Effect of Letrozole on Hyaluronic Acid Concentration of the Endometrial Wash

Received: 31-Jul-2020 Accepted: 21-Aug-2020 Published: 14-Dec-2020

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

Females

Physical and Clinical Exam + Hormonal assay

CD2 Basal U/S Exam

20 control group with natural cycle without any stimulation

20 patient group with Letrozole and FSH

Day of Trigger
U/S Exam Endometrial Thickness, Pattern, Number and Size of Ovarian Follicles
Endometrial Wash for Hyaluronic Acid Concentration

Pregnancy Test
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 [19]).

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±4.31 kg/m² (Mean± 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±19.7 pg/ml) than the control group (146.1±26.2 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 E₂ 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

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

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

<table>
<thead>
<tr>
<th>Hyaluronic acid (pg/ml)</th>
<th>Treated Group</th>
<th>Control Group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>(Mean ± SD)</td>
<td>158.7±19.7</td>
<td>146.1±26.2</td>
<td>0.031</td>
</tr>
</tbody>
</table>
**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

AlJuboory, et al. [http://doi.org/10.28969/IJEIR.v10.i1.r2](http://doi.org/10.28969/IJEIR.v10.i1.r2)
Figure (6): Correlation between hyaluronic acid and endometrial thickness in group A

P-VALUE: 0.043

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,
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.

### Acknowledgment

We would like to acknowledge Al Nahrain University, Baghdad, Iraq.

### Funding

This work received no funding.

### 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.

**References**


Endocrinology. 2019 Feb 1;35(2):133-7. [Online Article Link]


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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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She received her MBChB. From the College of Medicine at the University of Baghdad in 1996. Her M.Sc. in Applied Embryology and her Ph.D. in Infertility and Clinical Reproduction were from the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University in 2007 and 2018 respectively. She worked as a rotator in the Baghdad health department from 1996-1999. She worked in Gyn. & Obs. in Alsamawa general hospital and Babylon hospital from 2000-2003. She worked at the Babylon University, College of Medicine, anatomy and embryology department from 2003-2004. She has been working as a specialist physician and a consultant clinic at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University from 2008-2015. Currently, she is an assistant professor and specialist in infertility and clinical reproduction. She is the head of the clinical reproductive physiology department from 2019 till now. She has more than 25 published articles in national and international journals.

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She was born in Babylon, Iraq in 1976. She received the M.B.Ch.B. from College of Medicine, the University of Babylon in 1999, the Diploma in Obstetrics & Gynecology in 2005, Iraqi Board in Obstetrics & Gynecology (FIBMS) in 2006, the Part I MRCOG in 2006, and the Certificate of minimal access surgery from the Iraqi Ministry of Health in 2017. Currently, she is an Assistant Professor and the Head of the department in Obstetrics & Gynecology department, College of Medicine, University of Babylon. She is also a lecturer and supervisor of Ph.D. students in the High Institute of Infertility and Assisted Reproductive Technologies since 2016. She is a member of the medical education committee at the University of Babylon, College of Medicine, and a member of the scientific committee of Obs. & Gyne. department at the University of Babylon, College of Medicine. She participated in more than, 25 Symposiums, 3 International Symposiums, 18...
National Conferences, 15 International Conferences, 16 Workshops, and 9 Training Courses. She published more than 21 articles both local and international. She has more than 200 diagnostic & operative hysteroscopy operations & More than 200 diagnostic and operative laparoscopies performed for infertility patients.

**How to cite:**

AlJuboory SKH; Jwad MA; Al-Hilli N. Effect of Letrozole on Hyaluronic Acid Concentration of the Endometrial Wash; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2020); 10(1): 20-34. Doi: [http://doi.org/10.28969/IJEIR.v10.i1.r2](http://doi.org/10.28969/IJEIR.v10.i1.r2)

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