



Evaluation of Plasminogen Activator Inhibitor-1 (PAI-1) Levels in Blood Serum and Follicular Fluid (Predictive Marker) and its Effect on Oocyte Maturity in Iraqi Infertile Women Undergoing ICSI

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ABSTRACT

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Plasminogen Activator Inhibitor-1 expression in serum and follicular fluid plays a crucial role in regulating fibrinolysis during the pregnancy period by blocking the action of other proteins called plasminogen activators. The aim is to assess the Plasminogen Activator Inhibitor-1 level in blood serum and follicular fluid as a predictive marker of pregnancy. This study included Sixty-nine infertile females undergone ovarian stimulation with antagonist protocol and received recombinant follicle-stimulating hormone, at the cycle day two. The serum and follicular fluid were collected from each female on the day of oocyte retrieval, for PAI estimation using the Elisa kit. The data were analyzed using version 23.0 SPSS and Microsoft Office 2010. The serum PAI-1 levels at the day of oocyte retrievals were significantly lower in positive pregnancy women in comparison with non-pregnant women ($p < 0.05$), Yet no significant follicular fluid PAI-1 levels were noted in comparison with non-pregnant women. There was a negative significant correlation ($p > 0.05$) between serum and follicular fluid PAI-1 levels on the day of oocyte retrieval according to oocyte ICSI outcome. The evaluation of serum PAI-1 levels can be used as a promising parameter for the prediction of pregnancy outcomes of ICSI patients. It is assumed that the pregnancy rate was affected by PAI-1 level during ICSI cycles. The concentration of PAI-1 in serum and follicular fluid at the retrieval day is not correlated with the oocyte's maturity.

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KEYWORD

Plasminogen Activator Inhibitor-1 PAI-1, ICSI , Oocyte maturity.

1. Introduction

According to World Health Organizations, Females and males, both of them are equally responsible of infertility causes. Globally, there are 148-186 million individuals have infertility (World Health Organization, 2022 [1]). Environmental and lifestyle factors such as smoking, excessive alcohol intake, obesity and exposure to environmental pollutants have been associated with lower fertility rates (Van Heertum and Rossi, 2017[2]; Silvestris et, al. 2018[3]). The prevalence of primary infertility worldwide in women aged 24-44 about 2 out of 100 women (Mosconi et al., 2021[4]).

The pregnancy is a state characterized by hypercoagulation with decline of fibrinolytic activity, which caused by an increase of Plasminogen Activator Inhibitor-1 (PAI-1) expression. During implantation, expressed the PAI-1 in extravillous interstitial trophoblasts and

vascular trophoblasts. However, PAI-1 is responsible for degradation extra cellular matrix (ECM), then inhibition of trophoblasts invasion. PAI-1 represents a biomarker for reproductive diseases and may be a treatment target in the future (Ye et al., 2017 [5]; Agersnap et al., 2022[6]).

Follicular fluid (FF) is a crucial external environment for the growth and development of oocytes. An in-depth analysis of specific components found in follicular fluid from women undergoing assisted reproductive therapy can advance our understanding of intracellular signal transduction and help us find potential oocyte health biomarkers (Xingxing et al., 2020[7]).

Ovulation is a time-coordinated tightly regulated proteolytic event. PAI-1 is a major of physiological inhibitor, primarily produced by granulosa cells in human follicular fluid pre ovulatory follicles, allowing localized degradation and regulating ovulation. While ovulation process is rendered redundant in IVF cycles;

nevertheless, PAI-1 levels in follicular fluid may influence maturity of oocyte within the follicle (**Chimote N M and Chimote B, 2022[8]**). Any defect in pathological disturbance for PAI-1 levels may lead to several pregnancy problems (**Pihusch et al., 2005[9]**). Expression of Plasminogen Activator Inhibitor-1 in serum and follicular fluid has critical role in embryo implantation and successful pregnancy outcome, which plays an essential role in regulating fibrinolysis during pregnancy period by blocks or inhibits the action of other protein called plasminogen activators, tissue type plasminogen activator (t-PA) and urokinase type plasminogen activator (u-PA) (**Ye et al., 2017[5]**).

2. Patients and Methods

The study covered the period from October 2022 to February 2023 and involved 69 infertile females with a mean age of (33.03 5.97) years who were enrolled in Assisted Reproductive Technology (ART) programs to enter

ICSI programs at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University (Baghdad/Iraq). Infertility factors including ovulatory cycles, blocked tubes, moderate endometriosis, and normal ovarian reserve are considered inclusion criteria for all infertile females. While uterine pathology such as leiomyoma, fibroids, adenomyosis, congenital uterine anomalies, müllerian malformations, polyps, and inadequate ovarian reserve were used as exclusion criteria for female patients.

Samples collection:

All infertile females' blood samples and follicular fluid were collected on the day that the oocytes were picked up. The blood samples (5ml) were taken from the cubital veins, and the follicular fluid (5ml) was then poured into a clean, plain gel tube for the serological test and allowed to clot at room temperature. The serum was centrifuged at a speed of 3000rpm for 10 minutes, and the follicular fluid

was centrifuged at a speed of 2000rpm for 10 minutes. Then placed in Eppendorf tube stored at (-20C°) and use for measurement of PAI-1 levels. Every female participant underwent an interview and provided information on age, length of infertility (years), BMI (kg/m²), type of infertility, causes of infertility, hormonal assay, virology assay, biochemistry assay, complete blood samples, ovulation detection, analysis of the husband's seminal fluid, and ultrasonography for females. Pregnancy test done for all female after 14 day of embryo transfers.

ELISA Assay:

Measurement the concentration of plasminogen activator inhibitor type 1 (PAI-1) (ng/mL), serum and follicular fluid levels by using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Diagnostic/ USA).

3. Results

- **Baseline Characteristics of level of PAI-1 enrolled in the Present Study:**

As Show in the Table (1). Serum PAI-1 levels range (3.47 – 16.62 ng/mL), and the range of follicular fluid PAI-1 levels (1.09–16.88 ng/mL).

- **The Oocytes characteristics and Fertilization Rate (FR%) in females in this study**

The oocyte characteristics of infertile females is shown in Table (2), involved mean number of oocytes, mean number of Germinal Vesicle oocytes GV, mean number of immature Metaphase I oocyte MI, mean number of mature Metaphase II oocytes MII, mean number Abnormal oocytes and mean number of Fertilization Rate % FR between females' groups.

(Table 1): Baseline characteristics of serum and follicular fluid PAI-1 levels.

Parameters	Range	Mean $\pm$ SD
Serum PAI-1 (ng/mL)	3.47 – 16.62	6.12 $\pm$ 1.98
Follicular fluid PAI-1 (ng/mL)	1.09 – 16.88	6.77 $\pm$ 2.56

SD: Standard deviation; PAI-1: Plasminogen activator inhibitor

(Table 2): Baseline ICSI outcome of females involved in the current study.

Parameters	Range	Mean $\pm$ SD
Total oocytes count	1-33	10.87 $\pm$ 6.01
Germinal vesicles	0-9	1.71 $\pm$ 0.94
Metaphase I (MI)	0 - 6	1.34 $\pm$ 0.98
Metaphase II (MII)	0-16	7.91 $\pm$ 4.11
Abnormal oocytes	1-7	2.30 $\pm$ 1.59
Fertilization rate % (	25-100	75.48 $\pm$ 19.44

SD: Standard deviation; MII: Metaphase II; MI: metaphase I.

- Correlations between serum and follicular fluid PAI-1 with oocytes ICSI outcome**

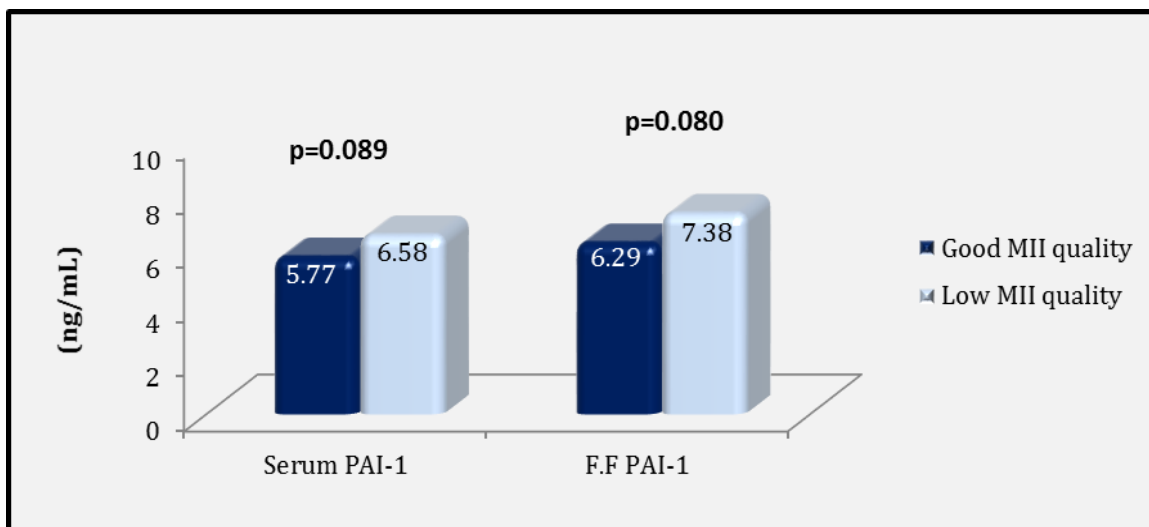
Correlation between serum PAI-1 levels at the day of oocytes retrievals and follicular fluid PAI-1 levels at the day of oocytes retrieval was non-significant correlation from the total

oocytes count, MII oocytes, MI oocytes, Germinal vesicles GV oocytes and Fertilization Rate with low Pearson's correlation coefficient and high *p* value as demonstrated in Table (3). The serum and follicular PAI-1 levels comparison can be illustrated in figure 1.

Table 3: Correlations between serum and follicular fluid PAI-1 with oocytes ICSI outcome.

Parameters		Serum PAI-1	Follicular fluid
Total oocytes	r	0.017	-0.047
	p	0.891 NS	0.703 NS
MII Oocyte	r	0.077	0.104
	p	0.532 NS	0.399 NS
MI Oocyte	r	-0.132	-0.028
	p	0.279 NS	0.821 NS
Germinal vesicles	r	-0.021	-0.060
	p	0.866 NS	0.625 NS
Fertilization Rate	r	-0.061	0.095
	p	0.618 NS	0.438 NS

MI: Metaphase II; MI: metaphase I; r: Pearson's correlation coefficient; NS: Non significant ($p > 0.05$).



(Figure 1): Comparison of serum and follicular fluid PAI-1 levels between women with good and low MII oocytes quality.

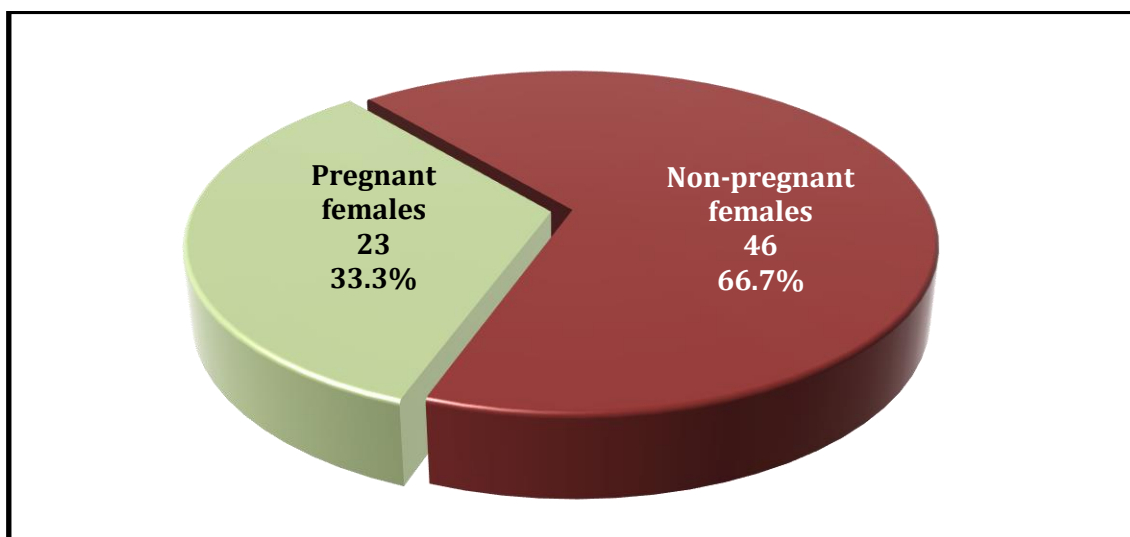
- **Pregnancy Rate (PR) in women enrolled in the present study**

The pregnancy outcome that was based on biochemical pregnancy test and the results were showed 23 women out of 69 had positive pregnancy test and making Pregnancy Rate equal to 33.3 % as shown in Figure (2).

- **Comparison of serum and follicular fluid PAI-1 levels between pregnant and non-pregnant women**

The comparison of serum PAI-1 levels at the day of oocytes retrievals

and follicular fluid PAI-1 levels at the day of oocytes retrievals between pregnant and non-pregnant women were demonstrated in Table (4) and Figure (3). According to the findings there were significantly lower serum PAI-1 levels in positive pregnancy women (5.41 ± 0.88 vs. 6.95 ± 0.56 & $p=0.013$); however, there was insignificantly lower follicular fluid PAI-1 in pregnant women (6.22 ± 1.97 vs. 7.61 ± 0.76 & $p=0.115$).

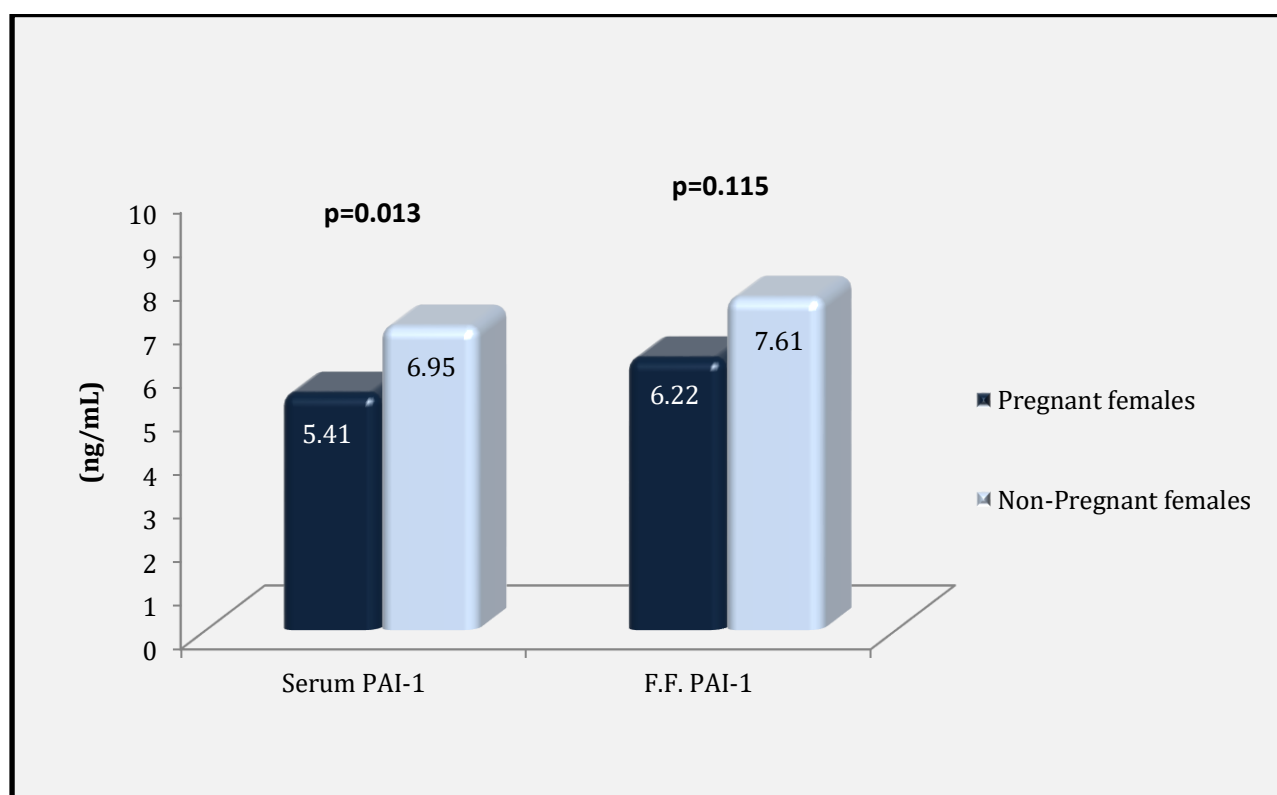


(Figure 2): Pie chart showing the frequency distribution of infertile women according to the results of biochemical pregnancy test (Pregnancy rate).

(Table 4): Comparison of serum and follicular fluid PAI-1 levels between pregnant and non-pregnant women.

Parameters	Pregnant women No.=23	Non-pregnant women No.=46	p value
Serum PAI-1	5.41 ± 0.88	6.95 ± 0.56	0.013 T
Follicular fluid	6.22 ± 1.97	7.61 ± 0.76	0.115 T

PAI-1: Plasminogen activator inhibitor; T: Independent sample t test; S: Significant ($p \leq 0.05$); NS: Non significant ($p > 0.05$).



(Figure 3): Comparison of serum and follicular fluid PAI-1 levels between pregnant and non-pregnant females.

4. Discussion

- **Baseline Characteristics of level of PAI-1 enrolled in The Present Study:**

As shown in a Table (1). The serum results were in line with the normal value PAI-1 levels present in plasma at concentration (2-46 ng/mL). These findings are consistent with the findings obtained by (Abbassi-Ghanavati et al., 2009 [10]; Tjärnlund-Wolf et al., 2012[11]).

Moreover, Zhang X et al agrees with this in terms of significant association between the PAI-1 levels and the risk of women infertility (Zhang X et al (2020[12])).

- **The Oocytes characteristics and Fertilization Rate (FR%) in women in this study**

As shown in Table (2), the present study shows the overall fertilization rate (FR) was (75.48 ±19.44). Which is identical to the previous studies representing the most efficient ART state which that fertilization rates obtained after ICSI treatment within

antagonist protocol are reported between (70% - 80%) (Cardona-Barberán et al., 2020[13]; Fatimah et al., 2019[14]). When a comparison was done between the fertilization rates in the groups, the statistical analysis recorded no significant difference between them, because of the similarity in the characteristics of females in the groups regarding age, ovarian reserve and the unique ICSI technician.

- **Pregnancy Rate (PR) in females enrolled in the present study**

As compared to the results of pregnancy rate (33.3 %) in this study, which is very close to Hassan et al study, a results published in which overall rate of pregnancy after ICSI was 32.3 (Hassan et, al. 2020[15]). In this study the result showed agreement with previous study which confirmed that human PAI-1 levels are very closely related to embryonic development and pregnancy success rates in pregnant females (Chen et al., 2015[16]).

- **Correlations between serum and follicular fluid PAI-1 with oocytes ICSI outcome**

As shown in Table (3), there was no significant correlation between serum and follicular fluid PAI-1 levels at the day of oocytes retrievals with oocytes ICSI outcome ($p > 0.05$). In serum PAI-1 levels (MII $p = 0.532$) than (MI $p = 0.279$) and (GV $p = 0.866$), and follicular fluid PAI-1 levels was (MII $p = 0.399$) than (MI $p = 0.821$) and (GV $p = 0.625$). The findings of the current study, agreed with Hassan's *et al* study which noticed no significant differences in total oocytes count, abnormal oocytes, germinal vesicles oocytes, metaphase II oocytes MII, metaphase I oocytes MI between females' groups (Hassan et, al. 2020[15]).

- **Comparison of serum and follicular fluid PAI-1 levels between pregnant and non- pregnant women**

As shown the Table (4) and Figure (3). According to the findings there were significantly lower serum PAI-1

levels in pregnant females (5.41 ± 0.88 vs. 6.95 ± 0.56 & $p = 0.013$). The PAI-1 measured between women and the corresponding linear rhythmic rise in serum PAI-1 levels is a key determinant of transfer embryos within the same cycle or to cryopreserve them and delay the embryo transfer until a later. Therefore, periodicity in serum PAI-1 levels is a reliable predictive indicator for estimating the likelihood of a clinical pregnancy following an IVF cycle.

- **Mechanism of PAI-1 in pregnancy**

Lower inhibitor (PAI-1) level observed at the time of is presumably necessary for active proteolysis of extracellular matrix components in order to prepare a “pocket” or “cavity” for the incoming embryo to implant. Thereafter, when the supposed ‘implantation window’ closes, persistence in proteolytic activity may cause difficulty in the development of implanted embryo. Therefore, the observed high levels in PAI-1 at day7

and day14 may logically account for the keeping of embryo and prevention of possible pathological invasion of embryos within the uterus. The PAI-1 levels recorded a continuous rise till the sixth week of gestation, which is in line with earlier reports of an increase in serum PAI-1 levels with gestational age (Mehta B N *et al.*, 2014[17]).

5- Conclusion

- The lower PAI-1 level in blood serum at the retrieval day is a promising parameter to predict pregnancy of females undergoing ICSI cycles. Also, the follicular fluid PAI-1 levels on the retrieval day were insignificant in pregnant females.
- Concentration of PAI-1 in serum and follicular fluid at the retrieval day is not correlated with the oocyte's maturity.

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Author Contribution

Samhar F. Alwan, performed the study, Rana A. R. Alsaadi, Hayder A. L. Mossa, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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