Assessment of EMMPRIN level in serum and follicular fluid and studying its relation to age, BMI and pregnancy outcomes in ICSI cycle

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Abstract

Background: Implantation is an important and critical process that have many complicated molecular and cellular steps between maternal and embryonic sides.

The aim of the study: to evaluate the relationship of EMMPRIN levels in follicular fluid and serum of intracytoplasmic sperm injection patients with oocyte. oocyte characteristics, embryo grading, and pregnancy outcome

Patients and Methods: The current study is across sectional study included 58 women. All of those women were prepared for ICSI and received recombinant follicle stimulating hormone (FSH) for stimulation of ovary at the second day of the cycle. Serum and follicular fluid samples were collected at ova pick up day and at the day of embryo transfer, another serum sample was collected. EMMPRIN (Basigin) levels in these samples were evaluated. Transvaginal ultrasound was done for all women for estimation of oocyte quality, size and foe evaluation of endometrial thickness at both days of oocyte retrieval and embryo transfer.

Result: The pregnancy rate of the current study was 27.6 %. The statistical analysis showed a low significant level of EMMPRIN in serum and follicular fluid at both days of oocyte retrieval and embryo transfer in women who get pregnant ($p < 0.05$). The mean endometrial thickness at both days of ova pickup and embryo transfer of pregnant women was significantly higher than that of non-pregnant women, ($p = 0.022$, $p = 0.047$) respectively. There was no significant correlation ($p > 0.05$) between serum and follicular fluid EMMPRIN to women age, BMI. There was a positive and significant correlation ($p < 0.001$) between serum EMMPRIN at day of ova pick up and serum EMMPRIN at day of embryo.

Conclusion: Serum and follicular fluid levels of EMMPRIN (Basigin) are not correlated to age, BMI of women undergo ICSI procedure and it may be considered a predictor marker for ICSI outcome.

Keywords: Basigin; EMMPRIN; ICSI; follicular fluid; ICSI.

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Introduction

One of the common and widely utilized procedure for the management of infertile patients are intracytoplasmic sperm injection (ICSI) (Esteves et al., 2018). Implantation to success required afunctional endometrium and any defect in the receptivity of endometrium may lead to implantation failure which represents about one third of causes of repeated failure of implantation (Chen et al., 2020). The extracellular matrix (ECM) provide support for cells of uterus which undergo constant degradation and remodeling in order to maintain the homeostasis of tissue and any defect in this homeostasis affect the implantation process. A functional endometrium is required for the initial interaction of embryo with endometrium

(Governini et al., 2021). There are several proteases enzymes that required for remodeling and degradation of extracellular matrix and matrix metallo-proteinases members (MMP) and their tissue inhibitors (TIMPs) considered one of the most important proteases (Scolari et al., 2017). EMMPRIN which is also called Basigin considered a potent inducer of MMP and has an essential role in expression and regulation of MMP. The aim of the study was to evaluate the relationship of EMMPRIN levels in follicular fluid and serum of intracytoplasmic sperm injection patients with oocyte. oocyte characteristics, embryo grading, and pregnancy outcome

Subjects material and method

Serum and follicular fluid samples of 58 infertile women who undergo ICSI procedure were collected at the infertility centre of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University/ Baghdad/ Iraq, from November 2021 till March 2022. The protocol that used by all the patient is antagonist protocol. All of them have their basal hormonal level of FSH, LH, TSH, and PRL. In the fertility clinic, the basic fertility work-up including history, physical examination, ovulation assessment, evaluation of patency and function of both tubes, uterine cavity assessment, hormonal measurement, exclusion of uncontrolled endocrine disorders and seminal fluid analysis was performed for all couples.

Women underwent agonist protocol, those with embryo grade III, serum AMH hormone level less than 1ng/ml, females who have systemic diseases or uncontrolled endocrine diseases, congenital anomalies of reproductive tract or big uterine fibroid, moderate to severe endometriosis, hydrosalpinx and male with severe oligoasthenozoospermia, azoospermia were all excluded from the study. Serum and follicular fluid samples were collected at the day of ova pick up and another serum sample was collected at the embryo transfer day and stored in (-20) for deep freezing for estimation of EMMPRIN then after. Transvaginal ultrasound was used for evaluation of oocyte size and for measurment of endometrial thickness on both days of oocyte retrieval and embryo transfer. EMMPRIN were measured evaluated in all the samples by Human EMMPRIN ELISA kit (Mybiosource /USA). After transferrinf the embryo by 14 days b-hCG level was measured and if it was > 5 mIU/Ml, this refer to the presence of the biochemical pregnancy. Depending on the presence of biochemical pregnancy post ICSI procedure, women was divided into two groups. Group 1 involved that get pregnant and the other group those who failed to get pregnancy.

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion).

Results:

The overall pregnancy rate of this cross sectional study was 27.6 % depending on biochemical pregnancy test as shown in **figure 1**. There was no statistical significant association between the EMMPRIN level in serum and follicular fluid with age and BMI of women underwent ICSI cycle ($p > 0.05$). As shown in **table 1.** Pregnant women had a significant higher endometrial thickness at day of oocyte retrieval 9.43 ±1.32 mm versus 8.70 ±0.96 mm, respectively ($p = 0.022$). and at day of embryo transfer 10.22 ±1.31 mm versus 9.60 ±0.99 mm, respectively ($p = 0.047$) in comparison to non-pregnant women. The results demonstrated that EMMPRIN level in serum and follicular fluid at ova pickup day and EMMPRIN level in serum at day of embryo transfer have a significant low level ($p < 0.05$) in females who get pregnant compared to non-pregnant women.

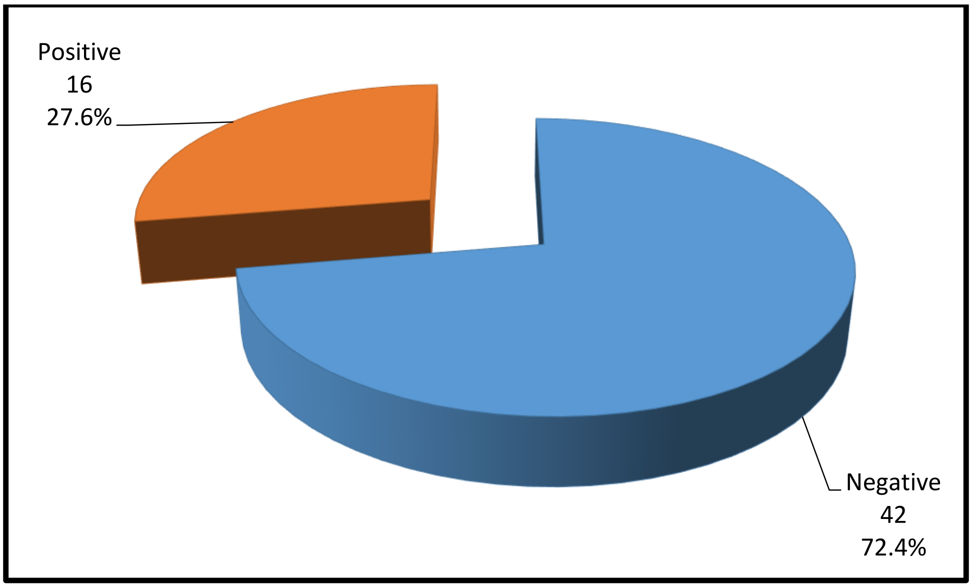

Figure 1: Pie chart showing the frequency distribution of infertile women enrolled in this study according to the results of biochemical pregnancy test

Table 1: The endometrial thickness and both day of ova pick up and embryo transfer

Characteristic	Total n = 58	Positive pregnancy n = 16	Negative pregnancy n = 42	P
Endometrial thickness at day of ova pick up				
Mean ±SD	8.90 ±1.11	9.43 ±1.32	8.70 ±0.96	0.022 I *
Range	7 -12	7.8 -12	7 -11	
Endometrial thickness at day of embryo transfer				
Mean ±SD	9.77 ±1.11	10.22 ±1.31	9.60 ±0.99	0.047 I *
Range	8 -12.2	8.3 -12.2	8 -12	

Table 2: Comparison of EMMPRIN levels in serum and follicular fluid between pregnant and non-pregnant women

Characteristic	Total n = 58	Positive pregnancy n = 16	Negative pregnancy n = 42	P
EMMPRIN level in serum at oocyte retrieval day				
Mean ±SD	60.51 ±37.01	44.10 ±35.98	66.76 ±35.86	0.036 I *
Range	9.34 -181.8	9.34 -107.96	17.83 -181.81	
EMMPRIN level in follicular fluid at oocyte retrieval day				
Mean ±SD	46.38 ±37.11	28.78 ±14.32	53.09 ±40.90	0.024 I*
Range	4.04 -236.9	4.04 -57.36	5.13 -236.92	

EMMPRIN level in serum at embryo transfer day				
Mean ±SD	57.88 ±34.08	42.32 ±29.92	63.80 ±34.02	0.031 I *
Range	3.43 -174.3	3.43 -90.21	15.43 -174.3	

n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; *: significant at p ≤ 0.05

Table 3: Correlations of serum and follicular fluid EMMPRIN to women age, BMI

Characteristic	Serum EMMPRIN at day of ova pick up		Follicular fluid EMMPRIN at day of ova pick up		Serum EMMPRIN at day of ET	
	r	p	r	p	r	P
Age	0.080	0.553	0.120	0.368	0.051	0.703
BMI	0.082	0.538	0.097	0.469	0.108	0.420

There was a positive and significant correlation between serum EMMPRIN at day of ova pickup and serum EMMPRIN at day of embryo transfer (r= 0.915; p < 0.001) as shown in **figure 2**. indicating that increasing the serum EMMPRIN level at day of ova pickup are considered as predictors of the higher serum EMMPRIN level at day of embryo transfer. In addition to that, the R^2 value was 0.836, this indicate that the association was strong and the correlation line can explain 83.6 % of concordance between X and Y co-ordinates.

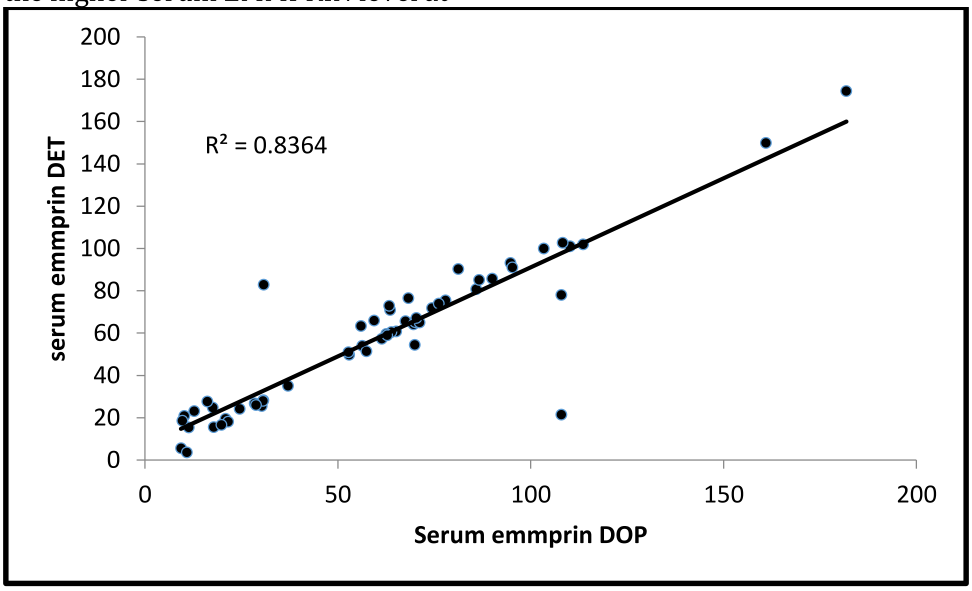

Figure 2: Scatter graph showing the correlation between serum EMMPRIN at day of ova pickup and serum EMMPRIN at day of embryo transfer

Table 4 demonstrated that the area under curve (AUC) was larger than 0.7; this mean that the accuracy of EMMPRIN level in serum and follicular fluid at both day of ova pick up and embryo transfer were accepted; while estimation of EMMPRIN level in serum at embryo transfer day considered as a weak marker to predict the ICSI outcome because the area under the curve was fewer than 0.7.

Measuring the level of EMMPRIN in both serum and follicular fluid together increase the EMMPRIN predictive power to predict the presence of biochemical pregnancy as the follicular fluid sensitivity greater than that of serum sample specificity better than that of follicular fluid.

Table 4: The parameters of ROC curve

Characteristic	Serum EMMPRIN At day of ova pick up	Follicular fluid EMMPRIN At day of ova pick up	Serum EMMPRIN At day of embryo transfer
Cutoff	≤17.6	≤40.4	≤24.65
AUC	0.71	0.738	0.667
95 % CI	0.576 to 0.822	0.606 to 0.845	0.531 to 0.785
P	0.018 *	< 0.001 **	0.055
Sensitivity %	50.0	87.5	50.0
Specificity %	100.0	64.3	88.1
Accuracy %	71.0	73.8	66.7

AUC: area under the curve; CI: confidence interval; *: significant at p ≤ 0.05; **: significant at p ≤ 0.01

Discussion

The thickness of endometrium is considered as a mirror for receptivity of endometrium and an indicator for the prognosis of the transferred embryo of women undergone ICSI procedure. The endometrial thinning is considered one of the risk factors that affect the process of implantation and lead to failure (Liu et al., 2018). The optimal endometrial thickness for achieving a conception is controversial for the clinicians. In general, the endometrium is considered sub-optimal for the transferred embryo if it is less than 8 mm by ultrasound examination resulting in decrease the probability of pregnancy (Makrigiannakis et al., 2021). Table 2 showed that serum EMMPRIN at both day of ova pickup and embryo transfer, follicular fluid EMMPRIN at day of ova pick up in pregnant women were all have a significant low level of EMMPRIN compared to those who fail to get pregnancy (p < 0.05), this similar to what was found by Takmaz et al., (2019). One of the causes of low EMMPRIN level in women with positive pregnancy, first of all, could be using of different methods for estimation of EMMPRIN level which may be vary from the methods that utilized for evaluation of EMMPRIN level in the previous studies that performed on animal model which clarified the high expression of EMMPRIN in women who get pregnancy (Dang et al., 2013). The other reason of this result might return to various expression of EMMPRIN in epithelial cells and stromal fibroblast during the luteal phase (Li & Nowak, 2020), So all these causes might affect the level of expression of EMMPRIN. Figure 2 clarified that there was a positive and significant relation between serum EMMPRIN at day of ova pick up and serum EMMPRIN at day of embryo transfer (r= 0.915; p < 0.001). This mean that increased serum EMMPRIN at day of embryo transfer can be predicted when serum EMMPRIN at day of ova pick up is elevated. In addition, the correlation was strong which indicates that evaluation of serum EMMPRIN at oocyte retrieval day can be enough and may be substituted for the estimation of EMMPRIN level in serum at embryo transfer day. This elevated level of EMMPRIN at ova pick up and embryo transfer days similar to result of study of Li & Nowak, (2020) and what was found by Turgut et al., (2014) that a significant decrease in the expression of EMMPRIN in the secretory phase was seen in endometrial cells of uterus in women recurrent failure in implantation in comparison to female with normal fertility. This result clarifies the critical EMMPRIN (Basigin) role in the process of implantation.

Conclusion

This study confirmed the validity of measurement of EMMPRIN in serum and follicular fluid as an indicator to predict the ICSI outcome. However, no association was found of serum and follicular fluid EMMPRIN with females age, BMI and evaluation of serum EMMPRIN at ova pick up can be sufficient and substituted for the measurement of EMMPRIN in serum at embryo transfer day.

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