



The significance of PlGF in the progression of the ovarian hyperstimulation syndrome in patients undergoing an in vitro fertilization procedure.

Aliyah Dhahir Habeeb ¹, Lubna Amer Al-Anbari ¹, Rehab Sh. Al-Maliki ¹

¹High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq.

aliyah.dhahir@ierit.nahrainuniv.edu.iq

ABSTRACT

Received:
07-May-2022
Accepted:
30-May-2022
Published:
12-Jun-2022

Placenta growth factor is, VEGF family member, a Pleiotropic Factor affects a variety of cell types and regulates various biological responses. PlGF is similar in structure and function to VEGF, and it amplifies VEGF's angiogenic actions. Ovulation stimulation during cycles of in vitro fertilization (IVF) for the management of infertility results in ovarian hyperstimulation syndrome (OHSS), an iatrogenic side effect. Considering potential roles of PlGF and its receptor (sFlt-1) in angiogenesis, the association of these factors with OHSS among study women during controlled ovarian stimulation have evaluated in the present study. Comparative cross-sectional study including of 60 women who go through controlled ovarian stimulation. On oocyte retrieval day, their serum and follicular fluid were taken. The concentrations of PlGF and sFlt-1 were assessed using ELISA. Eighteen patients presented with ovarian hyperstimulation syndrome (OHSS) and 42 patients were no OHSS. There was significantly higher serum PlGF in women with OHSS patients as compared to women with no OHSS, 141.4 ± 11.8 versus 91.9 ± 5.4 respectively ($p < 0.001$). Furthermore, Follicular fluid PlGF there was significantly higher in women with OHSS patients as compared to women with no OHSS, 163.4 ± 7.2 versus 91.1 ± 5.5 respectively ($p < 0.001$). Additionally Follicular fluids PlGF/sFlt ratio significantly higher in women with OHSS patients as compared to women with no OHSS, 0.034 ± 0.01 versus 0.026 ± 0.01 respectively ($p < 0.001$). On the contrary there was significantly lower serum and follicular fluid sFlt in ovarian hyper-stimulated patients. These statistics show that serum and FF PlGF and PlGF to sFlt ratio higher in women with ovarian hyperstimulation syndrome (OHSS) patients as compared to women with no OHSS. So, the PlGF may be played an important a role in angiogenesis dysregulation.

How to cite:

Aliyah Dhahir Habeeb; Lubna Amer Al-Anbari ; Rehab Sh. Al-Maliki; The significance of PlGF in the progression of the ovarian hyperstimulation syndrome in patient undergoing an in vitro fertilization procedure; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2022); 12 (1): 68-85

Doi:

<http://doi.org/10.28969/IJEIR.v12.i1.r6.22>

KEYWORD

EMMPRIN, Basigin, Serum and Follicular fluid, ICSI

1. Introduction

Ovulation induction during cycles of in vitro fertilization (IVF) results in the iatrogenic condition known as ovarian hyperstimulation syndrome (OHSS). Although OHSS involves 12-25 percent of IVF cycles, severe forms of OHSS occur in 0.5-5 percent of cycles for assisted reproductive technology (ART), and in its critical form, is known to cause maternal death **(Naredi, Talwar, and Sandeep, 2014 [1])**. The formation of a lot of follicles, elevated estradiol (E2) levels, and larger ovaries are all symptoms of OHSS **(Naredi, Talwar, and Sandeep, 2014 [1])**. High luteinized bilaterally enlarged cystic ovaries are a hallmark of OHSS. Vascular hyperpermeability, one of the secondary effects, can, in rare cases, be fatal **(Nelson, 2017 [2])**. The mild type of OHSS's morbidity is probably underestimated because its symptoms may go unrecognized. Moderate OHSS symptoms include abdominal distention, nausea,

vomiting, and decreased appetite. Bleeding from an ovarian rupture, acute respiratory distress syndrome, thrombosis, and hepatorenal failure are just a few of the disorders that drive 1.9% of patients to the hospital overall. The critical OHSS could be lethal **(Sun et al., 2021 [3])**.

A rise in capillary permeability, which causes fluid to shift from extravascular to intravascular regions, is a hallmark of OHSS. By rising vascular permeability, VEGF significantly contributes to the pathophysiology of OHSS **(Namavar Jahromi B.et al.,2018 [4])**. VEGF is generated by the granulosa cells, as well as the human chorionic gonadotropin (hCG), which encourages its production. Higher levels of VEGF are linked to severe OHSS **(Namavar Jahromi et al., 2018 [4])**. Higher vascular permeability and fluid extravasation are the proposed mechanisms for this condition, which force fluid to leak to enter the lungs and peritoneum, modifications in the coagulation of the blood that raises the

probability of thrombosis, hemoconcentration with reduced organ **(Naredi, Talwar and Sandeep, 2014 [1])**. The exogenous dose of hCG, which is the last stage in initiating oocyte maturation, is the most significant implication for the progression of OHSS after controlled ovarian stimulation **(Namavar Jahromi et al., 2018 [4])**

VEGF encourages the creation of new blood vessels and boosts capillary hyperpermeability by interacting with its VEGF receptor 2 **(Wise et al., 2018 [5])**. The renin-angiotensin system and immunological system have been proposed as pathogenetic precursors to OHSS in addition to enhanced capillary permeability and extensive follicular angiogenesis, both induced by VEGF **(Naredi, Talwar, and Sandeep, 2014 [1])**. Pro-inflammatory cytokines such as tumor necrosis factor and interleukin-1b, IL-6, and IL-8 have been linked to mediating the immediate phase reaction that results in vascular leakage and the third space loss of

OHSS **(Naredi, Talwar, and Sandeep, 2014 [1])**. These cytokines' serum levels have been observed to be higher in OHSS patients **(Naredi, Talwar, and Sandeep, 2014 [1])**.

PlGF is an angiogenic factor that facilitates the pro-angiogenic action of VEGF. Theca cells and granulosa cells both expressed PlGF and VEGFA. PlGF and VEGFA levels have been at their lowest in follicular fluid preceding to hCG. After 36 hours of hCG exposure, plGF levels have risen sevenfold to their highest levels after being low for the previous 36 hours. Twelve hours after hCG, VEGFA levels in follicular fluid tripled before dropping to intermediate levels **(Bender, Trau, and Duffy, 2018 [6])**.

VEGF promotes the growth of new blood vessels in addition to vascular hyperpermeability though interacting with VEGF receptor 2. In order to increase the amount of VEGF-A accessible to activate VEGFR2, placental growth factor PlGF competes with VEGF-A for binding to VEGFR-1

(Ceci et al., 2020 [7]). Through a number of ways, PlGF has been demonstrated to synergistically enhance VEGF angiogenic activities in the systemic microcirculation under pathological situations: (1) increasing the quantity of VEGF accessible to connect and stimulate VEGFR-2 by shifting VEGF from VEGFR-1 (2) heterodimerizing with VEGF (VEGF/PlGF), causing the VEGFR-2/VEGFR-1 heterodimer receptor complex to activate and transmit angiogenic signals (3) directly reinforcing VEGFR-1, which boosts VEGFR-2 activity by transphosphorylating VEGFR-2, increases VEGFR-2 activity (Freitas-Andrade et al., 2012 [8])

Previous research has connected OHSS to PCOS, a history of the condition, being youthful, slim women, and a high antral follicle count (AFC). Other risk factors have included a past of allergies, higher AMH values, high gonadotropin dosages, and higher serum E2 levels (Corbett et al., 2014

[9]). High blood E2 levels produced by ovarian follicles may lead to an excess generation of vascular endothelial growth factor (VEGF) and inflammatory factors after rises in human chorionic gonadotropin (HCG) in IVF/ICSI cycles (Sun et al., 2021 [3]). Patients undertaking reproductive treatment have a higher risk of OHSS when their AMH values are >3.4 ng/ml, AFC is >24, and estradiol levels are >3,500 pg/ml.

Ovarian enlargement, release of vasoactive chemicals, ascites, and hypovolemia brought on by an abrupt extravasation of fluid into the interstitial space are the primary events in the pathophysiology of OHSS. (Namavar Jahromi et al., 2018 [4]).

OHSS can be classified as early or late depending on when it first manifests, which can predict the prognosis. The symptoms starting nine days after the hCG trigger dosage are due to the exaggerated ovarian response and the exogenously administered hCG's precipitating influence on the ultimate

follicular maturation. On the other hand, endogenous hCG stimulation from an early pregnancy causes OHSS to manifest after this time. So, late OHSS is probably going to be longer and more severe (Naredi, Talwar, and Sandeep, 2012 [1])

2. Subject material and method

The High Institute of Infertility Diagnosis and Assisted Reproductive Techniques at Al-Nahrain University in Baghdad, Iraq, was where this study took place. The time frame of the study is from October 2021 to September 2022. It is cross-sectional comparative research. Specifically 60 women, 30 infertile women with PCOS, and 30 infertile women without PCOS were chosen. Each group was included women that were subjected to ovarian stimulation by antagonist protocol. The indications for Controlled ovarian hyperstimulation in the PCOS group were male factor and tubal factor.

Women without PCOS were undergone COS in preparation for ICSI, and these group was matched to the PCOS group by age and BMI prior to enrollment. All involved women provided their written, informed consent. Women were treated using a GnRH antagonist protocol to prevent premature ovulation. Combining recombinant FSH and/or HMG was used to stimulate the ovaries. When there was a possibility of ovarian hyperstimulation or a history of poor response, the standard stimulation procedure was altered.

Considering the potential roles of serum and follicular fluid sflt and PlGF in ovarian function, the association of these factors and also PlGF/sFlt-1 ratio with the OHSS by dividing patients according to Rotterdam criteria have been evaluated in the present study. PCOS women were fulfilling two from the three following criteria based on the Rotterdam criteria: Polycystic ovary on ultrasound (at least 12 follicles 2-9

mm in diameter per ovary), chronic anovulation or oligovulation, clinical or biochemical Hyperandrogenism. The inclusion criteria include infertile women who were diagnosed as PCOS in the presence of at least 2 of the Rotterdam criteria, based on the Rotterdam Consensus Meeting (2003). When at least three follicles are 18 mm with serum E2 measured, two ampules of recombinant hCG 250 mg/0.5 ml prefilled syringe are given and to trigger and induce maturation of the oocyte. Then, 34-36 hours after the hCG injection, oocyte retrieval took place with transvaginal ultrasound guidance under general anesthesia or spinal anesthesia. Embryo transfer was performed transcervical on a patient with a dorsal lithotomy, guided by ultrasound, and the embryo was placed 15-20mm from the fundus. Clinical pregnancy was detected by performing a B-hCG test after two weeks of embryo transfer, followed by a transvaginal

ultrasound two weeks later to ensure the viability of the embryo.

1- Collecting and preserving the samples

Blood samples and follicular fluid were collected from each woman enrolled in the ICSI procedure. Blood sample was obtained from the patients at cycle day 2. Blood samples was taken for hormone analysis (LH, FSH, E2, testosterone, prolactin) and AMH for infertile women aged 40 years and more assay by MiniVidas. At the day of pickup, the blood sample was taken and allowed to coagulate for 10 minutes, then centrifuged at 3000 rpm for 20 min to separate the serum. The samples were aliquot and quickly frozen and stored at -20C° until assayed. Follicular fluid was collected, and it was utilized for analysis. Following the oocyte's removal, the fluid was centrifuged at 3000 rpm for 10 minutes to take out debris and granulosa cells. Blood or flushing solution were not taken; only

the initial clear follicular fluid associated with the existence of an oocyte was used for analysis. The supernatant was separated into aliquots and kept at 20°C before being analyzed.

2- Analysis of serum and follicular fluid parameters

According to the manufacturer's instructions, this kit uses an enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technique to measure placental growth factor and sFlt-1. PlGF and sFlt-1 had sensitivities of 2.54 and 0.25 ng/ml, respectively. Additionally, the intraassay and interassay coefficients of variation (CV) for PlGF and sFlt-1 were 8% and 10%, respectively.

3- Statistical analysis

According to the manufacturer's instructions, this kit uses an enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technique to measure placental growth factor and sFlt-1.

PlGF and sFlt-1 had sensitivities of 2.54 and 0.25 ng/ml, respectively. Additionally, the intraassay and interassay coefficients of variation (CV) for PlGF and sFlt-1 were 8% and 10%, respectively.

3. Results

Demographic features between studied groups were showed in **(Table 1)** There were no significant differences in mean patients' age, BMI, and duration of infertility type and cause of infertility ($p=0.109$, $p=0.125$, $p=0.220$, $p=0.136$, $p=0.389$) respectively between PCOS and non-PCOS groups. Furthermore, AMH level was significantly positive correlated with both serum PlGF ($r=0.588$) and follicular fluid PlGF ($r=0.565$). There was Positive correlations between basalE2 level and day of trigger E2 with serum PlGF, $r=0.425$ and $r=0.650$, respectively. Furthermore, There were Positive correlations between basal E2 level and day of trigger E2 with follicular fluid

PlGF, $r=0.555$ and $r=0.701$, respectively, while there was significant negative correlation between follicular stimulated hormone and both serum and follicular fluid PlGF, -0.345 and 0.555 respectively. In addition, there was a positive significant correlation between serum and follicular fluid PlGF with total retrieved oocytes count (chart 5), MII oocytes count and total transferred embryos.

Out of 60 infertile women with and without PCOS, eighteen patients presented with ovarian hyperstimulation syndrome (OHSS), and 42 patients were no OHSS. The PlGF and sFlt levels were also compared between two groups of women; according to the results, there was significantly higher serum PlGF in women with OHSS patients as compared to women with no OHSS, 141.4 ± 11.8 versus 91.9 ± 5.4 respectively ($p<0.001$). Furthermore, Follicular fluid PlGF there was significantly higher in women with

OHSS patients as compared to women with no OHSS, 163.4 ± 7.2 versus 91.1 ± 5.5 , respectively ($p<0.001$). Additionally, the Follicular fluids PlGF/sFlt ratio was significantly higher in women with OHSS patients as compared to women with no OHSS, 0.034 ± 0.01 versus 0.026 ± 0.01 , respectively ($p<0.001$). On the contrary, there was significantly lower serum and follicular fluid sFlt in ovarian hyper-stimulated patients **(Table 3)**.

Receiver Operative Characteristic curve (ROC curve) has been used to calculate the serum and follicular fluid of both biomarkers cut-off values as a predictor of ovarian hyper-stimulation with acceptable sensitivity, specificity, and accuracy. The cut-off value of serum PlGF was ≥ 105.3 (ng/l) with sensitivity=72.2 %, specificity=85.7 %, accuracy =79 % and good area under curve (AUC) =0.814, while the cut-off value of follicular fluid PlGF was ≥ 112.2 (ng/l) with sensitivity=94.4 %, specificity=85.7 %, accuracy =79 % and good area under curve (AUC) =0.814.

specificity=85.7 %, accuracy =90 % and excellent area under curve (AUC) =0.814 .The cut-off values of both serum and follicular fluid sFlt were ≤ 3.36 and ≤ 3.49 ng/ml, respectively;

however, sFlt cutoff values were invalid or unreliable because of the low area under the curve (AUC< 0.700) with low sensitivity and specificity as shown in (Table3).

Table (1): Comparison of demographic features between PCOS and non-PCOS groups

Parameters	PCOS patients n=30	Non-PCOS patients	p-value
Age (years) (Mean ±SD)	29.27 ± 4.09	30.10 ± 7.33	0.109 NS
Body mass index (BMI)			
BMI (Kg/m²)	30.47 ± 4.30	29.13 ± 6.93	0.125
Normal weight, n. (%)	4 (13.3%)	9 (30%)	0.114 NS
Overweight, n. (%)	20 (66.7%)	15 (50%)	
Obese, n. (%)	6 (20%)	6 (20%)	
Duration of infertility			
Duration of infertility	7.63 ± 2.37	8.60 ± 3.55	0.220
< 5 years n. (%)	3 (10%)	2 (6.7%)	0.322 NS
5-10 years n. (%)	21 (70%)	18 (60%)	
>10 years n. (%)	6 (20%)	10 (33.3%)	
Type of infertility n. (%)	Primary=20	Primary=25	0.136
	Secondary=10	Secondary=5	
Causes of infertility n. (%)	Male causes=28 (93.3%)	Male causes=26 (86.7%)	0.389 NS
	Female causes=2 (6.7%)	Female causes=4 (13.3%)	

SD: Standard deviation; PCOS: Polycystic ovary syndrome: NS: p-value ≥ 0.05 (Not significant); S: p-value < 0.05 (Significant); BMI: Body mass index.

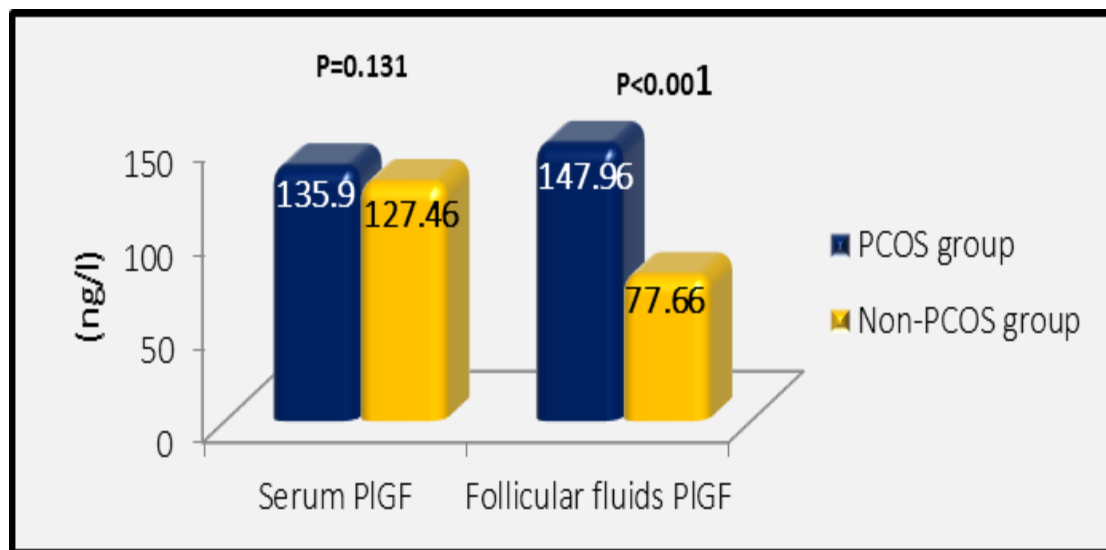
(Table 2): Comparison of PlGF and sFlt levels according to ovarian response

Parameter	OHSS women (n.=18)	Non- OHSS women (n.=42)	p-value
Serum PlGF (ng/l)	141.4 ± 11.8	91.9 ± 5.4	<0.001 S
Follicular fluid PlGF (ng/l)	163.4 ± 7.2	91.1 ± 5.5	<0.001 S
Serum sFlt (ng/ml)	3.46 ± 0.43	3.87 ± 0.32	0.047 S
Follicular fluid sFlt (ng/ml)	4.39 ± 0.66	3.49 ± 0.48	0.017 S
Follicular fluids PlGF/sFlt ratio	0.034 ± 0.01	0.026 ± 0.01	<0.001 S

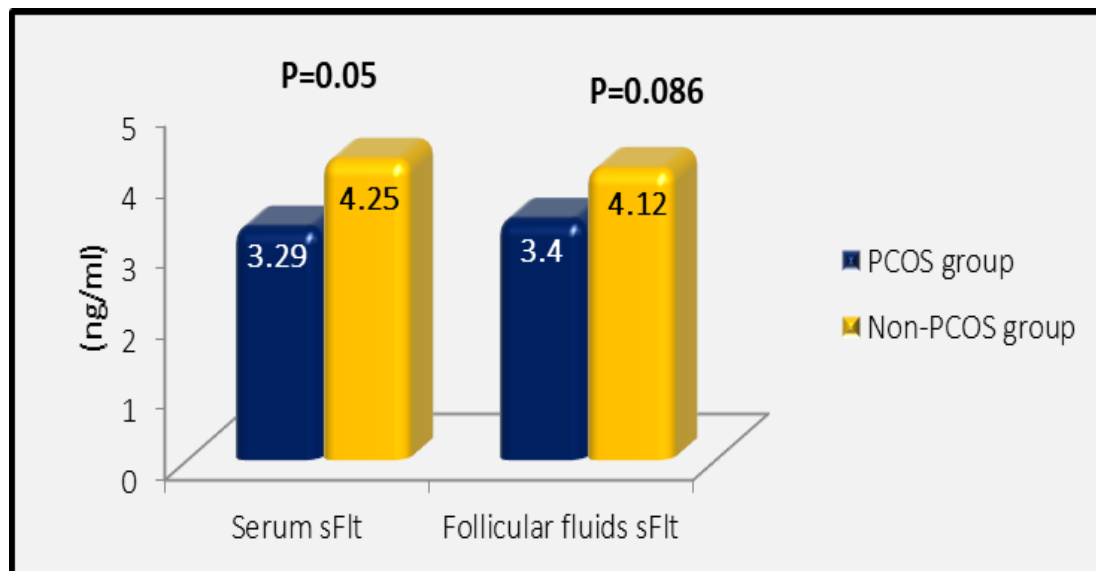
(Table 3): Receiver operative characteristics of serum & follicular fluid PlGF and sFlt discriminatory ability to predict early ovarian hyper-stimulation (OHSS)

Receiver operative characteristics	Serum PlGF	Follicular fluid PlGF	Serum sFlt	Follicular fluid sFlt
Cut off value	≥ 105.3 (ng/l)	≥ 112.2 (ng/l)	≤ 3.36 (ng/ml)	≤ 3.49 (ng/ml)
Area under curve	0.814	0.926	0.671	0.538
Sensitivity	72.2%	94.4%	72.2%	55.6%
Specificity	85.7%	85.7%	66.7%	52.5%
Accuracy	79%	90%	69.5%	54%
p-value	< 0.001 S	< 0.001 S	0.037 S	0.640 NS

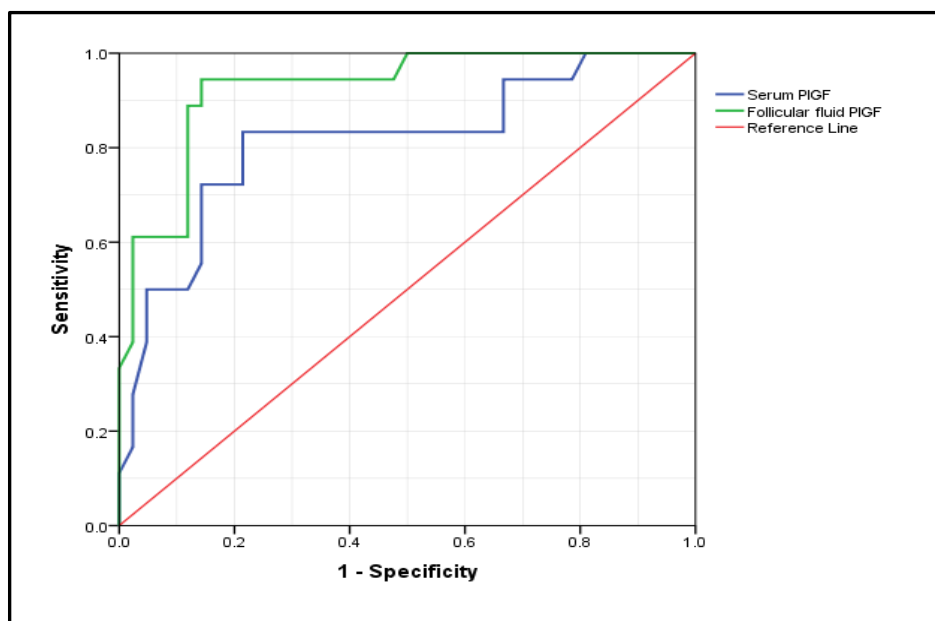
NS: Not significant ($p > 0.05$); S: Significant ($p \leq 0.05$).



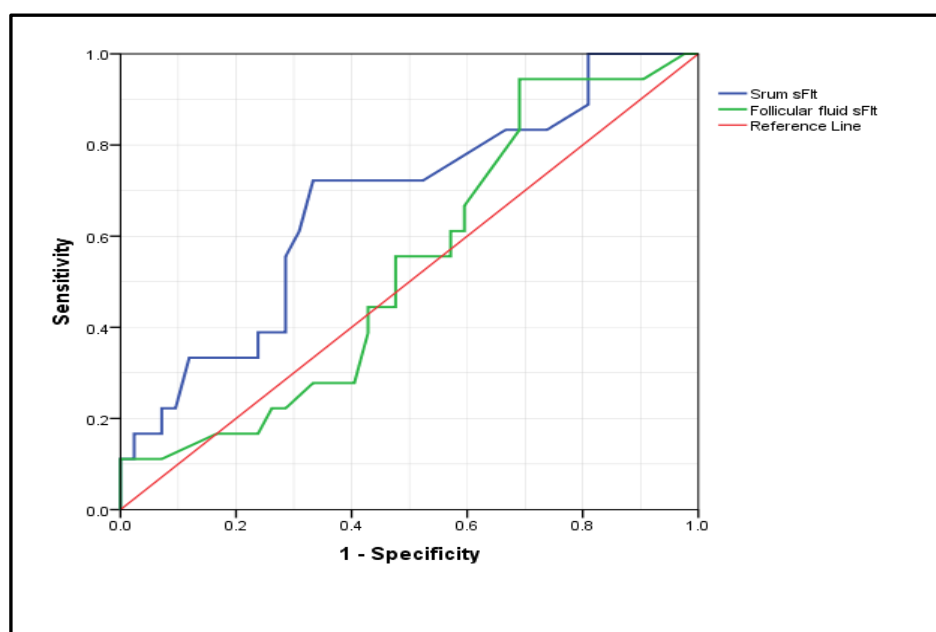
(Figure 1): Comparison of PlGF levels between PCOS & non-PCOS women



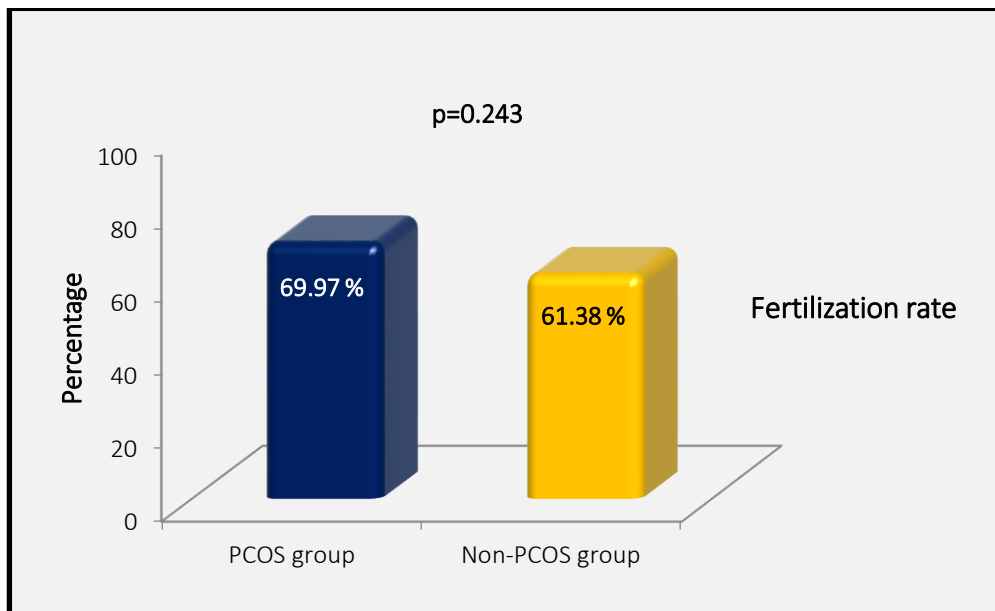
(Figure 2): Comparison of sFlt levels between PCOS & non-PCOS women



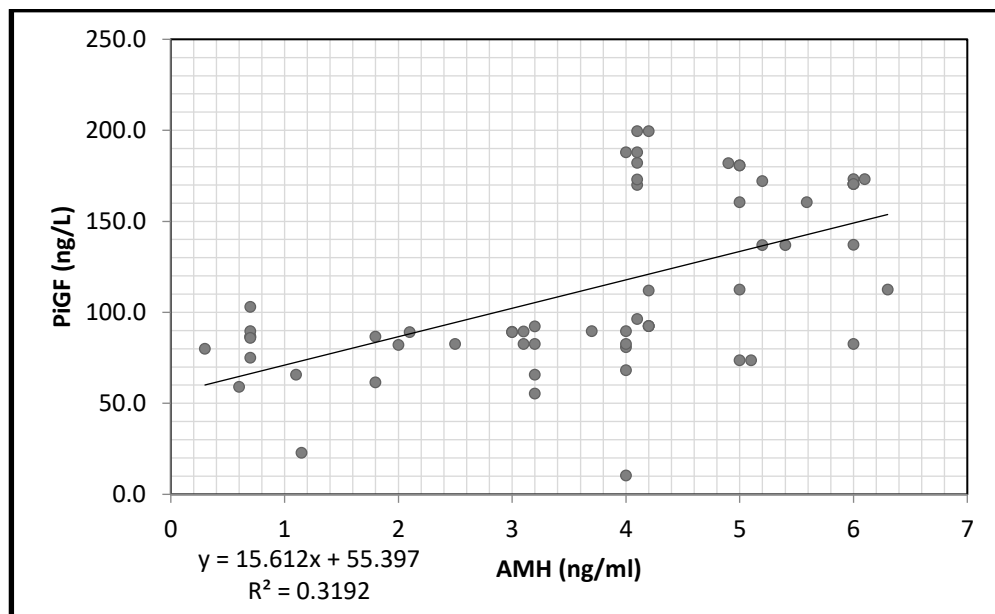
(Figure 3): ROC curve of serum & follicular fluid PIGF



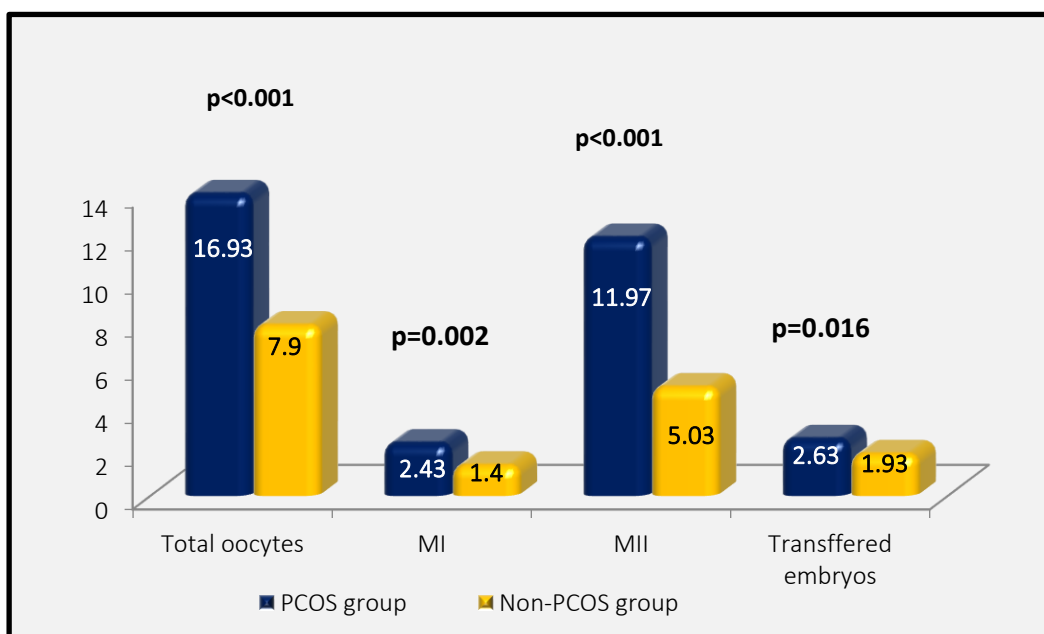
(Figure 4): ROC curve of serum & follicular fluid sFlt



(Figure 5): Comparison of fertilization rate between PCOS & Non-PCOS women



(Figure 6): Correlations between follicular fluid PlGF & AMH



(Figure 7): Comparison of ICSI characteristics between PCOS & Non-PCOS women

4. Discussion:

In the current study, there has been significantly higher serum PIGF in women who have OHSS as compared to women who do not have OHSS ($p < 0.001$) with cut-off value ≥ 105.3 ng/l was obtained with 72.2 % sensitivity and 85.7 % specificity as well as 79% accuracy. While no studies have documented connections between PIGF and OHSS in the past but, a previous study demonstrated that during ovarian stimulation, vascular

endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2) mRNA expression grows and that with the delivery of hCG, each expression reaches its peak (Soares et al., 2008). An in vitro study revealed VEGF-A to be the major motivation of enhanced vascular permeability in OHSS. When VEGF-A binds to VEGFR 1 and 2 (VEGF-R1 and VEGF-R2), the receptors are phosphorylated to activate them and transmit the signal to other cell signaling pathways. On the other hand, it has been found that the

PlGF binds to VEGFR-1 and its soluble variant sFLT-1(antiangiogenic) but not VEGFR-2 **(Albonici et al., 2019 [10])**. Furthermore, PlGF has been demonstrated to synergistically boost the angiogenic activity of VEGF via switching VEGF from VEGFR-1, increasing the amount of VEGF available to bind and activate VEGFR-2, heterodimerizing with VEGF (VEGF/PlGF), which detonates and transmits angiogenic signals through the VEGFR-2/VEGFR-1 heterodimer receptor complex, and additionally, directly engagement VEGFR-1 which, through transphosphorylation of VEGFR-2, enhances VEGFR-2 activity **(Freitas-Andrade et al., 2012 [8])** from all these give a suggestion that PLGF enhances VEGF-induced vascular permeability causing OHSS in PCOS women .

Follicular fluid PlGF has been significantly higher in women with OHSS patients as compared to women with no OHSS ($p<0.001$) with a cut-off value of follicular fluid PlGF was $\geq$

112.2 (ng/l) with 94.4 %, sensitivity, and 85.7 %, specificity as well as 90 % accuracy. Granulosa cells and theca cells both expressed PlGF and VEGFA. Prior to hCG, PlGF and VEGFA levels in follicular fluid were at their lowest. After 36 hours of hCG injection, plGF levels increased sevenfold to their highest levels. **(Bender et al., 2018 [6])**. On the other side, other study found follicular fluid VEGF, ANG II, and serum VEGF levels were significantly higher in OHSS. Pan et al. demonstrated that Angiotensin II induces PlGF gene expression and protein secretion in vascular endothelial cells **(Pan et al., 2010 [11])**. From all these data give a suggestion that the PlGF may have a role in the progression of OHSS and its usefulness as a predictor of early and/or late OHSS .

Additionally, the Follicular fluids PlGF/sFlt ratio is significantly higher in women with OHSS patients as compared to women with no OHSS. Placental growth factor competes with

VEGF-A for binding to sFlt (soluble form of VEGFR-1) so that more VEGF-A is available to activate VEGFR2; thus, by interacting with VEGFR-2, VEGF stimulates new blood vessel development and vascular hyper-permeability (Ceci et al., 2020 [7], Alzaidi, Z et al., 2021 [12]). Based on these data, PlGF/sFlt increases as a result of an increase in the PlGF level.

5- Conclusion

These data provide evidence that serum and FF PlGF and PlGF/sFlt ratio is higher in women with ovarian hyperstimulation syndrome (OHSS) patients as compared to women with no OHSS. The serum and follicular fluid sFlt were lower in women with in ovarian hyper-stimulated patients. This gives a suggestion that PlGF may be played an essential task in angiogenic dysregulation.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted

Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution Aliyah Dhahir Habeeb performed the study, Lubna Amer Al-Anbari, and Rehab Sh. Al-Maliki 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.

References:

- [1] Naredi, N., Talwar, P. and Sandeep, K. (2014). VEGF antagonist for the prevention of ovarian hyperstimulation syndrome: Current status. Medical Journal Armed Forces India, 70 (1), pp.58–63. doi:10.1016/j.mjafi.2012.03.005.
- [2] Nelson, S.M. (2017). Prevention and management of ovarian hyperstimulation syndrome. Thrombosis Research, 151, pp.S61–S64. doi:10.1016/s0049-3848

(17)30070-1.

[3] Sun, B., Ma, Y., Li, L., Hu, L., Wang, F., Zhang, Y., Dai, S. and Sun, Y. (2021). Factors Associated with Ovarian Hyperstimulation Syndrome (OHSS) Severity in Women With Polycystic Ovary Syndrome Undergoing IVF/ICSI. *Frontiers in Endocrinology*, [online] 11, p.615957. doi:10.3389/fendo.2020.615957.

[4] Namavar Jahromi, B., Parsanezhad, M.E., Shomali, Z., Bakhshai, P., Alborzi, M., Moin Vaziri, N. and Anvar, Z. (2018). Ovarian Hyperstimulation Syndrome: A Narrative Review of Its Pathophysiology, Risk Factors, Prevention, Classification, and Management. *Iranian Journal of Medical Sciences*, [online] 43 (3), pp.248–260. Available at: <https://pubmed.ncbi.nlm.nih.gov/29892142/>

[5] Wise, L.M., Stuart, G.S., Real, N.C., Fleming, S.B. and Mercer, A.A. (2018). VEGF Receptor-2 Activation Mediated by VEGF-E Limits Scar Tissue Formation Following Cutaneous Injury. *Advances in Wound Care*, [online] 7 (8), pp.283–297. doi:10.1089/wound.2016.0721.

[6] Bender, H.R., Trau, H.A. and Duffy, D.M. (2018). Placental Growth Factor Is Required for Ovulation, Luteinization, and Angiogenesis in Primate Ovulatory Follicles. *Endocrinology*, [online] 159 (2),

pp.710–722. doi:10.1210/en.2017-00739.

[7] Ceci, C., Atzori, M.G., Lacal, P.M. and Graziani, G. (2020). Role of VEGFs/VEGFR-1 Signaling and Its Inhibition in Modulating Tumor Invasion: Experimental Evidence in Different Metastatic Cancer Models. *International Journal of Molecular Sciences*, [online] 21 (4). doi:10.3390/ijms21041388.

[8] Freitas-Andrade, M., Carmeliet, P., Charlebois, C., Stanimirovic, D.B. and Moreno, M.J. (2012). PlGF knockout delays brain vessel growth and maturation upon systemic hypoxic challenge. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, [online] 32 (4), pp.663–675. doi:10.1038/jcbfm.2011.167.

[9] Corbett, S., Shmorgun, D., Claman, P., Cheung, A., Sierra, S., Carranza-Mamane, B., Case, A., Dwyer, C., Graham, J., Havelock, J., Healey, S., Hemmings, R., Liu, K., Motan, T., Murdock, W., Smithson, D., Vause, T., Wong, B. and Gysler, M. (2014). The Prevention of Ovarian Hyperstimulation Syndrome. *Journal of Obstetrics and Gynaecology Canada*, [online] 36 (11), pp.1024–1033. doi:10.1016/S1701-2163 (15) 30417-5.

[10] Albonici, L., Giganti, M.G., Modesti, A.,

Manzari, V. and Bei, R. (2019). Multifaceted Role of the Placental Growth Factor (PlGF) in the Antitumor Immune Response and Cancer Progression. *International Journal of Molecular Sciences*, 20 (12), p.2970. doi:10.3390/ijms20122970.

[11] Pan, P., Fu, H., Zhang, L., Huang, H., Luo, F., Wu, W., Guo, Y. and Liu, X. (2010). Angiotensin II upregulates the expression of placental growth factors in human vascular endothelial cells and smooth muscle cells. *BMC Cell Biology*, 11 (1), p.36. doi:10.1186/1471-2121-11-36.

[12] Alzaidi, Z., Yildiz, Ş.M., Saatçi, Ç. et al. The effect of cytokine leukemia-inhibitory factor (LIF) and interleukin-11 (IL-11) gene expression on the primary infertility related to polycystic ovary syndrome, Tubal factor, and Unexplained infertility in Turkish women. *Egypt J Med Hum Genet* 22, 85 (2021). <https://doi.org/10.1186/s43042-021-00201-9>