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Evaluation of Endometrial Receptivity and Pregnancy Outcome in view of Assessment of Serum and Follicular Fluid Level of CD44 Following Administration of Hyaluronic Acid to Infertile Women

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Abstract

Background: Successful implantation needs a receptive endometrium, a functional and normal embryo at the stage of blastocyst developmental and a synchronized dialogue between embryonic tissues and maternal tissues. Local angiogenesis is an essential pre-requisite for implantation and pregnancy.

Aim of the study: the current study will potentially shed light on Rejuvenation or supplementation of Hyaluronic Acid (HA) and participation in vascular events involved in successful pregnancy further deciphering some pathological processes responsible for implantation failure

Patients and methods: This study included 63 infertile women (32 women as study group receiving hyaluronic acid and 31 control group) who were subjected to ovulation induction programs with GnRH antagonist protocols for IVF/ICSI procedures. Blood samples were taken by Vacutainer tubes and centrifuged according to the manufacturer's instructions for the preparation of serum samples. Serum samples for CD44 were obtained from each patient when the basal hormonal status was determined. The second time point when the samples were obtained on the day of ova pickup, after that the Grade 1(G1) embryos will be transferred inside uterine cavity. Then performance of endometrial receptivity parameters or patterns evaluation and patients were followed up to do pregnancy test after14 days after embryo transfer.

Results: There was no significant difference in mean serum CD44 between study group and control group at cycle day 2 ($p > 0.05$), but at day of Human Chorionic Gonadotropin (hCG), there was highly significant difference in mean serum CD44 between study group and control group ($p < 0.01$); the level being higher in study group. Regarding follicular fluid, there was highly significant difference in mean serum CD44 between study group and control group ($p = 0.010$); the level being higher in study group.

Conclusion: Treatment with hyaluronic acid resulted in significant increase in serum and follicular fluid CD44 and this increment causes significant improvement in local blood endometrial blood flow as reflected by reduction in resistive index and pulsatility index.

Key words: Endometrial Receptivity, Pregnancy outcome, CD44, Hyaluronic Acid.

Introduction

Implantation of Embryo, a unique biological process, represents the most essential step of the reproductive process. Successful implantation needs a receptive endometrium, a functional and normal embryo at the stage of blastocyst developmental and a synchronized dialogue between embryonic tissues and maternal tissues. The endometrium is receptive to implantation of blastocyst during a temporally and spatially restricted window, called the implantation window. This period is between 6–10 days after the LH surge and persists for about 48 h (1). Local angiogenesis is an essential pre-requisite for implantation and pregnancy (2). Several diagnostic ways have been suggested to "measure" or "estimate" the endometrial receptivity. These ways included ultrasound measures (uterine and subendometrial blood flows in addition to endometrial thickness) and local expression of compounds that are vasoactive; both depend on the local angiogenesis that happen around the period of implantation.

Hyaluronic acid, also called hyaluronan, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues (3). It is unique among glycosaminoglycans in that it is nonsulfated synthesis of Hyaluronic acid and its organization into an extracellular matrix are processes involved in successful ovulation and fertilization in most mammals, including human (4, 5). Hyaluronic acid (HA) has been reported to participate in the regulation of vascular development in a number of physiological processes (6). Specifically, high molecular weight HA has been shown to inhibit angiogenesis, whereas its enzymatic degradation products are by nature pro-angiogenic (7). On the basis of this information it hypothesized that HA may be involved in vascular modifications associated with implantation (8,9). Therefore, the current study will potentially shed light on Rejuvenation or supplementation of HA and participation in vascular events involved in successful pregnancy further deciphering some pathological processes responsible for implantation failure.

Methods

The present study proposal was conducted with the infertile female patients that were admitted to the High Institute for Infertility Diagnosis and ART's Clinics, other IVF centers and candidate for IVF/ICSI. After the Approval of the ethical committee that must be obtained prior to the initiation of the study. Inclusion criteria were: age of women between 18 and 39 years at the time of screening; basal serum follicle stimulating hormone level (FSH) <10 mIU/mL in the early follicular phase; good physical and mental health and recurrent implantation failure. The exclusion criteria were: ovarian cysts, endocrinopathies such as hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease and clinically suspected Cushing's syndrome, uterine pathology such as leiomyoma, adenomyosis, congenital uterine anomalies and tubal hydrosalpnix, endometriosis cases, hematological diseases, old age above 40 years, azoospermic cases (Testicular Biopsy). This study included 63 infertile women (32 women as study group receiving hyaluronic acid and 31 control group) who were subjected to ovulation induction programs with GnRH antagonist protocols for IVF/ICSI procedures. Blood samples were taken by Vacutainer tubes and centrifuged according to the manufacturer's instructions for the preparation of serum samples. Serum samples for CD44 were obtained from each patient when the basal hormonal status was determined. The second time point when the samples were obtained on the day of ova pickup, after that the G1 embryos will be transferred inside uterine cavity. Then performance of endometrial receptivity parameters or patterns evaluation and patients were followed up to do pregnancy test after14 days after embryo transfer.

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Categorical variables were presented as number and percentage while numeric variables were expressed as mean, range and standard deviation. Independent samples t-test was used to compare means of numeric variables between study group. Cutoff values were calculated using receiver operator characteristic (ROC) curve analysis. The level of significance was set at ≤ p 0.05.

Results

The current study included 63 infertile women who were randomly allocated into two groups, study group (n = 32) and control group (n = 31). The demographic characteristics of those women are shown in table 4.1. The mean age of all enrolled infertile women was 32.51 ±5.61 years and there was no significant difference in mean age between study group and control group, 33.16 ±5.17 years versus 31.84 ±6.04 years, respectively (p = 0.356). The mean body mass index (BMI) of all enrolled infertile women was 28.02 ±2.76 kg/m2 and there was no significant difference in mean age between study group and control group, 27.83 ±2.97 kg/m2 versus 28.22 ±2.56 kg/m2, respectively (p = 0.581).

The Comparison of serum and follicular fluid levels of CD44 between study group and control group at cycle day 2 is shown in table 2. There was no significant difference in mean serum CD44 between study group and control group at cycle day 2 (p > 0.05), but at day of hCG, there was highly significant difference in mean serum CD44 between study group and control group (p < 0.01); the level being higher in study group. Regarding follicular fluid, there was highly significant difference in mean serum CD44 between study group and control group (p = 0.010); the level being higher in study group.

The comparison of ultrasound findings and biophysical profile between study group and control group is shown in table 3. There was highly significant difference in mean endometrial thickness between study group and control group, 8.03 ±0.75 mm versus 6.41 ±1.39 mm, respectively (p < 0.001); the thickness being greater in study group. There was highly significant difference in mean pulsatility index and resistive index between study group and control group (p < 0.001); the level being less in study group.

The comparison of biochemical pregnancy rate between study group and control group is shown in figure 1. Overall, out of 63 infertile women, successful biochemical pregnancy was reported in 25 cases (39.7 %) and the rate of positive biochemical pregnancy of study group was higher than that of control group, 62.5 % versus 16.1 %, respectively in a highly significant manner (p < 0.001).

The differences in mean CD44 serum level at day of hCG and follicular fluid CD44 between study and control groups suggest the existence of cutoff values for these parameters that can predict positive biochemical pregnancy in infertile women subjected to ICSI. Therefore, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in figures 2 and 3 and table 4. Regarding serum CD44 at day of hCG, the cut off value was > 0.545 with a sensitivity level of 96 %, a specificity level of 55.3 % and an accuracy level of 73.7 %. Regarding follicular fluid CD44, the cut off value was > 0.774 with a sensitivity level of 72 %, a specificity level of 78.9 % and an accuracy level of 73.5 %.

Discussion

In the current study, the level of serum CD44 in study group was comparable to that of control group; even though, the amount of change in serum CD44 in study group was substantially higher in comparison with control group since the amount of change was 2.7 times and the difference in change was highly significant (p < 0.001) indicating that treatment with hyaluronic acid greatly increases serum CD44. CD44 is a widely-distributed type I transmembrane glycoprotein that binds hyaluronic acid (HA) in most cell types (10). Implantation failure is an important impediment to increasing success rates in assisted reproductive technologies. Knowledge of the cascade of morphological and molecular events at implantation remains limited. Cell surface CD44 and hyaluronate (HA) have been reported in the uterus, but a role in intercellular interaction at implantation remains to be evaluated (11). However, experimental work has shown that CD44–HA binding could be employed by embryos during initial docking, but the persistence of HA in epithelial cells might be detrimental to later stages of implantation by retarding attainment of stable attachment (11). Actually, after thorough search in the available published articles, we failed to find an article with similar study design in order to compare our results with it and therefore, our study provide a novel insight to the clinical value of using

hyaluronan in improving embryo implantation and pregnancy outcome during ICSI cycles.

In addition, in our study, the mean follicular fluid CD44 of study group was higher than that of control group in a highly significant manner and the same argument concerning VEGF can be applied here, and we favor the suggestion that the higher level of follicular fluid CD44 in study group is partly due to the effect of hyaluronic acid treatment because its level in study group is nearly double that of control group (1.05 versus 0.55). In the current study there was highly significant difference in mean endometrial thickness between study group and control group; the thickness being greater in study group. In this study in addition, there was highly significant difference in mean pulsatility index between study group and control group; the level being less in study group. Moreover, there was highly significant difference in mean resistive index between study group and control group; the level being less in study group.

Taking all these finding together, one can conclude that the significant improvement in vascular blood flow of endometrium, reflected by less impedance to vascular flow (significantly lower resistive index and significantly lower pulsatility index), resulted in significant increase in endometrial thickness. And the rise in blood flow in the study group in comparison with control group is due to hyaluronic acid treatment mediated by greater concentration of CD44.

In the current study, the rate of positive biochemical pregnancy of study group was higher than that of control group, 62.5 % versus 16.1 %, respectively in a highly significant manner ($p < 0.001$). The only possible explanation for this highly significant discrepancy in biochemical pregnancy rate in study group is attributable to treatment with hyaluronic acid with resulted in significant increase in endometrial thickness and better endometrial blood flow (less resistive index and less pulsatility index) and hence better endometrial receptivity and significantly higher embryo implantation rate. Based on current reuslts we were able to indentify cutoff values for these predictive parameters (CD44 at hCG, and follicular fluid CDE44); the cutoff values were: > 0.545 and > 0.774, respectively with variable levels of accuracies. In line with our observation, the interaction between haluronan and CD44 was the main player in improving embryo implantation and pregnancy outcome according to a previous report (12).

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Table 1: Demographic characteristics of infertile women participating in the current study.

Characteristic	Total *n* = 63	Study group *n* = 32	Control group *n* = 31	*p*
Age (years)				
Mean ±SD	32.51 ±5.61	33.16 ±5.17	31.84 ±6.04	0.356 I
Range	20 -42	21 -40	20 -42	NS
BMI (kg/m²)				
Mean ±SD	28.02 ±2.76	27.83 ±2.97	28.22 ±2.56	0.581 I
Range	22.84 -35.8	22.84 -33.67	23.42 -35.8	NS
Normal, *n* (%)	8 (12.7 %)	6 (18.8 %)	2 (6.5 %)	
Overweight, *n* (%)	37 (58.7 %)	16 (50.0 %)	21 (67.7 %)	---
Obese, *n* (%)	18 (28.6 %)	10 (31.3 %)	8 (25.8 %)	

n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p > 0.05

Table 2: Comparison of serum levels of vascular markers between study group and control group at cycle day 2.

Characteristic	Total *n* = 63	Study group *n* = 32	Control group *n* = 31	*p*
Cycle day 2 CD44 (ng/ml)				
Mean ±SD	0.63 ±0.21	0.65 ±0.21	0.61 ±0.21	0.405 I
Range	0.33 -0.99	0.33-0.99	0.37-0.99	NS
CD44 at day of hCG (ng/ml)				
Mean ±SD	0.68 ±0.28	0.84 ±0.28	0.50 ±0.11	< 0.001 I
Range	0.37 -1.37	0.46 -1.37	0.37 -0.67	HS
Follicular fluid CD44 (ng/ml)				
Mean ±SD	0.81 ±0.35	1.05 ± 0.31	0.55 ±0.14	< 0.001 I
Range	0.4 -1.7	0.57 -1.7	0.4 -0.77	HS

n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p > 0.05

Table 3: Comparison of ultrasound findings and biophysical profile between study group and control group.

Characteristic	Total *n* = 63	Study group *n* = 32	Control group *n* = 31	*p*
Endometrial thickness (mm)				
Mean ±SD	7.21 ±1.38	8.03 ±0.75	6.41 ±1.39	< 0.001 I
Range	4 -9	7 -9	4 -8	HS
Pulsatility index				
Mean ±SD	1.17 ±0.63	0.69 ±0.28	1.68 ±0.48	< 0.001 I
Range	0.4 -2	0.4 -2	1 -2	HS
Resistive index				
Mean ±SD	1.01 ±0.30	1.25 ±0.24	0.77 ±0.07	< 0.001 I
Range	0.6 -1.5	0.6 -1.5	0.7 -0.9	HS

n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p > 0.05; S: significant at p ≤ 0.05; HS: highly significant at p ≤ 0.01

Table 4: The characteristics of ROC curve analysis regarding CD44 at day of hCG and follicular fluid CD44 cutoff values that can predict a positive pregnancy test.

Characteristic	CD44 hCG	FF CD44
Cutoff	> 0.545	> 0.774
AUC	0.737	0.735
95% CI	0.611 - 0.840	0.608 - 0.838
p	< 0.001	< 0.001
Sensitivity %	96.0	72.0
Specificity %	55.3	78.9
Accuracy %	73.7	73.5

FF: follicular fluid; PI: pulsatility index; RI: resistive index; ET: endometrial thickness; AUC: area under curve; CI: confidence interval

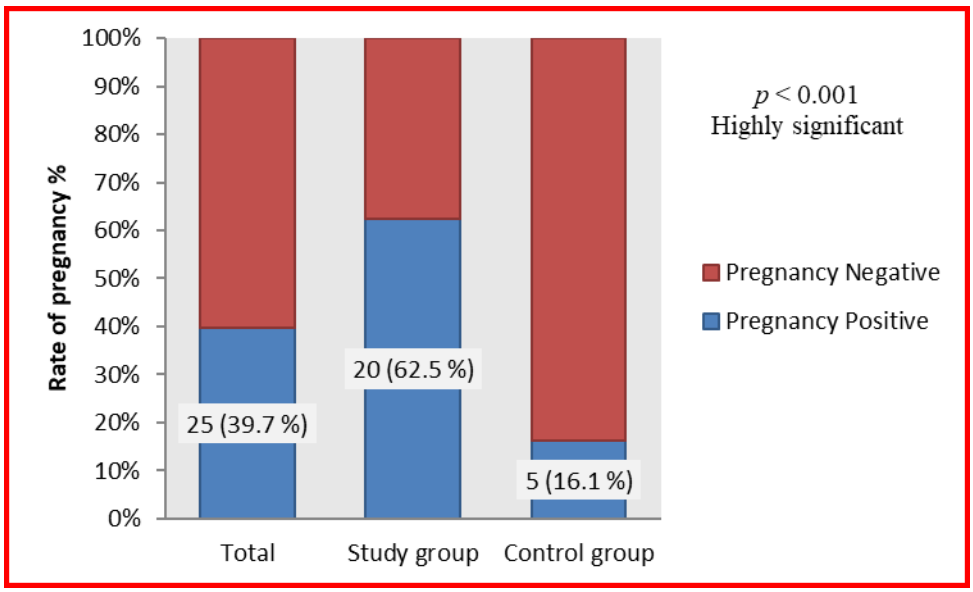

Figure 1: Comparison of biochemical pregnancy rate between study group and control group.

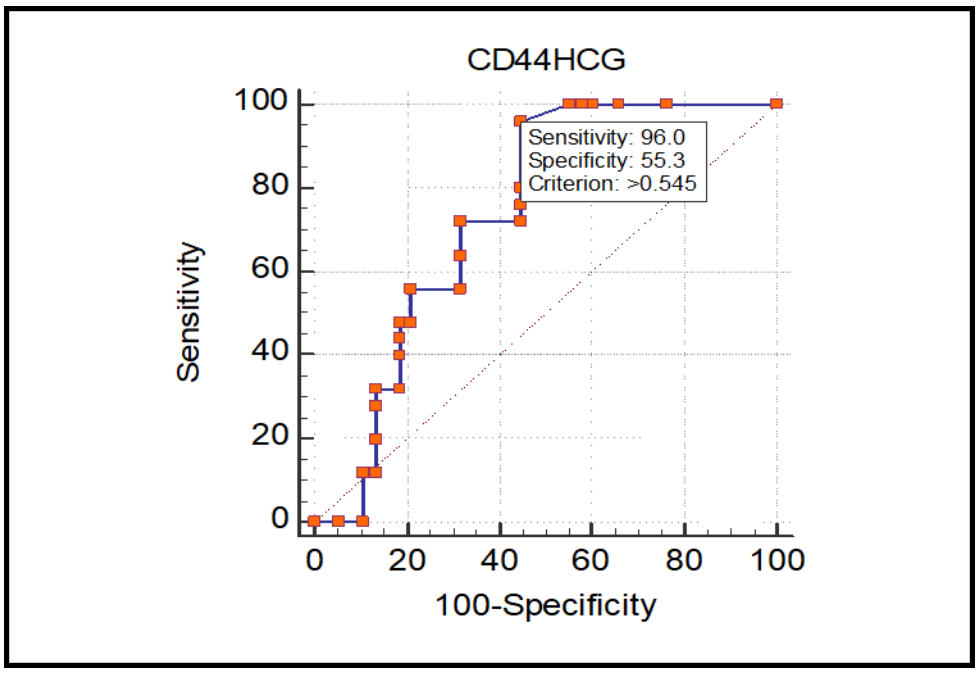

Figure 2: Receiver operator characteristic (ROC) curve analysis to find the best serum CD44 cutoff value at day of hCG that c an predict positive pregnancy outcome in terms of sensitivity and specificity.

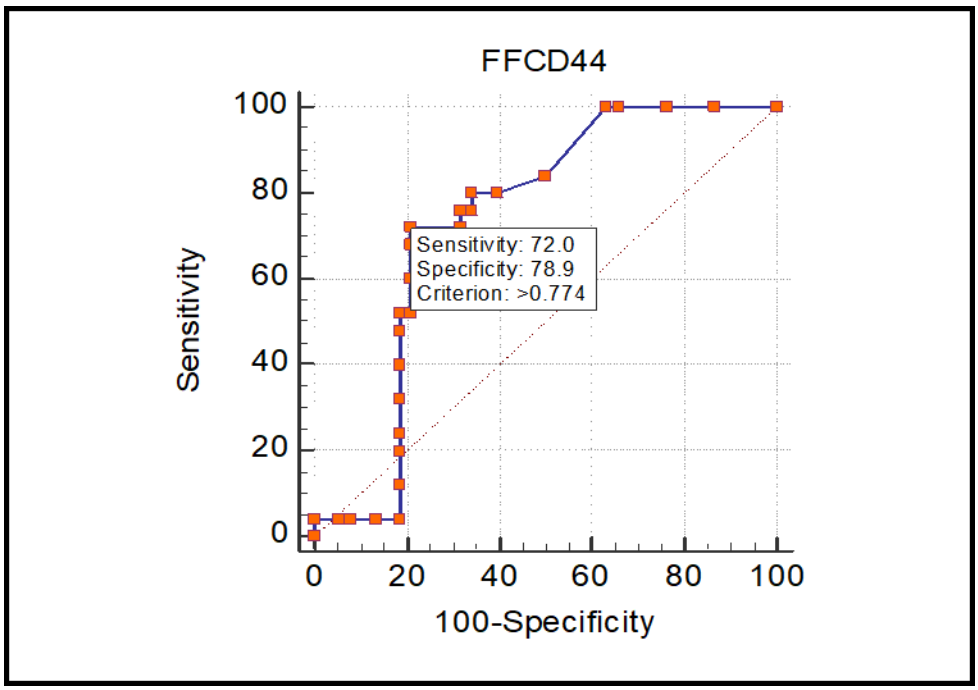

Figure 3: Receiver operator characteristic (ROC) curve analysis to find the best follicular fluid CD44 cutoff value that can predict positive pregnancy outcome in terms of sensitivity and specificity.