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Interleukin-15 and Glypican-1 as Predictive Biomarkers for Ovarian Response and Pregnancy Success in ICSI Cycles

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ABSTRACT: Introduction: Ovarian microenvironment plays importance role in determining ovarian response and reproductive outcomes in intra cytoplasmic sperm injection (ICSI). Several inflammatory and growth-associated biomarkers such as IL-15 and glypican-1 can be used to estimate ovarian response and pregnancy outcome. This study aims to assess the concentration of IL-15 and glypican-1 in serum and follicular fluid in patients undergoing in vitro fertilization and correlate the markers' presence with ovarian response and pregnancy outcome.

Aim of the study: The study aimed to evaluate serum and follicular fluid levels of glypican and IL-15 in women undergoing ICSI and to determine the correlation with ovarian response, oocyte development and pregnancy outcomes.

Subjects and Methods: A total of 120 infertile women undergoing ICSI were enrolled and stratified according to ovarian response into three groups of 40 participants each: poor responders, normal responders, and hyper-responders. Follicular fluid concentrations of IL-15 and glypican-1 were quantified by ELISA on the oocyte retrieval day and embryo transfer day. Associations between hormonal profiles, oocyte characteristics, and pregnancy outcomes were evaluated using nonparametric tests and correlation analyses.

Results: It was found that the concentrations of studied markers significantly differed between three groups ($P < 0.001$). Both biomarkers were found to positively correlate with hormonal activity and characteristics of the follicle/oocyte. Nevertheless, most of

the biomarkers did not serve as reliable predictors of pregnancy outcome. It was found that serum glypican-1 on the oocyte retrieval day has low predictive value ($AUC = 0.625$), and only follicular fluid CRH in normal responders group showed better predictive accuracy ($AUC = 0.769$).

Conclusions: IL-15 and glypican-1 have a positive association with follicle/oocyte development and ovarian response, yet do not serve as predictors of pregnancy success.

1. INTRODUCTION

Successful outcomes in intra cytoplasmic sperm injection (ICSI) depend not only on ovarian response but also on the quality of the follicular microenvironment, which plays a critical role in oocyte maturation, fertilization, and embryo development [1].

In this context, inflammatory cytokines and growth factors have been suggested as novel biomarkers to assess ovarian function and ICSI results [2,3]. The Interleukin-15 (IL-15) is a pro-inflammatory cytokine that involve in immune cells regulation and activation [4]. IL-15 responsible for some processes such as follicle development and implantation.

The alteration in the IL-15 concentration can be connected to the change in ovarian function [5,6]. Glypican-1 is a heparan sulfate proteoglycan that participates in cell signaling and growth factor regulation [7]. It is involved in key biological processes such as cellular proliferation, differentiation, and extracellular matrix interactions. Within the ovarian microenvironment, glypican-1 may contribute to follicular growth and remodeling, thereby influencing oocyte maturation and fertilization potential [7,8]. Despite the biological relevance of IL-15 and glypican-1 evidence suggests that individual biomarkers may have limited predictive value when used in isolation [6,9].

Therefore, evaluating these markers in both serum and follicular fluid, and in relation to ovarian response and reproductive outcomes. Accordingly, the aim of this study was to investigate the levels of IL-15 and glypican-1 in serum and follicular fluid among women undergoing ICSI and to assess their association with ovarian response, oocyte characteristics, and pregnancy outcomes. This study also seeks to determine their potential value as predictive biomarkers for ICSI success.

2. SUBJECTS AND METHODS

This was a prospective comparative study carried out at the infertility center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq. A total of 120 infertile women undergoing ICSI cycles were enrolled between January 2025 and January 2026. Recruitment took place at the same center, and every participant provided written informed consent prior to enrollment. Ethical clearance was issued by the Institutional Review Board of Mansoura University, Egypt (approval code: MDP.24.10.168), which also reviewed the protocol for methodological validity.

Patients were stratified into three equal arms on the basis of ovarian response: poor responders ($n = 40$), normal responders ($n = 40$), and hyper-responders ($n = 40$).

2.1. Subjects

Biological samples were obtained at three predefined time points: serum collected on the oocyte retrieval day (ORD), follicular fluid aspirated at the time of oocyte retrieval, and serum collected on the embryo transfer day (ETD). All samples were centrifuged at 5,000 rpm for 10 minutes ; the resulting serum and follicular fluid supernatants were separated and stored at -20°C until further analysis.

Inclusion criteria

Inclusion criteria: Women subjected to ICSI, Poor, normal, and hyper responders, (PCOS cases included), and Men having normozoospermia.

Exclusion criteria

Presence of major systemic disease, History of recent infections, Treatment with immunosuppressives, Infertility in men (azoospermia and sperm retrieval)

Clinical Evaluation

All subjects were subjected to: A comprehensive medical, reproductive history and Physical and gynecological examination.

2.2. Study design

Calculation of body mass index (BMI)

Baseline evaluation of hormones including AMH, FSH, LH, estrogen (E2), prolactin (PRL), and thyroid-stimulating hormone (TSH), Transvaginal ultrasound scan and further testing where necessary to evaluate their reproductive health.

Procedure for Ovarian Stimulation and ICSI (Day3 Embryo Transfer Protocol)

A flexible GnRH antagonist protocol was used for controlled ovarian stimulation. Gonadotropin administration began on day 2 of the menstrual cycle with recombinant fsh, and the starting dose was individualized according to each patient's baseline characteristics. final oocyte maturation was triggered with hcg once at least two follicles had reached a mean diameter of 17–18 mm. Oocyte retrieval was performed transvaginally under ultrasound guidance 34–36 hours after the trigger. Following fertilization, embryos were cultured and examined morphologically and mature MII oocytes were selected for ICSI. Fertilization was proved by two pronuclei appearance 2PN. Further monitoring of embryo development was conducted until cleavage stage Day3. Embryos were transferred at day 3 post fertilization depending on the state of their development. Pregnancy determination was done 14 days after embryo transfer.

2.3. Biochemical Analyses

IL-15 and glypican-1 levels in serum and follicular fluid samples were determined using commercially available ELISA kit (ELK Biotechnology, USA) according to the manufacturer's guidelines. The analysis was carried out employing conventional ELISA techniques. The optical density values were recorded at 450 nm wavelength using microplate reader and concentration calculated using calibration curves.

Assessment of Oocytes and Embryos

Maturation of oocytes was assessed following the process of denudation; they were further classified into germinal vesicle (GV), metaphase I (MI), and metaphase II (MII).

2.4. The rate of fertilization was done by:

Fertilization rate (%) = $\frac{\text{Number of fertilized oocytes (2PN)}}{\text{Number of injected MII oocytes}} \times 100$

Quality of embryos and their cleavage efficiency were evaluated using morphological evaluation.

2.5. Statistical Methods

Statistical analysis was conducted using SPSS version 23.0. Distributional normality was assessed with the kolmogorov–smirnov and shapiro–wilk tests. continuous variables were reported as mean $\pm$ standard deviation when normally distributed and as median with interquartile range otherwise. Between group comparisons relied on non-parametric tests, and associations between variables were examined using spearman's rank correlation. a two-sided p-value below 0.05 was treated as statistically significant.

3. RESULTS

3.1. Distribution of IL-15 and Glypican-1 Levels

Table 1 refers to the calculation of the data normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, the results demonstrated that serum and follicular fluid levels of IL-15 and glypican-1 were not normally distributed $P < 0.05$. All variables were expressed as medium and interquartile range, and non-parametric statistical were applied for subsequent analyses.

Table 1: Tests of Normality for IL-15 and Glypican-1 Levels

Marker	Sample Type	Kolmogorov	DF	<i>p</i>	Shapiro Wilk	DF	<i>p</i>	Interpretation
IL-15	Serum (ORD)	0.092	90	0.047	0.963	90	0.011	Non-normal
	Follicular Fluid	0.092	90	0.046	0.964	90	0.015	Non-normal

	Serum (ETD)	0.126	90	0.001	0.947	90	0.001	Non-normal
Glypican-1	Serum (ORD)	0.127	90	0.001	0.954	90	0.003	Non-normal
	Follicular Fluid	0.146	90	<0.001	0.899	90	<0.001	Non-normal
	Serum (ETD)	0.135	90	<0.001	0.925	90	<0.001	Non-normal
df: degrees of freedom, ORD: Oocyte retrieval day, ETD: Embryo transfer day, Data were considered non-normally distributed when $p < 0.05$								

3.2. Comparison between IL-15 and Glypican-1 in Poor, Normal, and Hyper Comparison between IL-15 and Glypican-1 in Poor, Normal, and Hyper Responders

There was a significant difference in the concentration of serum and follicular fluid of IL-15 and glypican-1 in poor, normal, and hyper responders ($p < 0.001$, Table 2). For the level of IL-15, there was a higher amount of IL-15 in the hyper responders followed by the poor responders while having the least concentration of IL-15 in normal responders. The level of glypican-1 was also found to be significantly different among the groups where the highest level of glypican-1 was seen in hyper responders while poor responders had the lowest amount of glypican-1 in serum.

Table 2: Serum and Follicular Fluid Levels of IL-15 and Glypican-1 Across Ovarian Response Groups

Marker	Sample	Poor (n = 40)	Normal (n = 40)	Hyper (n = 40)	p-value
IL-15	Serum (ORD)	297.47 (47.09)	184.93 (57.30)	388.86 (82.61)	<0.001*
	Follicular Fluid	304.21 (31.27)	154.77 (99.61)	353.65 (86.67)	<0.001*
	Serum (ETD)	207.86 (69.93)	216.99 (66.08)	359.69 (32.95)	<0.001*
Glypican-1	Serum (ORD)	6.14 (5.76)	9.46 (3.89)	17.76 (5.38)	<0.001*
	Follicular Fluid	10.42 (2.17)	9.63 (2.82)	15.53 (5.16)	<0.001*
	Serum (ETD)	8.81 (4.73)	10.60 (6.44)	17.81 (8.52)	<0.001*
Data are presented as median (interquartile range), ORD: Oocyte retrieval day, ETD: Embryo transfer day, significant Statistically ($p < 0.05$)					

3.3. Association of IL-15 and Glypican-1 with Pregnancy Outcomes

The overall pregnancy rate among the study population was 37.5%. Positive pregnancy rates were higher in hyper responders (47.5%) and normal responders (42.5%) compared to poor responders (22.5%), with a statistically significant difference among groups ($p = 0.05$), as presented in Table 3. When comparing biomarker levels between women with positive and negative pregnancy outcomes, no significant differences were observed for IL-15 in serum or follicular fluid ($p > 0.05$). Similarly, glypican-1 levels did not show significant differences in most measurements, except for serum glypican-1 at oocyte retrieval day, which demonstrated a statistically significant association with pregnancy outcome ($p = 0.048$).

Table 3: Pregnancy Outcomes Across Ovarian Response Groups

Outcome	Total (n = 120)	Poor (n = 40)	Normal (n = 40)	Hyper (n = 40)	p-value
Positive Pregnancy	45 (37.5%)	9 (22.5%)	17 (42.5%)	19 (47.5%)	0.05*

Negative Pregnancy	75 (62.5%)	31 (77.5%)	23 (57.5%)	21 (52.5%)	
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3.4. Predict of Glypican-1 for pregnancy outcomes

The receiver operating characteristic (ROC) curve test was performed to determine the predictive power of glypican-1 for predicting pregnancy outcomes, Table 4. Glypican-1 concentration in serum samples taken at the time of egg retrieval was found to have weak predictive power, with a cut-off point higher than 6.39, an area under the curve (AUC) of 0.625 (95% CI: 0.517-0.725), sensitivity of 85.3%, and specificity of 37.5%. This indicates that despite the link between glypican-1 and pregnancy outcomes, its predictive efficacy as an independent marker is quite weak.

Table 4: Comparison of IL-15 and Glypican-1 Levels According to Pregnancy Outcome

Marker	Sample	Positive Pregnancy (n = 45)	Negative Pregnancy (n = 75)	p-value
IL-15	Serum (ORD)	307.61 (199.25)	289.62 (91.98)	0.485
	Follicular Fluid	300.37 (168.10)	294.48 (92.91)	0.764
	Serum (ETD)	267.72 (139.21)	227.31 (142.35)	0.396
Glypican-1	Serum (ORD)	10.52 (8.77)	9.05 (9.21)	0.048*
	Follicular Fluid	10.65 (6.62)	10.71 (3.12)	0.635
	Serum (ETD)	13.14 (9.24)	11.97 (6.84)	0.148

3.5. Correlation of IL-15 and Glypican-1 with Hormonal Profiles and Oocyte Characteristics

Both IL-15 and glypican-1 demonstrated significant positive correlations with key reproductive hormones, including AMH, estradiol, and LH, and negative correlations with FSH ($p < 0.05$, Table 5), suggesting their association with ovarian activity. Additionally, both markers showed positive correlations with oocyte characteristics, including total oocyte count, MII oocytes, and fertilized oocytes, indicating their involvement in follicular development and oocyte maturation.

Table 5: Correlation of IL-15 and Glypican-1 with Hormonal Profiles

Marker	Sample	AMH (r)	p-value	E2 Trigger (r)	p-value	LH (r)	p-value	FSH (r)	p-value
IL-15	Serum (ORD)	0.304	0.004*	0.319	0.002*	0.384	<0.001*	-0.138	0.195
	Follicular Fluid	0.250	0.018*	0.290	0.006*	0.309	0.003*	-0.066	0.538
	Serum (ETD)	0.587	<0.001*	0.632	<0.001*	0.484	<0.001*	-0.319	0.002*
Glypican-1	Serum (ORD)	0.632	<0.001*	0.500	<0.001*	0.389	<0.001*	-0.364	<0.001*
	Follicular Fluid	0.380	<0.001*	0.424	<0.001*	0.419	<0.001*	-0.170	0.109
	Serum (ETD)	0.530	<0.001*	0.430	<0.001*	0.414	<0.001*	-0.250	0.018*

3.6. Correlation between IL-15 and Glypican-1 and Oocyte Features

Correlation analysis showed that there was a Significant association were observed between the measured biomarkers and oocyte parameters (Table 6). On the day of oocyte retrieval, serum IL-15 showed a positive correlation with the total oocyte count, the number of MII oocytes, injected oocytes, and 2PN embryos ($r = 0.285-0.319$, $p < 0.01$), whereas follicular fluid IL-15 correlated inversely with the same indices ($r = -0.210$ to -0.290 , $p < 0.05$). At the embryo transfer stage, serum IL-15 demonstrated stronger positive correlations across all of these parameters ($r = 0.515-0.632$, $p < 0.001$).

Glypican-1 levels in both serum and follicular fluid were positively associated with every oocyte parameter assessed. The strongest associations were seen for serum glypican-1 on the retrieval day, which correlated most closely with the total oocyte count and the number of 2PN embryos ($r = 0.632$, $p < 0.001$).

4. DISCUSSION

to examine the association in this study between IL-15 and glypican-1 and ovarian responsiveness as well as to establish whether they could serve as predictors of pregnancy outcome in patients undergoing ICSI. Interestingly, the study shows that both markers have a significant connection with ovarian response, hormonal levels, and oocyte parameters. However, their ability to predict pregnancy outcomes appears to be limited.

As a pro-inflammatory cytokine, IL-15 exhibited considerable differences between the three groups studied and had strong connections with different hormones and oocyte parameters. These findings suggest that IL-15 contributes meaningfully to the regulation of the follicular microenvironment [10,11].

Positive associations of serum IL-15 were observed concerning oocyte characteristics while negative associations of IL-15 were found in relation to oocyte characteristics in follicular fluid [12,13].

Systemically high levels of IL-15 can reflect physiological mechanisms while locally increased IL-15 levels may indicate inflammation that can affect the quality of oocytes negatively. On the contrary, glypican-1 demonstrated a consistent pattern, namely the presence of significant positive correlations in relation to hormone profile and oocyte characteristics. Glypican-1 promotes follicle development and maturation of oocytes through extracellular signal regulation [7,14].

As it was already found, despite having strong biological associations, none of IL-15 and glypican-1 showed good predictive value in relation to pregnancy results [15,16].

Neither IL-15 nor glypican-1 levels differed in pregnant and non-pregnant women meaning that they are more related to follicle development than fertilization or implantation [6,17]. However, glypican-1 had a statistically significant association with pregnancy in serum samples at the moment of oocyte retrieval; however, the performance of this marker was modest. This fact leads to another idea, specifically, although biomarkers reflecting activity of ovaries and the development of follicles can help understand how fertile a woman is, they cannot guarantee pregnancy success.

Pregnancy depends on numerous factors including the quality of embryos, the endometrium, and successful implantation. From the clinical point of view, both IL-15 and glypican-1 have a potential to serve as valuable indicators of ovarian response [18].

They will help assess follicular microenvironment and provide information about ovarian responsiveness. However, their performance as predictors of pregnancy outcomes remains poor, which means that they should not be used individually.

CONCLUSION

From this study, we can say that IL-15 and glypican-1 are important regulators of follicular response and hormonal balance in ICSI treatment. Glypican-1 was consistently positively correlated with the studied indicators of follicular maturation, while IL-15 was found to be differentially expressed at the follicular and systemic levels. Glypican-1 could predict successful follicular maturation and oocyte quality but had little influence on pregnancy outcomes, while IL-15 failed to correlate with pregnancy outcomes. Thus, biomarkers related to the function of ovaries do not always prove efficient for predicting the success of fertility treatments. The presented data suggest that reproductive success is controlled by a wide variety of factors that go far beyond follicular maturation. Therefore, future investigations should pay attention to the integration of many clinical factors into predictive models.

Ethical approval and consent to participate:

The work was carried out at the infertility center of the High Institute for Infertility Diagnosis and assisted reproductive technologies, al-nahrain university in baghdad, iraq. ethical clearance for the protocol was obtained from the institutional review board of mansoura university, egypt, under approval number mdp.24.10.168. all study procedures followed the principles set out in the Declaration of Helsinki, including its later revisions. Each participant signed a written informed consent form before any study-related activity was performed, and personal identifiers were removed prior to data processing and statistical analysis.

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this work.

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