



Correlation of Pregnancy-Associated Plasma Protein (PAPP-A) in Serum and Follicular Fluid with Oocyte and Embryo Quality in PCOS and non-PCOS Women Undergoing ICSI Cycle

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ABSTRACT

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Pregnancy-Associated Plasma Protein (PAPP-A) is a zinc metalloproteinase in the insulin growth factor system (IGFs) produced by the syncytiotrophoblast region of the placenta. It plays a critical function in the cleavage of IGFBP4. In the ovary IGFs, it regulates follicular and oocyte maturation, and steroidogenesis. While in polycystic ovarian syndrome (PCOS) Hyperinsulinemia and hyperandrogenemia it causes follicular environment changes and early ovulation resulting in lower oocyte and embryo quality in patients and this will decrease the success of pregnancy in women enrolled in the ICSI cycle. The present study aimed to assess the relationship of PAPP-A levels in serum and follicular fluid in women with PCOS and non-PCOS with oocyte and embryo quality in women undergoing ICSI cycle. 45 infertile Iraqi women were enrolled. Women with PCOS had to meet at least two of the three criteria set by the Rotterdam ESHRE/ASRMS criteria, the age of the included women ranged between 20-45 years. In non-PCOS patients, PAPP-A has higher level in serum and follicular fluid but without a statistically significant difference matching with PCOS group. In addition, there was no significant correlation between PAPP-A levels in serum and follicular fluid with oocytes and embryo characteristics. However, PAPP-A levels are higher in serum and follicular fluid in women with positive pregnancy but without significant differences. PAPP-A had no correlation with oocyte and embryo quality.

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KEYWORDS

Infertility, Polycystic Ovarian Syndrome, Pregnancy-Associated Plasma Protein, Intra Cytoplasmic Sperm Injection, Oocyte and Embryo Characteristics

1. Introduction

Infertility is one of the most frequent health problems in the world, resulting from genetic or pathological diseases, prompting ongoing research and studies in the field of assisted reproductive technology (ART) to find the best treatment options possible (**Mongkolchaipak, S** ^[1]). Despite the efforts of fertility clinics worldwide in the field of ART, there are an estimated 13% of couples influenced by infertility. Therefore, a successful pregnancy needs high-quality oocytes and embryos (**Chen, et al.** ^[2]). Polycystic Ovarian Syndrome (PCOS) is the most frequent endocrine condition and the predominant cause of female infertility, according to studies conducted around the world (**Deshpande and Gupta** ^[3]). PCOS, affects 5–15 % of women and result of ovulatory failure (**Ecklund and Usadi** ^[4]). The clinical expression varies widely but commonly includes oligo- anovulation or anovulation, hyperandrogenism (either clinical or biochemical), and the presence of polycystic ovaries on ultrasonography (**Fauser, et al.** ^[5]). In PCOS patient premature granulosa cell luteinization

and disruption of the intrafollicular environment are caused by increased ovarian hyperandrogenism and hyperinsulinemia, resulting in poor follicular development and oocyte maturation (**Ecklund and Usadi** ^[4]). Pregnancy-Associated Plasma Protein A (PAPP-A) is a glycoprotein and a highly selective metalloproteinase that attaches to glycosaminoglycans on the cell surface and associated in the cleavage of IGFBP, specifically IGFBP4,5, resulting in an increase in bioactive IGF. PAPP-A also acts as a growth-promoting enzyme within the tissue, producing bioactive IGF when it comes into contact with IGF receptors (**Oxvig, C** ^[6]). IGFs are major intraovarian regulators of follicle growth, selection, cellular differentiation, and steroidogenesis, as well as oocyte maturation and cumulus expansion, according to members of the IGF family (**Kwintkiewicz and Giudice** ^[7]). The proteolytic activity of PAPP-A toward IGFBP-4 was detected in human follicular fluid. The IGF system appears to contribute to the reported resistance to follicle-stimulating hormone (FSH) activity and the persistence of an androgen-dominant environment in the

arrested follicles in PCOS, a condition characterized by small antral follicle arrest resulting in anovulation (**Kwintkiewicz and Giudice** ^[7] , **Öztürk, et al.** ^[8]). This study is going to assess the correlation between PAPP-A levels and oocyte and embryo characteristics in serum and follicular fluid of women undergoing Intracytoplasmic Sperm Injection (ICSI) cycle.

2. Materials and Methods

This study involved 45 infertile females who were enrolled in assisted reproductive technology (ART) programs through undergoing ICSI cycles at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq, during the period from September 2020 to May 2021. All couples were subjected to the basic fertility work-up of the fertility center, which includes case history, physical examination, ovulation detection, assessment of the tubal and uterine cavity, and semen analysis. The morphological evaluation of oocytes retrieved from infertile women and their resulting embryos was performed in the ICSI lab of the High Institute

for Infertility Diagnosis and Assisted Reproductive Technologies. While the measurement of PAPP-A in serum and follicular fluid was done using the Enzyme-Linked Immunosorbent Assay (ELISA) kit (Shanghai Biological, China). The blood samples were collected by venipuncture from each infertile woman on the both days of oocyte retrieval and embryo transfer. They were then centrifuged for 15 minutes at 1000 g. The serum was removed and stored at -20°C or -80°C. The Follicular Fluid (FF) was obtained from the first retrieved follicle to avoid contamination of blood and flushing medium, and collected in a plane tube. It was frozen at -20°C for approximately 20 minutes before being analyzed. The measurement of PAPP-A serum and follicular fluid levels was assessed by the ELISA kit.

3. Statistical Analysis

The data were investigated using Statistical Package for Social Sciences (SPSS) version 22.0 and Microsoft office 2007. The descriptive statistics including frequency, mean and standard deviation were measured to

describe the data. The groups were compared by applying independent sample t-test (unpaired t-test between two groups), paired sample t-test (same variable between two periods e.g. pick up and embryo transfer) and chi-square (for non-continuous data or percentage). The grade of relationship between continuous variables was calculated by Pearson's correlation coefficient (r) and the outcomes were considered statistically significant when p value was less than 0.05.

4. Results

A total number of 45 infertile women were nominated in this cross-sectional comparative study, 23 patients were diagnosed with polycystic ovary syndrome (PCOS group) and 22 patients without polycystic ovary syndrome (non-PCOS group), as shown in **Figure 1**.

4.1. Comparison of Mean Age and BMI Between PCOS and non-PCOS Patients

The comparison of mean patients age and Body Mass Index (BMI) between PCOS and non-PCOS patients were presented in **Table 1**. The results expressed as mean $\pm$ standard

deviation. There was a significant difference in BMI ($p=0.01$) between the two groups with higher BMI in PCOS patients, nevertheless there was no significant difference in mean patients age ($p=0.213$) between PCOS and non-PCOS patients.

4.2. Comparison of Hormonal Levels Between PCOS and non-PCOS Groups

The comparison of hormonal levels between PCOS and non-PCOS groups were established in **Table 2**. There were significant differences between the two groups about Antimullerian Hormone (AMH) ($p=0.008$) and E2 at day of trigger ($p=0.012$) with higher levels in PCOS group, but there was no significant difference in Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and basal Estradiol (E2) levels between PCOS and non-PCOS groups ($p > 0.05$).

4.3. Comparison of Pregnancy-Associated Plasma Protein Between PCOS and non-PCOS Patients

In the current study, at the day of oocytes pickup and at the time of embryo transfer, we

measured serum and follicular fluid (PAPP-A) levels, according to the results there were higher serum and follicular fluid (PAPP-A) levels in non-PCOS patients without statistically significant difference between PCOS and non PCOS groups ($p > 0.05$), as shown in **Table 3**.

4.4. Relationships Between Serum and Follicular PAPP-A Levels with Oocytes and Embryo Characteristics

The relationships between serum PAPP-A levels with patients' oocytes characteristics, and embryos characteristics were shown in **Table 4** and **Table 5** and the results also revealed no significant correlations between PAPP-A levels with all parameters (low Pearson's correlation coefficient and $p > 0.05$).

4.5. Comparison of Pregnancy-Associated Plasma Protein A (PAPP-A) Levels Between Pregnant and non-Pregnant Patients

The comparison of serum PAPP-A levels between pregnant and non-pregnant patients was presented in **Table 6**, as a result there was

higher serum and follicular fluid PAPP-A levels in women with positive pregnancy but without significant differences between pregnant and non-pregnant ($p > 0.05$).

5. Discussion

In the present study, there was no significant difference in mean patient age ($p=0.213$) between PCOS and non-PCOS patients. While the BMI was higher in PCOS patients and significant difference ($p=0.01$) between PCOS and non-PCOS patients, Furthermore, the statistical analysis of basal hormonal profile level in this research (FSH, LH, and E2) showed no significant differences ($P > 0.05$) between the studied groups. On the other hand, the granulosa cells that surround parenteral and antral follicles produce AMH, which plays a crucial role in follicle growth and maturation. AMH serum levels have been proposed as a marker for PCOS in several investigations (**Bako, et al.** ^[9]). The current study presented a significant difference in AMH levels ($p=0.008$) between the PCOS and non-PCOS groups at the day of trigger, with higher levels in the PCOS group.

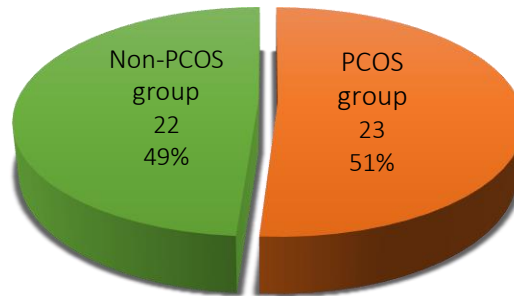


Figure (1): Comparison of demographic features between PCOS and non-PCOS patients

Table (1): Comparison of Age and BMI between PCOS and non-PCOS group

Parameters	PCOS patients (n=23)	Non-PCOS patients (n=22)	p-value
Age (years) (Mean $\pm$ SD)	33.3 $\pm$ 4.95	34.4 $\pm$ 5.5	0.213
BMI (Kg/m ²) (Mean $\pm$ SD)	28.16 $\pm$ 3.1	25.25 $\pm$ 4.06	0.010*

*: p value < 0.05 (significant)

Table (2): Comparison of hormonal levels between PCOS and non-PCOS groups

Hormone	PCOS group (Mean $\pm$ SD)	non-PCOS group (Mean $\pm$ SD)	p-value
FSH (mIU/ml)	6.11 $\pm$ 1.99	6.4 $\pm$ 1.84	0.663
LH (mIU/ml)	7.68 $\pm$ 6.79	4.8 $\pm$ 2.29	0.097
AMH (ng/ml)	3.02 $\pm$ 2.2	1.39 $\pm$ 0.86	0.008*
E ₂ (pg/ ml)	59.3 $\pm$ 26.9	55.78 $\pm$ 45.97	0.826
E ₂ at trigger time (pg/ ml)	1661 $\pm$ 1112	733 $\pm$ 626	0.012*

SD: Standard deviation; *: p-value < 0.05 (significant).

Table (3): Comparison of PAPP-A levels between PCOS and non-PCOS patient

PAPP-A level (ng/ml)	PCOS group (Mean $\pm$ SD)	non-PCOS group (Mean $\pm$ SD)	p-value
Serum PAPP-A levels at day of oocytes pickup	4.02 $\pm$ 3.73	7.96 $\pm$ 5.84	0.079
Serum PAPP-A levels at day of embryo transfer	14.48 $\pm$ 6.52	16.46 $\pm$ 9.63	0.171
Follicular fluid PAPP-A levels	1.55 $\pm$ 1.18	1.59 $\pm$ 0.90	0.916

Table (4): Relationships between PAPP-A Levels in serum and follicular fluid with Oocyte characteristic

Parameters		PAPP-A level in serum at oocytes pickup day	PAPP-A level in serum at ET day	Follicular fluid PAPP-A level
Total oocytes count	<i>R</i>	-0.112	0.015	-0.037
	<i>p-value</i>	0.461	0.922	0.811
MI	<i>R</i>	-0.087	0.017	-0.149
	<i>p-value</i>	0.575	0.911	0.334
MI	<i>R</i>	-0.133	0.056	0.053
	<i>p-value</i>	0.389	0.719	0.727
GV	<i>R</i>	-0.059	-0.055	-0.243
	<i>p-value</i>	0.706	0.729	0.113
Abnormal oocytes	<i>R</i>	-0.049	-0.130	-0.058
	<i>p-value</i>	0.753	0.398	0.709
Maturity index	<i>R</i>	0.038	0.009	-0.017
	<i>p-value</i>	0.803	0.952	0.914

Table (5): Correlations between PAPP-A Levels in serum and follicular fluid with embryo characteristic

Parameters	PAPP-A level in serum at oocytes pickup day		PAPP-A level in serum at ET day	Follicular fluid PAPP-A level
Total embryo count	<i>R</i>	0.222	0.049	0.002
	<i>p-value</i>	0.146	0.755	0.985
G I	<i>R</i>	0.232	-0.038	0.026
	<i>p-value</i>	0.128	0.806	0.866
GII	<i>R</i>	-0.002	0.005	0.053
	<i>p-value</i>	0.994	0.974	0.726
GIII	<i>R</i>	-0.082	-0.222	0.049
	<i>p-value</i>	0.602	0.145	0.755
Fertilization rate	<i>R</i>	0.189	0.109	0.051
	<i>p-value</i>	0.243	0.483	0.748

R: Pearson`s correlation coefficient

Table (6): Comparison of PAPP-A levels between pregnant and non-pregnant patients

PAPP-A level (ng/ml)	Positive pregnancy (n=17)	Negative pregnancy (n=28)	P-value
Serum PAPP-A levels at day of oocyte pickup	6.83 ± 10.85	5.62 ± 4.86	0.619
Serum PAPP-A levels at day of embryo transfer	14.88 ± 12.81	14.67 ± 10.47	0.968
Follicular fluid PAPP-A levels	1.77 ± 1.35	1.49 ± 0.83	0.404

p-value < 0.05 (significant).

Likewise, as (**Wiweko, et al.** ^[10]) proposed there were also statistically significant differences between the PCOS and non-PCOS patient in AMH levels. PAPP-A is a zinc-binding metalloproteinase that shows an important role in the IGF pathway (**Kiliç and Güler** ^[11], **Boldt et al.**, ^[12]). In this study, the level of PAPP-A was compared between the PCOS and non-PCOS groups, and the results presented that non-PCOS patients had higher serum and follicular fluid PAPP-A levels but without statistically significant differences ($p > 0.05$). While (**Wetta, et al.** ^[13]) showed that women with a PAPP-A value of less than 0.4 MoM were more expected to develop PCOS. Women with PCOS had a lower PAPP-A level than normal. However, there was no relation between infertility and PAPP-A levels that are less than 0.4 MoM. Although (**Öztürk, et al.** ^[8]) showed that the patients with PCOS revealed higher PAPP-A levels than healthy women, other studies suggested that PAPP-A levels increase throughout a normal pregnancy. There is a sharp increase of about 24-fold between the end of the first trimester and the end of the second trimester, followed

by a slower, 2-fold increase from the end of the second trimester up to delivery (**Leguy, et al.** ^[14]). These results reveal that the PAPP-A level increases in pregnant more than in non-pregnant women, mainly in the first trimester. Furthermore, in the current study, the relationships between serum and follicular fluid PAPP-A levels with patient oocyte characteristics and embryo characteristics, maturity index, and fertilization rate at days of pick-up and embryo transfer presented no significant correlations between PAPP-A levels with all parameters (low Pearson's correlation coefficient and $p > 0.05$). Other studies observed there was no significant correlation between PAPP-A levels, fertilization and good quality embryos in two groups of women undergoing ART (**Firouzabadi, et al.** ^[15]). These statistics suggested that PAPP-A levels are responsible for the follicular maturity index but not for the oocyte quality and pregnancy potential. PAPP-A levels in the blood start to increase soon after implantation and continue to increase during pregnancy, with a doubling period of 3–4 days during the first trimester and maximal levels at

term, PAPP-A looks to be the dominating IGFBP-4 proteinase in serum of pregnancy. (Kirkegaard, et al. ^[16] , Fialova and Malbohan ^[17]). Herein, we show higher PAPP-A levels in serum and follicular fluid in women with positive pregnancy but without significant differences between pregnant and non-pregnant ($p > 0.05$).

6. Conclusions

From this study, it was concluded that the higher level of PAPP-A in serum and follicular fluid in women with positive pregnancy but without significant differences can be used as a marker in predicting pregnancy after ICSI cycles. Secondly, non-significant difference was noted between PCOS and non-PCOS groups with higher PAPP-A levels in serum and follicular fluid on both days of oocyte retrieval and embryo transfer. In addition, there is no correlation between PAPP-A and oocytes and embryo characteristics.

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Author Contribution

Altimimi, ZS, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Jwad, MA, and Mohammed, AA.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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