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Original Article

Predictive value of follicular fluid insulin like growth factor-1 in IVF outcome of normo-ovulatory women

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ABSTRACT

Introduction: Insulin like growth factor-1 (IGF-1) has been found to stimulate follicular development, estrogen and progesterone production and oocyte maturation. This study aims to detect the changes in the follicular fluid IGF-1 levels in normo-ovulatory women undergoing in vitro fertilization (IVF) cycle and evaluate the possibility of IGF-1 to be a predictive marker for IVF outcome in normo-ovulatory women.

Materials and methods: This prospective cross-sectional study was performed among 88 IVF normo-ovulatory women who were categorized according to IVF pregnancy outcome (clinical pregnancy) into two groups: 55 pregnant women and 33 non-pregnant women. Follicular fluid IGF-1 levels have been measured at the time of oocyte retrieval by utilizing enzyme linked immune-sorbent assay (IGF-1 ELISA kit, Diametra, Inc, Italy). The relationship between follicular fluid IGF-1 (FF IGF-1) levels and the number of oocytes retrieved, number of mature oocytes, number of embryos, fertilization and clinical pregnancy rate were assessed. The demographic data were similar between two groups as regards age and BMI.

Results: FF IGF-1 levels were significantly higher in pregnant women than in non-pregnant women (90.11 ± 44.31 ng/ml, 69.07 ± 30.55 ng/ml) respectively ($p < 0.05$). There is no significantly correlation between FF IGF-1 levels and any IVF outcomes (retrieved oocyte number, mature oocyte number, fertilized oocyte number, embryos number, percent of mature oocytes, cleavage rate and fertilization rate). In addition to that there is no difference in terms of age and body mass index (BMI).

Conclusion: Our study demonstrated that higher FF IGF-1 levels predict higher pregnancy rates in normo-ovulatory women undergoing IVF cycles.

1. Introduction

Infertility is a disease, defined by the failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse [1].

Infertility has a global prevalence of 9% [2]. Infertility is considered as a major health care problem of different communities [3] and according to World Health Organization (WHO) 60–80 million couples worldwide currently suffer from infertility [4]. Hope is present for infertile couples because the cause of infertility in 85–90% of cases can be diagnosed, and about 50–60% can be treated successfully [5].

Assisted reproductive technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), are widely used to solve human infertility, and have provided great benefits for millions of couples who have struggled with infertility disorders [6].

There are many models including different types of prognostic factors for IVF procedures – age, embryo morphology, number of transferred embryos, type of treatment, diagnosis, serum biochemical markers of the endometrium, etc [7]. It has been proven that during the ART cycles ovulation is controlled by IGF-1 [8].

Insulin-like growth factor 1 (IGF-1) is a 7.7 kDa [9] single-chain polypeptide -70 aminoacids [10]- encoded by chromosome 12 [9] and has endocrine, paracrine, and autocrine effects [11]. IGF-1 is

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an anabolic hormone mainly synthesized in the liver and local expressed in peripheral tissues [12] and the secretory site seems to determine its actions [13]. IGF 1 is a protein that cells use to communicate with other cells and their environment [14].

IGFs may all be implicated in initiation and maintenance of follicle growth [15]. The involvement of the IGF system as intraovarian regulators of folliculogenesis has been intensively studied in a variety of mammal species [16]. Multiple studies have demonstrated the importance of insulin-like growth factors (IGFs) in the amplification of FSH action [17]. IGF-1 synergizes with gonadotropins in stimulating granulosa cell function and promotes follicular survival and development [18]. Locally produced and secreted IGF-1 may affects the mechanisms of folliculogenesis, inducing a larger number of follicles to grow [19]. In particular, the insulin-like growth factor (IGF) family is known to affect mammalian embryo development [20].

The aim of this study is to determine the role of follicular fluid IGF-1 levels (FF IGF-1 Levels) on oocyte development and IVF outcome (pregnancy rate) as a possible predictive indicator to IVF outcome for normo-ovulatory women undergoing IVF cycle.

2. Materials and methods

A total of 88 women were recruited (mean age 28.2 ± 5.0 years, body mass index 26.63 ± 4.43), we categorized those women in two groups according to the primary IVF outcome (clinical pregnancy). Group I (cases) consisted of 55 women that after IVF became pregnant and group II (controls) was formed by 33 women that after IVF attempt did not get pregnant (Fig. 1).

IGF-1 concentrations were assessed in the follicular fluid of all 88 women submitted to IVF as the treatment for infertility at the Human Reproduction Unit, Orient Hospital, Syria, between January 2016 and June 2016.

The research project was approved by the Ethics Committee and registered at Damascus University researches.

2.1. Exclusion criteria

- Women elder than 38 years of age
- Women with polycystic ovary syndrome PCOs (as defined according to the Rotterdam consensus)
- Women laparoscopically diagnosed with endometriosis
- Women with poor ovarian reserve or any other hormonal disorders
- Women with previous endocrine disorders
- Women with pathologies of ovarian or fallopian tubes (e.g. Adenomyosis, Hydrosalpinx)
- Women with any autoimmune disorders or thrombotic diseases disorders

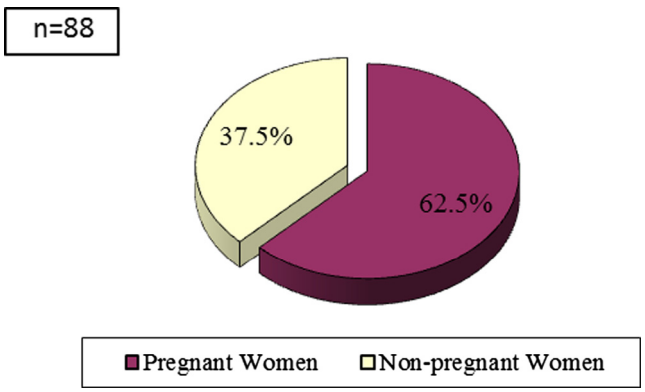

Fig. 1. The percentage of normo-ovulatory women according to study groups.

- Patients with non-obstructive azoospermia were excluded

The patients were similar as regards age, basal FSH, cause of infertility (male factor), and BMI, all patients were on long protocol using GnRH agonist, ovulation Induction was by HMG or rFSH and the dose was adjusted according to Response. When 3 leading follicles reached 18 mm, 10,000 IU of HCG was given and, oocytes retrieval was scheduled 35 h later. Ultrasound guided embryo transfer (ET) of 2–3 embryos was performed after 46–48 h. Luteal phase support was achieved with 400 mg vaginal micronized progesterone twice daily till day of pregnancy test which was done after 14 days post ET. Clinical pregnancy (presence of gestational sac in ultrasound) was done after 3 weeks post ET [21].

Follicular fluid obtained on the day of oocyte was aspirated from all follicles (>15) mm, and then each sample was immediately centrifuged at 3000*g* for 15 min at room temperature to separate out the cellular contents and debris, and each supernatant was transferred to sterile polypropylene tubes and stored at −20 °C until assayed. Samples contaminated with blood were discarded.

IGF-1 levels were detected in follicular fluid samples by enzyme-linked immunosorbent assay (IGF-1 ELISA kit, Diametra, Inc, Italy).

Main Outcomes were measured:

- Oocyte Maturity rate: Total No. of mature oocytes/Total No. of all oocytes
- Oocyte quality is determined through their morphological shape e.g. granulation
- Cleavage rate: Total No. of informed embryos/Total No. of fertilized oocytes
- Fertilization rate: Total No. of zygotes (2 pro nucleus)/Total No. of mature oocytes MII

3. Statistical analysis

The Data was analyzed for statistical significance using SPSS 13.0.

The normality of all studied parameters was evaluated by Kolmogorov–Smirnov test. All values were expressed as mean ± stan-

Table 1
Clinical characteristics of normo-ovulatory women.

	Pregnant women group (n = 55)	Non-pregnant women group (n = 33)	P-value
Age (year)	28.9 ± 4.7	27.2 ± 5.2	NS
BMI (kg/m^2)	26.63 ± 4.41	26.65 ± 4.52	NS
Retrieved oocyte no.	14.58 ± 9.34	15.42 ± 7.26	NS
Mature oocyte no.	9.45 ± 6.59	9.55 ± 5.45	NS
Fertilized oocyte no.	7.55 ± 5.58	5.85 ± 3.93	NS
Embryos no.	4.78 ± 1.47	4.30 ± 1.72	NS
Percent of mature oocytes (%)	64.65 ± 13	60.55 ± 14.68	NS
Cleavage rate	78.32 ± 25.16	84.06 ± 21.61	NS
Fertilization rate (%)	55.14 ± 33.63	40.73 ± 18.35	0.026

NS = non-significant; values are given as mean ± S.D.

Table 2
Follicular fluid Insulin like Growth Factor-1 Levels in pregnant and non-pregnant women.

	Pregnant women group	Non-pregnant women group	P-value
FF IGF-1 (ng/ml)	90.11 ± 44.31	69.07 ± 30.55	0.018

Values are given as mean ± S.D.

Table 3
Correlation between follicular fluid IGF-1 levels and IVF outcomes.

	Pregnant women group (n = 55)		Non-pregnant women group (n = 33)	
	r	P value	r	P value
Retrieved oocyte no.	0.459	0.459	−0.089	0.622
Mature oocyte no.	0.469	0.469	−0.031	0.866
Fertilized oocyte no.	0.618	0.618	0.006	0.973
Embryos no.	0.209	0.209	0.011	0.952
Percent of mature oocytes (%)	0.636	−0.065	0.211	0.238
Cleavage rate	0.686	0.056	0.098	0.960
Fertilization rate (%)	0.601	0.072	−0.009	0.586

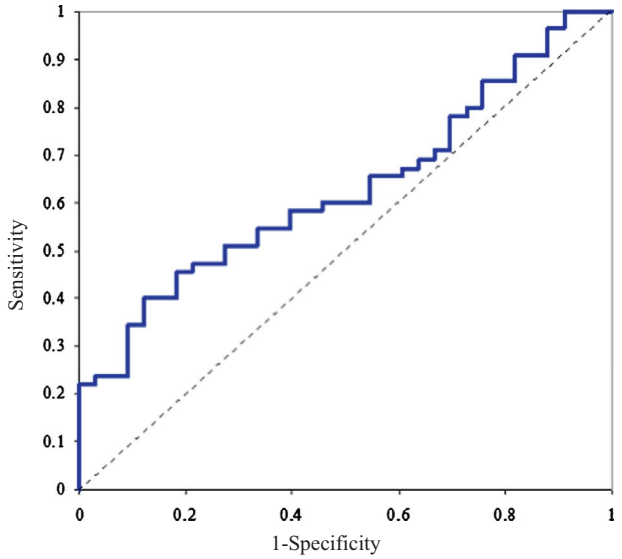

Fig. 2. ROC curve for FF IGF-1 levels in normo-ovulatory women.

Table 4
ROC curve data of FF IGF-1 levels in normo-ovulatory women.

FF IGF-1 (ng/ml)	Specificity (%)	Sensitivity (%)
91.701	81.80	45.50
94.090	81.80	43.60
97.266	81.80	41.80
99.388	81.80	40.00
100.087	84.80	40.00
100.863	**87.90**	**40.00**
101.858	87.90	38.20
102.738	87.90	36.40
104.820	87.90	34.50
108.723	90.90	34.50
111.938	90.90	32.70

dard deviation. Student's *t*-test was used to compare between means while the correlations among the studied parameters were evaluated with Pearson's correlation coefficients. Receiver operating characteristics (ROC) analysis was done to obtain cutoff values and area under curve (AUC) and 95% confidence intervals for IGF-1 determination of pregnancy was evaluated by ROC analysis.). A p value < 0.05 was considered as statistically significant in all cases.

4. Results

Table 1 presents the demographic characteristics of normo-ovulatory women from the two groups (pregnant women & non-pregnant women) and compares between terms of age and body mass index, in addition to the IVF characteristics which could have an influence in our results.

There were no big differences between the two previous groups of women in terms of age and BMI.

FF IGF-1 levels were significantly higher in pregnant women group than in non-pregnant women group (90.11 ± 44.31 ng/ml, 69.07 ± 30.55 ng/ml) respectively ($p < 0.05$) (Table 2).

We did not identify any differences between both groups of women regarding IVF outcomes (retrieved oocyte number, mature oocyte number, fertilized oocyte number, embryos number, percent of mature oocytes cleavage rate and fertilization rate) (Table 3).

FF IGF-1 levels had a predictive value of pregnancy. The predictive potency of FF IGF-1 levels was tested by the receiver operating characteristic (ROC) procedure. In normo-ovulatory women, the area under the curve was 0.629 (Fig. 2) and p value was 0.043.

Several cutoff values of FF IGF-1 levels in both pregnant and non-pregnant women were analyzed in terms of sensitivity and specificity from the ROC curve data.

Table 4 shows that the best adjustment between specificity (87.9%) and sensitivity (40%) was obtained with a cutoff value of 100.863 ng/ml in normo-ovulatory women.

5. Discussion

Various prognostic factors were required in order to determine the suitable patient therapy plan and to evaluate the success of therapy [8].

Our study rested on the stimulatory effects of IGF-1 on oocyte maturation and embryo development [22]. IGF-I stimulates both proliferation and differentiation of granulosa cells, stimulates steroidogenesis in thecal cells [23] and promotes preantral follicle growth [24]. Primary follicle development is dependent on the availability of growth factors (GFs), especially insulin-like growth factor-1 (IGF-1) [25].

Our study shows successfully that follicular fluid levels of IGF-1 were significantly higher in pregnant women group than non-pregnant group, which is in agreement with Mendoza et al. and Mehta et al.

Mendoza et al. study showed that the mean intrafollicular concentration of IGF-1 was also higher in patients who became pregnant as compared with those who failed to establish pregnancy [26].

Mehta et al. reported significantly higher FF IGF-1 levels in pregnant women group versus non-pregnant women group [27] as our study showed. In addition to all of that, both studies confirmed the relationship between FF IGF-1 and pregnancy rate.

According to our study higher levels of FF IGF-1 predict higher pregnancy rates.

Insulin-like growth factor-I (IGF-I) is present in follicular fluid and is synthesized by granulosa cells [22] and the increased IGF1 concentrations can modify the regulatory system of ovarian folliculogenesis to enable: (1) the activation and development of more preantral follicles, (2) the maintenance of larger pools of small antral follicles, (3) the recruitment of more follicles within

the cohort of developing follicles, and (4) the selection of two or more dominant follicles within a follicular wave [8]. All this could explain how FF IGF-1 could influence on the folliculogenesis, hence on the pregnancy rate in the future.

Our study and Mehta BN et al.'s study [27] have another result in common. Both studies found that there was no correlation between FF IGF-1 and cleavage rates neither in pregnant women group nor in non-pregnant women group.

Unlike our finding Dorn C at el reported that follicular fluid levels of IGF-1 showed no association with pregnancy rate [28]. This may be explained by including PCOs patients in their study versus ours. With the increased understanding of the physiology of ovarian folliculogenesis and steroidogenesis, it is now understood that the ovarian microenvironment is very important for normal physiology. It has been proven that during the ART cycles ovulation is controlled by IGF 1 and ovulatory disorders can indirectly effect insulin, IGF and IGFBP levels [8]. So we excluded this group of patients in our study.

Cunha-Filho et al. study showed that IGF-1 was not associated with pregnancy after IVF [29]. This difference in results could return to the different protocols that were used in IVF. In Cunha-Filho et al. study performed the ovarian hyperstimulation with the ultra-short protocol, but in our study the long protocol was used.

The differences in terms of results presented by these investigators could be explained by the fact of the design, patient selection (studied population), inclusion criteria, and reproductive outcome chosen and, also, the controlled stimulation protocol utilized [29] as we mentioned previously in a clear manner.

In conclusion, our study demonstrated that higher FF IGF-1 levels predict higher pregnancy rates in normo-ovulatory women undergoing IVF cycles.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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