

Relation of Endometrial Hyaluronic Acid with Female Infertility in Women Undergoing Ovarian Stimulation Protocol

Sarab Khlalaf Al-Juboory¹, Mufeda Ali Jwad², Muayad Sraibet Abood³

¹M.B.Ch. B. HD Ob & Gyn, Kirkuk Health Directorate, Kirkuk, Iraq, ²Lecturer Dr, Clinical Infertility, Reproductive Technologies, Baghdad, Iraq, ³Assistant Prof, Pharmacology, Al-Nahrain University, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies Baghdad, Iraq

Abstract

A 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 aim of the study was to evaluate the level of hyaluronic acid (HA) in endometrial fluid at day of trigger in infertile women whom undergoing ovarian stimulation protocol. The study included a total of 60 women who were selected from those attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. All couples subjected to a full history taking, complete general examination, complete gynecologic examination and infertility workup including: husband's semen analysis, hormonal analysis, uterine cavity and tubal patency was performed. Different ovulation induction programs were used evaluation of endometrial thickness, pattern, size and number of mature follicles was performed by transvaginal sonography in the 2nd of menstrual cycle and cycle day 11-14 before HCG injection. In this study basal hormonal analysis were performed to the female in the 2nd or 3rd day of the menstrual cycle and study included aspiration of endometrial fluid for determination of hyaluronic acid level by ELISA technique. The study showed that mean of hyaluronic acid was elevated significantly in women received letrozole plus FSH (158.7±19.7 pg/ml) followed by women received Clomid plus FSH (160.8±13.5 pg/ml) and the lowest mean was recorded in the control group (146.1±26.2 pg/ml). The study showed positive correlation between hyaluronic acid and LH at day of triggering of ovulation in women received Clomid plus FSH (group B). The study showed positive correlation between hyaluronic acid and progesterone at day of triggering of ovulation in women received letrozole plus FSH. The study showed positive correlation between hyaluronic acid and number of follicles in women after receiving letrozole plus FSH. 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 4). The study also revealed that HA was significantly elevated in women who became pregnant after receiving letrozole + FSH (164.2 pg/ml) as compared with non pregnant women (152.1 pg/ml) while HA level was non significantly related with pregnancy occurrence after administration of Clomid+FSH. It was concluded that, hyaluronic acid was elevated significantly in women received ovulation stimulation drugs and letrozole was superior to clomiphene as a treatment for anovulatory infertility in women

Keywords: Hyaluronic acid; Female infertility; Letrozole; Endometrial.

Introduction

Infertility is a disease of the reproductive system, defined by the failure to achieve a pregnancy after at least one year of regular unprotected sexual intercourse in women < 35 years not using contraception and after six months in women > 35 years. The causes of infertility can be generally classified into four groups: male, female,

combined (both male and female) and unexplained. Some additional factors that may contribute to female infertility are behavioural factors such as diet, exercise, smoking, alcohol and drug use^(1,2). Combined infertility arises from the combination of male and female causes and it may be that each partner is independently fertile but the couple cannot conceive together without assistance. Nowadays,

progress in assisted reproductive technology (ART) has enabled the clinicians to treat many types of infertility⁽³⁾. Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation⁽⁴⁾. It is the window of time when the uterine environment is conducive to blastocyst acceptance and subsequent implantation⁽⁵⁾. Hyaluronic acid (HA) has also a significant role in establishing the cellular microenvironment conducive to the development of proliferative process⁽⁶⁾. The lack of HAS regulation genes causes abnormal production of HA and promotion of abnormal biological processes such as metastasis and pregnancy loss. An increasing number of reports suggests a role of hyaluronan (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⁽⁴⁾. The aim of the study was to evaluate the level of hyaluronic acid (HA) in endometrial fluid at day of trigger in infertile women whom undergoing ovarian stimulation protocol.

Patients and Method

A 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 60 women who were selected from those attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. All couples subjected to a full history taking, complete general examination, complete gynecologic examination and infertility workup including: husband’s semen analysis, hormonal analysis, uterine cavity and tubal patency was performed. Different ovulation induction programs were used evaluation of endometrial thickness, pattern, size and number of mature follicles was performed by transvaginal sonography in the 2nd of menstrual cycle and cycle day 11-14 before HCG injection. Pregnancy outcome was used as a main comparative parameter between selected groups. 40 patients undergone with one of two of ovulation stimulation programs [clomid

+ gonadotropin and letrozole + gonadotropin] had been chosen for each patient according to her age, history and hormonal assay. In this study basal hormonal analysis were performed to the female in the 2nd or 3rd day of the menstrual cycle and study included aspiration of endometrial fluid for determination of hyaluronic acid level by ELISA technique.

Results

In this study, majority of women enrolled in this study were belonged to rural area and below 30 years, parity below 1 and infertile between 2-4 years, as shown in Table 1.

Table 1: General characteristics of studied cases.

Parameters	No. (Total: 40)	Percentage
Residence		
Rural	24	60%
Urban	16	40%
Age Groups (Years)		
<30	22	55%
≥30	18	45%
Mean±SD	29.5±4.52	
Parity		
0	21	52.5%
1	10	25%
2-3	7	17.5%
4-5	2	5%
Duration of infertility (years)		
2-4	23	57.5%
5-7	13	32.5%
>7	4	10%
BMI	26.14±4.31	

The study showed that mean of hyaluronic acid was elevated significantly in women received letrozole plus FSH (158.7±19.7 pg/ml) followed by women received Clomid plus FSH (160.8±13.5 pg/ml) and the lowest mean was recorded in the control group (146.1±26.2 pg/ml), Table 2.

Table 2: Level of hyaluronic acid in the studied groups

Hyaluronic acid (pg/ml)	Women under ovulation stimulation programs		Control Group	P. value		
	Group A (received Letrozole +FSH)	Group B (received Clomid +FSH)		(Group A & B)	(Group A & Control)	(Group B & Control)
N	20	20	20			
(Mean±SD)	158.7±19.7	160.8±13.5	146.1±26.2	0.09	0.031	0.029

The study showed positive correlation between hyaluronic acid and LH at day of triggering of ovulation in women received Clomid plus FSH (group B) (Figure 1).

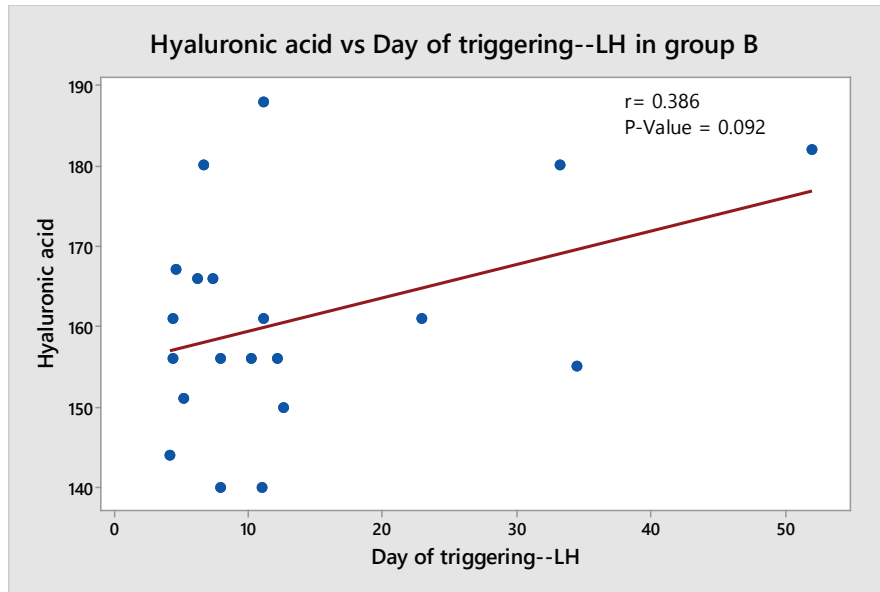


Figure 1: Correlation between hyaluronic acid and LH of group B at day of follicles triggering

The study showed positive correlation between hyaluronic acid and progesterone at day of triggering of ovulation in women received letrozole plus FSH (Figure 2)

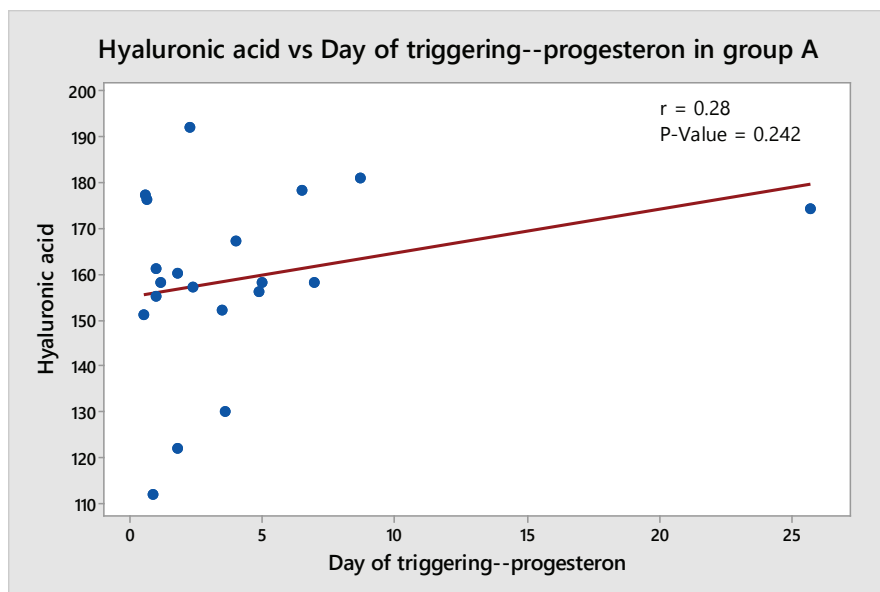


Figure 2: Correlation between hyaluronic acid and progesterone of group A at day of follicles triggering

The study showed positive correlation between hyaluronic acid and number of follicles in women after receiving letrozole plus FSH (Figure 3).

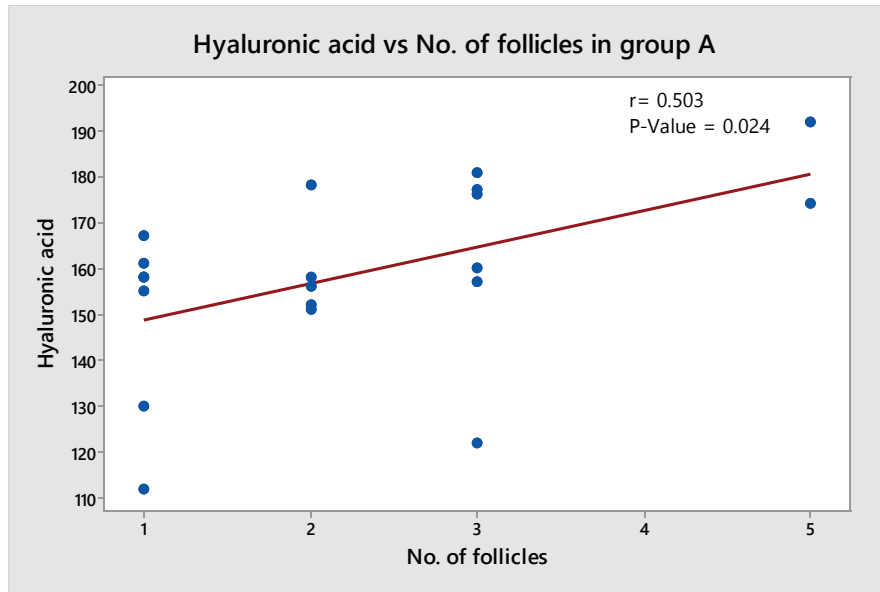


Figure 3: Correlation between hyaluronic acid and number of follicles in group A

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 4). The study also revealed that HA was significantly elevated in women who became pregnant after receiving letrozole + FSH (164.2 pg/ml) as compared with non pregnant women (152.1 pg/ml) while HA level was non significantly related with pregnancy occurrence after administration of Clomid+FSH.

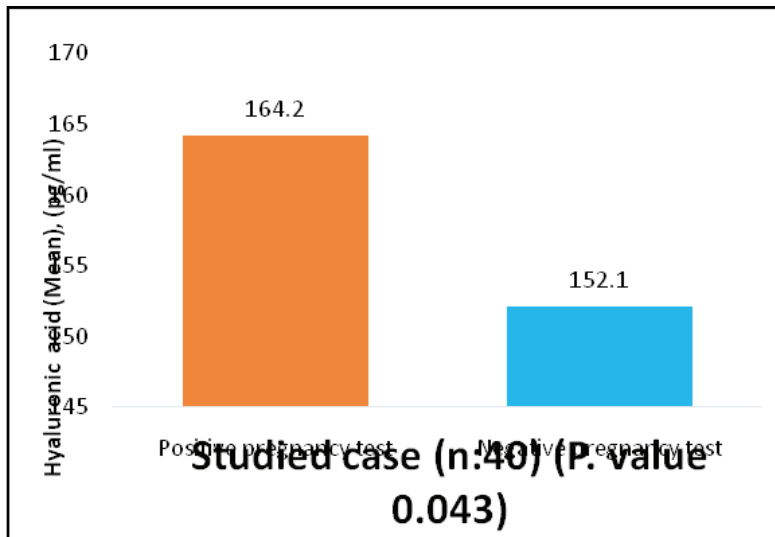


Figure 4: Relation of HA with stimulation outcomes

Discussion

In this study, majority of women enrolled in this study were belonged to rural area and below 30 years, parity below 1 and infertile between 2-4 years. These findings were close to that reported Ahmeid⁽⁷⁾, who

found that mean age of women under IVF was 30.36 year, his study also found that the mean of BMI was 25.9 (kg/m^2). Hameed and Ahmeid,⁽⁸⁾ in recent study included 45 women who enrolled in ART programs in infertility center for *in-vitro* fertilization (IVF) and

found that the mean age of infertile women was (31.80 ± 5.38 years), his study also found that the mean of BMI was 25.36 ± 1.99 (kg/m²). Al-Dujaily *et al*⁽⁹⁾ also found that the mean age of infertile women (31.0 year) and BMI 25.3 (kg/m²) Due to body mass index (BMI) has an adverse effect on reproduction, overweight women have a higher incidence of menstrual dysfunction and anovulation, possibly because of altered secretion of gonadotropin releasing hormone, sex hormone binding globulin, ovarian and adrenal androgen, and luteinizing hormone and also because of altered insulin resistance⁽¹⁰⁾. The study showed that mean of hyaluronic acid was elevated significantly in women received letrozole plus FSH (158.7±19.7 pg/ml) followed by women received Clomid plus FSH (160.8±13.5 pg/ml) and the lowest mean was recorded in the control group (146.1±26.2 pg/ml). Thus, studies on the distribution of HA are important to understand the histophysiological and pathological mechanisms underlying events of the female reproductive tract, such as endometriosis, tumors, and infertility⁽¹¹⁾. There was very little declared studies regarding the relation of hyaluronic acid with infertility and no previous studies done about the levels of HA in women who received letrozole plus FSH and who received Clomid plus FSH. A study done by Santos Simões *et al*⁽¹²⁾ indicated that there was an elevated concentration of hyaluronic acid in women endometrium in proliferative phase and higher HAS1 and HAS2 reactivity when compared with normal women. In addition, Nagyova⁽¹³⁾ 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 study, Gomes *et al*⁽¹⁴⁾ in study the immunoexpression 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, Fouladi-Nashta and coworkers reported spatiotemporal expression of RHAMM protein in mouse endometrium during the oestrous cycle and peri-implantation period, suggesting its possible role in endometrial receptivity⁽¹⁵⁾. HA has also been shown to improve the cryotolerance of blastocysts, which then leads to increased birth rates in cows⁽¹³⁾. Moreover HA was detected in oviductal fluids collected by catheterisation during the oestrous cycle in heifers and cows and was shown to be highest at ovulation⁽¹⁶⁾. In addition, Transcripts for HAS2 and

HAS3 have been found in the oviducts of several animal species⁽¹⁷⁾. Synthesis of HA is increased significantly in the uterus of mice on the day of implantation⁽¹⁸⁾, and HA differential expression in the human endometrium during the menstrual cycle implies its involvement in implantation. In the human uterus, peak expression of HAS and CD44 is in the mid-secretory stage⁽¹⁹⁾. There is a plethora of data suggesting the beneficial roles for HA in human embryo implantation⁽²⁰⁾. Artificially, Franik *et al.*,⁽²¹⁾ in previous study indicated that the supplementation of Whitten's medium with hyaluronic acid improves the development of 1- and 2-cell porcine embryos to the blastocyst stage

Conclusions

It was concluded that, hyaluronic acid was elevated significantly in women received ovulation simulation drugs and letrozole was superior to clomiphene as a treatment for anovulatory infertility in women.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk health directorate and all experiments were carried out in accordance with approved guidelines.

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