each patient according to her age, history of previous cycle response, and hormonal assay. Evaluation of thickness and pattern of the endometrium, size, and the number of mature follicles was performed by transvaginal ultrasonography on the 2nd day of the menstrual cycle and cycle day 11-14 before HCG injection (ovulatory phase). Endometrial wash was done on trigger day (day 11-14) for assessment of hyaluronic acid concentration. Pregnancy outcome was used as a main comparative parameter between selected groups and the study included aspiration of endometrial fluid for determination of hyaluronic acid

Figure (1): Methodology of the study

level by ELISA technique. The methodology is shown in *Figure 1*.

3. Aspiration of endometrial fluid

The present study included aspiration of endometrial wash fluid on the day of trigger by putting the patient in the lithotomy position. Cleaning of the vagina was done by normal saline wash. Using a sterile IUI catheter connected to a disposable syringe; three ml of sterile normal saline was injected slowly into the endometrial cavity after passing the cervical mucus and re-aspirated after thirty seconds. This volume was chosen after making a pilot study since a larger amount might spill into the ovarian tubes and may go farther into the peritoneal cavity causing peritoneal irritation, a lesser amount might not cover the entire cavity and the samples will not be sufficient for measuring the hyaluronic acid concentration. Samples were transferred into a plain sterile tube for assessment of HA concentration (*González-Ramos, et al [9]*).

4. Results

In this study, the majority of women enrolled have belonged to rural area (60%) and below 30 years old (55%), parity was 0-5 but the majority were complaining of primary infertility (60%) and the infertility duration from 2 years to >7 years, and with body mass index $26.14 \pm 4.31 \text{ kg/m}^2$ (Mean $\pm$ SD) as shown in *Table 1*. The study showed that mean of hyaluronic acid was elevated significantly in women who received letrozole plus Gonadotropin ($158.7 \pm 19.7 \text{ pg/ml}$) than the control group ($146.1 \pm 26.2 \text{ pg/ml}$) as shown in *Table 2*. The study showed no correlation between hyaluronic acid and LH at the day of triggering of ovulation in women who received letrozole plus Gonadotropin ($r: 0.03$ and $P > 0.05$), as shown in *Figure 2*. The study showed a positive correlation of hyaluronic acid with E_2 at the day of triggering of ovulation in women who received letrozole plus Gonadotropin, ($r: 0.28$ and $P = 0.22$), (*Figure 3*). The study showed a positive correlation between

Table (1): General characteristics of females enrolled in the present study

Characteristics	Number of cases (Total: 20)	Percentage (%)
Residence		
Rural	12	60%
Urban	8	40%
Age groups (years)		
<30	11	55%
≥30	9	45%
Mean± SD	29.5±4.52	
Parity		
0	10	50%
1	5	25%
2-3	4	20
4-5	1	5%
Duration of infertility (years)		
2-4	12	60%
5-7	6	30%
>7	2	10%
BMI (kg/m²) (Mean± SD)	26.14±4.31	
LH (mIU/ml)	8.67±4.54	
E2 (pg/ml)	280.5±191.4	
progesterone (ng/ml)	4.15±3.2	

Table (2): Level of hyaluronic acid in the studied groups

Hyaluronic acid (pg/ml)	Treated Group	Control Group	P-Value
N	20	20	
(Mean ± SD)	158.7±19.7	146.1±26.2	0.031

Figure (2): Correlation between hyaluronic acid and LH of group A at day of follicles triggering

Figure (3): Correlation between hyaluronic acid and E₂ of group A at day of follicles triggering

Figure (4): Correlation between hyaluronic acid and progesterone of group A at day of follicles triggering

Figure (5): Correlation between hyaluronic acid number of follicles in group A

Figure (6): Correlation between hyaluronic acid and endometrial thickness in group A

Figure (7): Relation of HA with stimulation outcomes

hyaluronic acid and progesterone at the day of triggering of ovulation in women received letrozole plus Gonadotropin, ($r=0.28$ and $P=0.24$), (*Figure 4*). The study showed a positive correlation between hyaluronic acid and the number of follicles in women after receiving letrozole plus Gonadotropin, ($r=0.503$ and $P=0.024$), (*Figure 5*). The study showed a positive correlation between hyaluronic acid and endometrial thickness in women who received letrozole plus Gonadotropin, ($r=0.219$ and $P=0.354$), (*Figure 6*). The study revealed that HA mean was elevated significantly ($P < 0.05$) in women who became pregnant after stimulation protocol compared with women who failed to be pregnant (164.2 vs. 152.1 pg/ml) as shown in *Figure 7*.

5. Discussion

It is important to understand the host physiological and pathological mechanisms underlying events of the female reproductive tract, such as endometriosis, tumors, and infertility

(*González-Ramos, et al* ^[9]). There were very few declared studies regarding the relation of hyaluronic acid with infertility and no previous studies were done about the levels of HA in women who received letrozole plus Gonadotropin and who received Clomid plus Gonadotropin. A study done by (*Simões, et al* ^[10]) indicated that there was an elevated concentration of hyaluronic acid in women endometrium in the proliferative phase and higher HAS1 and HAS2 reactivity when compared with normal women. In addition, (*Nagyova, E* ^[11]) indicated that HA levels were increased in the endometrial stroma during the secretory phase of the menstrual cycle and fall to very low levels at menstruation, suggestive of a role in implantation. In different studies, (*Gomes, et al* ^[12]) in the study the immune-expression of hyaluronic acid (HA) in the uterine horns of the mouse throughout the estrous cycle phases, their data showed that the highest concentration of HA in uterine horns occurred during diestrus compared with other phases. Moreover,

(*Mohey-Elsaeed, et al* ^[13]) reported spatiotemporal expression of RHAMM protein in mouse endometrium during the estrous cycle and peri-implantation period, suggesting its possible role in endometrial receptivity. HA has also been shown to improve the cryotolerance of blastocysts, which then leads to increased birth rates in cows (*Lane, et al* ^[14]). Moreover, HA was detected in oviductal fluids collected by catheterization during the estrous cycle in heifers and cows (*Simon, et al* ^[15]) and was shown to be highest at ovulation (*Stojkovic, et al* ^[16]). Synthesis of HA is increased significantly in the uterus of mice on the day of implantation (*Fouladi-Nashta, et al* ^[17]), and HA differential expression in the human endometrium during the menstrual cycle implies its involvement in implantation. In the human uterus, the peak expression of HAS and CD44 is in the mid-secretory stage (*Afify, et al* ^[18]). There is a plethora of data suggesting the beneficial roles for HA in human embryo

implantation (*Ahmeid M.* ^[7], *González-Ramos, et al* ^[9]).

6. Conclusions

It was concluded that hyaluronic acid was elevated significantly in women who received ovulation stimulation drugs and letrozole was a good treatment for anovulatory cycle women.

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Author Contribution

AlJuboory, SKH performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Jwad, MA, and Al-Hilli, N.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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She received the M.B.CH.B. from the College of medicine, University of Baghdad in 2002.

She worked in Mesan and Kirkuk until she occupied a position of senior house officer during her permanency in obstetrics and gynecology 2008-2010 and from 2010-2012 she was the manager of a primary healthcare center until the start of residency program in 2012 at Tikrit Teaching Hospital and graduated as a specialist in Obstetrics and Gynecology in 2014 with H.D of Obst. And Gyne. She worked as a specialist in Kirkuk General Hospital in Kirkuk city. Currently, she is attending in Al-Nahrain University, the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies.

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