Original Article

Can Hyaluronan Binding Assay Predict the Outcome of Intrauterine Insemination in Couples with Unexplained or Mild Male Factor Infertility?

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Abstract

Background: The purpose of this study was to evaluate the prognostic effect of Hyaluronan Binding Assay (HBA) which has been used as a method of sperm selection for intracytoplasmic sperm injection procedure, on the outcome of intrauterine insemination (IUI) in couples with unexplained or mild male factor infertility.

Methods: 77 infertile couples were enrolled in our study. On the day of IUI procedure, HBA test was performed by using fresh semen samples, and the rates of sperm binding to HBA were calculated. HBA values and semen parameters were compared. Fisher exact test was used to evaluate the relationship between HBA ratio and pregnancy status. Mann-Whitney U test was used to compare quantitative variables between pregnant and non-pregnant groups. The $p<0.05$ was considered statistically significant.

Results: In this study, HBA ratio was 69(29.25%) and pregnancy rate was 14.29%. A significant positive correlation between HBA and total motile sperm count, inseminating sperm count, progressive motility, morphology, and sperm concentration ($p<0.001$, $p<0.001$, p:0.007, $p<0.003$, p:0.003 respectively) was observed. Although HBA values in pregnant group were higher than those in non-pregnant group, this result did not reach the statistically significant level (HBA: 67(20%) for non-pregnant group, 80.5(21.3%) for pregnant group). Also, no relationship between HBA values and pregnancy status was found. Moreover, there was no significant correlation between pregnancy status and HBA ratios based on the suggested cut-off value of 60 in literature ($p=0.425$).

Conclusion: HBA does not predict the IUI outcome in couples with unexplained infertility or mild male factor infertility, but it can be used together with semen parameters to verify sperm quality.

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Introduction

Male infertility can be assessed by semen analysis using parameters such as sperm count, motility and morphology of the spermatozoa (1). However, these parameters are not able to accurately measure the fertilization capacity of the spermatozoa. In addition to semen analysis, other tests that provide more accurate information about sperm maturation and fertilization capacity are needed to lead the infertile couples to the appropriate treatment path. Hyaluronan Binding Assay (HBA) is originated from the idea of selective mature spermatozoa binding to hyaluronan (HA)

during the naturel process of fertilization. This test was first applied to ICSI procedure performed to treat male factor infertility. Studies evaluating HBA which applied ICSI procedures clearly indicated that sperm that are able to bind to HA show normal morphology without any DNA fragmentation, excessive cytoplasmic remnants or persistence of histone proteins (2, 3). Several other studies have also confirmed these findings (4-7). Sperm binding to HA demonstrates normal morphology as described in Kruger classification in which thawed spermatozoas were exposed to HA containing media and sperm velocity and long-term motility improved immediately (8, 9). In the literature, the three binding zones were described based on sperm binding to HA as excellent binding for >90%, medium binding for 60-90%, and low binding for <60% (2). This study recommends that IUI can be attempted in those where the binding was over 60% (2).

The objective of this study was to determine the prognostic effect of HBA on IUI cycles performed for treatment ofunexplained infertility and mild male factor infertility. Also, the correlation between pregnancy status and HBA ratios was evaluated based on the suggested cut-off value of 60 in aforementioned literature. In addition, the semen parameters with HBA values were compared in order to show any association between them.

Methods

This study is carried out as a prospective comparative study at Turgut Ozal University Hospital in Obstetrics and Gynechology Clinic between March 2012 and July 2013. The study consisted of 77 IUI cycles of 77 infertile couples with unexplained (UEI) or mild male factor infertility (MMI) irrespective of their history of previous IUI attempts. The study protocol was approved by the ethics committee at Turgut Ozal University and written informed consent was obtained from all couples. Demographic characteristics of participants including age, gravida, parity, body mass index (BMI), semen analysis, HBA values, IUI outcomes as well as a thorough general medical, obstetric, and gynecologic history were obtained from all patients and subsequently recorded for further evaluation.

All female patients underwent a detailed physical and gynecologic examination on the third day of their menstrual cycle. Couples with primary or secondary infertility were included in the study. All female participants aged 18-40 years with BMI ≥18 kg/m^2 and ≤30 kg/m^2 and the ones with a regular and spontaneous menstrual cycle of 21-35 days, normal pelvic examination, hysterosalpingography (HSG) with tubal patency, normal ovarian reserve test [antral follicle count (AFC) of 8-15, basal serum follicle stimulating hormone (FSH) levels <12 *IU/L*, estradiol (E2) levels <85 *pg/ml*)], and normal prolactin (PRL) and thyroid stimulating hormone (TSH) levels were included in the study.

Inclusion criterion for male participants for unexplained infertility group was having normal semen analysis described in 2010 World Health Organization (WHO) guidelines [volume ≥1.5 *ml*, sperm concentration $\geq 15\times10^6/ml$, progressive motility ≥32%, morphology ≥4% based on Kruger's strict criteria]. The mild male factor infertility group was described as having total motile sperm count >5 million without meeting the aforementioned criteria of normal semen analysis.

Women with history of breast, ovarian and endometrial cancer, stage 3-4 of endometriosis according to American Society for Reproductive Medicine (ASRM), any systemic or endocrine disorder, polycystic ovarian syndrome, sensitivity to ovarian situmulating agents were excluded from the study. Women without having an ovarian cyst >15 *mm* and endometrial thickness >5 *mm* identified on transvaginal ultrasound (TVUSG) on the third day of menstrual cycle received recombinant follicle-stimulating hormone (rFSH) (Gonal-F®; Serono, Istanbul), Puregon® (Merck Sharp Dohme, Istanbul, Turkey) with the starting dose of 75-100 *IU/day* adjusted upon patient's age, BMI and response to previous treatments. Ovarian response was evaluated by performing serial TVUSG examinations and serum E2 levels when necessary.

When providing at least one follicle ≥18 *mm*, recombinant human chorionic gonadotropin (rhCG) (Ovitrelle® 250 *μgr*, Serono, İstanbul) was administered subcutaneously and IUI procedure was carried out 35-36 *hr* later. In case of more than three follicles >14 *mm* or serume E2 levels >1500 *pg/ml* on the day of hCG administration, the cycle had to be cancelled.

On the day of IUI, semen samples were provided by masturbation after 3-5 days of sexual abstinence and left to be liquefied for 30 *min* at room temparature and sperm parameters were assessed according to WHO criteria. After using density gradient centrifugation for sperm preparation, 10 *μl* of sperm sample was placed on HBA kit (Bio

Coat, Washington, USA) which is comprised of a chamber with a molecular layer of hyaluronan covalently linked to it, and covered with a special transparent Cell-Vu grid coverslip. After duration of ten minutes at room temperature, sperm were observed to be bound to HA with a head-first orientation. Unbound sperm were found to be moving freely in the chamber. Subsequently bound and unbound sperm were counted under the microscope and the percentage of hyaluronan-binding sperm was calculated as the number of HA bound sperm divided by the total number of bound and unbound sperm. IUI procedure was carried out by using a soft catheter (Wallace, PM Group, Istanbul, Turkey) passed through the cervix into the uterine cavity and 0.3 *ml* of prepared sperm was injected into the cavity. Patients were allowed to rest for 15 *min* after the procedure. 15 days after IUI, serum β-HCG levels were measured to determine the existence of pregnancy unless women had their menstrual periods before that time. β-HCG levels >50 *mIU/ml* were accepted as positive result in favor of pregnancy and blood test was repeated 48 *hr* later. A clinical pregnancy was confirmed with an TVUSG examination for presence of gestational sac in the uterine cavity.

The continuous variables in the study were assessed by Shapiro-Wilk Test in order to show normality of statistical distribution graphically and normally distributed variables were reported as mean±standart deviation (SD). The median and interquartile range (IQR) were used when the data was not normally distributed. Mann-Whitney U test was used to compare quantitative variables between groups. Chi-square test was used to examine qualitative variables. Spearman correlation coefficient was used to show relationships between variables. Fisher exact test was used to evaluate the relationship between HBA and infertility-pregnancy status.

All analyses were performed using IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.); p-values less than 0.05 were considered significant.

Results

This study consisted of 77 infertile couples with unexplained (n=46) and mild male factor (n=31) infertility. Conception rate in couples was found as 14.29% (n=11). 36.4% of couples with pregnancy had mild male factor infertility (n=4) and 63.6% of them had unexplained infertility (n=7). There was no correlation between type of infertility and pregnancy status (p=0.731). The mean female age was 31.28±4.33 years, mean female BMI was 24.41±2.75 kg/m^2, the mean male age was 34.92± 4.75 years, the median total motile sperm count (TMC) was $19.90(20.4)\times10^6$, the median inseminating sperm count (IMC) was $5.81(4.9)\times10^6$, and the median HBA value was 69(29.25%). The mean endometrial thickness on the day of HCG administration was 8.41±1.46 *mm*, the median value of maximum follicle size was 18.5(1.0) *mm*, and the median duration of infertility was 24(24) months. In the study, pre and post-wash progressive sperm motility (PR) were found as 32.28±12.52% and 95.22±2.32%, respectively.

Table 1 shows the baseline characteristics of infertile couples and sperm parameters. When the HBA ratios among couples with and without pregnancies after the IUI procedure were compared, HBA ratio was found as 80.5(21.3%) and 67 (20%), respectively (Table 2). Even though HBA ratio was detected as higher in the pregnant group, this result did not quite reach the statistical significance.

As seen in table 2, characteristics of infertile couples (such as female age, male age, female BMI, duration of infertility, *etc*.) with or without pregnancy were evaluated and no significant difference between pregnant and non-pregnant groups was observed. Furthermore, duration of ovulation

Table 1. Baseline characteristics of infertile couples (n=77) and sperm parameters

Variable	Mean±SD	Median (IQR)
Female age (years)	31.28±4.33	--
Male age (years)	34.92±4.75	--
Endometrial thickness on the day of HCG (*mm*)	8.41±1.46	--
The number of follicles	--	1 (1)
Maximum follicle size (*mm*)	--	18.5 (1.0)
Duration of infertility (months)	--	24(24)
Duration of ovulation induction (day)	--	7(3)
Amount of gonadotropin (*IU*)	--	500(178.0)
Number of IUI	--	1(2)
Body mass index (female) (kg/m^2)	24.41±2.75	--
Total motile sperm count (million)	--	19.90(20.4)
Inseminating motile sperm count (million)	--	5.81(4.9)
Pre-wash progressive sperm motility (PR) (%)	32.28±12.52	--
Post-wash progressive sperm motility (PR) (%)	95.22±2.32	--
Sperm concentration (*million/ml*)	--	40(37.5)
Morphology (%)	--	3(2)
Hyaluronan binding ratio (%)	--	69(29.25)

SD: Standard deviation; IQR: Interquartile range

Table 2. Baseline characteristics of pregnant (n=11) and non-pregnant infertile couples (n=66) and sperm parameters

Variable	Pregnancy		
	Pregnant	Non-pregnant	p-value
Female age (years) *	32.5±3.87	31.13±4.42	0.65
Male age (years) *	33.5±3.11	35.09±4.93	0.35
Endometrial thickness on the day of HCG (*mm*) *	8.2±1.55	8.2±1.28	0.90
The number of follicles (n)	2 (0.75)	1(1)	0.60
Maximum follicle size (*mm*)	19.3(0.9)	18.35(1)	0.015
Duration of infertility (months)	15(20)	24(24)	0.35
Duration of ovulation induction (day)	8.5(5)	7(3)	0.58
Amount of gonadotropin (*IU*)	500(410)	500(150)	0.92
Number of IUI	0(2)	1(2)	0.72
Body mass index (woman) (kg/m^2) *	23.65±2.25	24.5±2.82	0.48
Total motile sperm count (million)	28.5(25)	20(20)	0.21
Inseminating motile sperm count (million)	7.6(7.5)	5.8(4.6)	0.93
Pre-wash progressive sperm motility (PR) (%) *	35.25±8.1	32±13.02	0.86
Post-wash progressive sperm motility (PR) (%) *	95.75±0.5	95.15±2.45	0.70
Sperm concentration (*million/ml*)	46(33.25)	40(38.25)	0.82
Morphology (%)	3(5)	3(2)	0.118
Hyaluronan binding ratio (%)	80.5(21.3)	67(20)	0.50

* Values are expressed as mean±standard deviation, rest is expressed as median (interquartile range)

induction, amount of gonadotropin used in IUI cycles, number of follicles, and endometrial thickness on the day of HCG administration were also compared between pregnant and non-pregnant groups, but none of the differences among them reached the statistical significance except maximum follicle size on the day of HCG administration [19.3(0.9) *mm*, 18.35(1) *mm*, respectively], (p=0.015) as reported in table 2.

The mean TMC value was found as 28.5(25)×10^6 in the pregnant group and 20(20)×10^6 in the non-pregnant group (Z=0.75; p>0.05). The mean IMC value was 7.6(7.5)×10^6 in the pregnant group and 5.8(4.6)×10^6 in the non-pregnant group. The differences in sperm parameters (progressive motility, morphology, TMC, IMC, *etc*.) between pregnant and non-pregnant groups were also not statistically significant (Table 2).

In the current study, no association between HBA ratios and patients' demographic features (female age, male age, duration of infertility, *etc*.) was detected. On the other hand, the results yielded a significant positive correlation between HBA and TMC, IMC, PR, morphology, and sperm concentration (r=0.491; p<0.001, r=0.486; p<0.001, r=0.319; p=0.007, r=0.384; p<0.003, r=0.351; p=0.003 respectively) (Table 3).

Table 3. Correlations between HBA and sperm parameters

Sperm parameters	HBA	
	r *	p-value
Total motile sperm count (million)	0.491	<0.001
Inseminating motile sperm count (million)	0.486	<0.001
Pre-wash progressive sperm motility (%) (PR)	0.319	0.007
Morphology (%)	0.384	0.003
Sperm concentration (*million/ml*)	0.351	0.003

*Correlation coefficient

Table 4. Conception rate with IUI based on HBA cut-off level of 60%

Pregnancy	HBA (%)		p-value
	<60	≥60	
Non-pregnant (*n, %*)	20(30.3)	46(69.7)	0.425
Pregnant (*n, %*)	2(18.18)	9(81.82)	

As shown in table 4, no significant correlation between pregnancy status and HBA ratios was found based on the suggested cut-off value of 60 in literature (2). Despite the fact that 81.82% of the pregnancies (n=9) occured at HBA values above 60, this finding was not statistically significant (p=0.425).

Discussion

In the literature, there are a few studies that evaluate the prognostic effect of HBA on IUI cycles. Wu et al. examined the association between HBA values and TMC, morphology, and pregnancy outcomes in IUI cycles and found no significant correlation among those variables. Consistent with Wu et al., this study also found no statistical relationship between HBA levels and pregnancy outcomes. However, our results revealed a significant correlation between HBA levels and TMC and morphology. Patients with positive pregnancy results exhibited higher TMC values of 28.5(25)×10^6 in our study, while TMC values were found greater than 10×10^6 in 90% of pregnant couples in Wu et al.'s study (10). In a related study, Boynukalin et al. also failed to demonstrate a difference between pregnant and non-pregnant groups in terms of HBA values (50.2±25.2%, 48.3±26.2%, respectively p>0.05) (11).

Roudebush et al. (12) evaluated the relationship between HBA levels and IUI outcomes among 39 infertile couples. In contrast with our findings, they found that the pregnant group had significantly higher HBA values than the non-pregnant group in IUI cycles. Based on their findings, Roudebush et al. concluded that HBA test could be used for selecting the functionally competent sperm in IUI cycles. In our study, HBA values in pregnant group were found to be close to those reported in Roudebush et al. (80.5(21.3%) *vs*. 82%, respectively). Contrary to Roudebush et al., HBA was found to be of limited value in predicting IUI outcome. The conflicting results can be attributed to differences in sample size and patient characteristics in these studies. When HBA threshold for IUI is taken as 60%, as suggested in Huszar et al. (2), 16.4% of couples with HBA levels equal or greater than 60% conceived with IUI and only 9% of couples with HBA values less than 60% succeeded at conception. However, these results were not statistically significant. The studies on HBA thresholds usually evaluate *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ISCI) cycles rather than IUI cycles (13-16) in the literature. In order to compare HBA levels with fertilization rates in IVF, Kovacs et al. (17) described three HBA zones according to HBA levels as 60%, 70%, and 80%. The authors find no significant difference between these groups (fertilization rate for HBA<60%: 53.9%, for HBA >80%:52.2%). In present study, significant positive correlation between HBA values and sperm count, PR, TMC, IMC, and morphology was reported. These findings are consistent with that of Tarozzi et al.'s (18) in which 60 infertile couples receiving IVF treatment were assessed and a high correlation between HBA values and morphology was found. Tarozzi et al. also demonstrated that high HBA levels were related to low levels of DNA fragmentation in sperm.

In a related study by Nijs et al. (19), HBA levels were found to be lower in the fertilization rate less than 50% group than those in the fertilization rate greater than 50% group (69.7%-79.2%, respectively).

In the current study, HBA levels in the pregnant group were found to be higher than those in the non-pregnant group [80.5 (21.3%) *vs*. 67 (20%), respectively], but the difference was not statistically significant. Therefore, it is concluded that high level of HBA or HBA>60% can not significantly predict the pregnancy ratios in IUI cycles.

Conclusion

In conclusion, our results reveal a strong positive correlation between HBA values and sperm count, PR, TMC, IMC, and sperm morphology. However, the predictive power of HBA for the IUI outcome in couples with unexplained infertility or mild male factor infertility does not reach a statistically significant level despite higher HBA values found in pregnant group. In order to establish the prognostic effect of HBA on IUI outcomes and its clinical use, further studies are needed to be performed.

Conflict of Interest

Authors declare that there is no conflict of interest.

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