Evaluation of Inhibin-B Levels as a predictive marker in a sample of Iraqi Women Undergoing ICSI

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***Abstract*—**Inhibin-B can be released by the granulosa cells of the follicle and is involved in the regulatory functions in developing follicle. Ovarian reserve is directly connected with reproductive capacity. Assisted reproduction has focused more on ovarian reserve function with the aim of improving minimal risk of ovarian hyperstimulation syndrome. A range of prognostic biochemical markers of ovarian reserve can include this important marker "Inhibin-B".

The aim behind this work was to investigate the ability to utilize Inhibin-B as a biomarker to evaluate the pregnancy rate in a sample of Iraqi couples undergoing intracytoplasmic sperm injection(ICSI). Sixty infertile women were selected for undergoing ICSI, the patient's ovarian stimulation were either by antagonist protocol or agonist protocol according to their clinical findings.

The results showed that both serum and follicular fluid levels of Inhibin-B showed no significant correlation with the clinical pregnancy outcome (P > 0.05).

It can conclude that serum or follicular fluid Inhibin-B as a biomarker is a poor predictor of fertility outcome with no significant difference when comparing pregnant and non-pregnant ladies. follicular fluid inhibin in combination with serum inhibin can predict 70 % of positive clinical pregnancy outcome.

Keywords—Inhibin-B ,Follicular fluid, Clinical Pregnancy Outcome , Iraqi Women, Intracytoplasmic Sperm Injection(ICSI).

Introduction

The lack of female fecundity could be due to multifactorial causes and the most common causes are issues with ovulation such as polycystic ovarian syndrome(PCOS)[(1)].

Other causes include endometriosis, tubal, obstruction, medication and certain health issues. Age of the female partner is also could be a common reason for female

infertility, women over 40 year have a smaller number of healthy eggs that tend to be benefit for conception[(2)] ,cleavage and implantation[(3)].

Intra-ovarian factors can conduct a direct role in modulating the sensitivity of follicular cells to gonadotropins and other factors of this system. Of these various locally produced factors involved in this process different members of the transforming growth factor beta(TGF-B) superfamily including activing's, and bone morphogenetic proteins[(4)].

Thus, the follicular fluid (FF) enactment as an environment where by which signal mediators are transported within the follicle between various cell types, as well in and out of the follicle. Oocyte quality and its capability to accomplish fertilization event and the development of the embryo is determining by some biochemical characteristics of the follicular fluid which may play an essential function for it [(5)].

The sixty-year journey to isolate inhibin drove to the recognition of a family of molecules where activins considered to be a member. It also led to identify a member of a special family of binding proteins that has the ability to regulate activin. These researches decipher the possible roles of these hormones in the physiology of the ovary and the pathophysiology of the reproductive system. Together, these molecules affect the development of gonads, germ cell proliferation, the formation of follicle, activation and growth of the follicle, ovulation process, and the function and activity of the corpus luteum function[(6)].

Inhibin-B, on the other hand, is released by the granulosa cells of the follicle and is involved in the regulatory functions in developing follicle[(7,8)].

Ovarian reserve is directly connected with reproductive capacity. Assisted reproduction has focused more on ovarian reserve function with the aim of improving minimal risk of ovarian hyperstimulation syndrome[9] .A range of prognostic biochemical markers of ovarian reserve include follicle stimulating hormone (FSH), estradiol (E2), Inhibin-B and Anti-Mullerian hormone (AMH)[10].

Subjects, Materials and Methods

Sixty infertile female ladies aged range between 18 - 41 years old, their body mass index (BMI) ranged from (20-28.5) were recruited from the clinic of infertility in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies (ART's) at Al-Nahrain University and from private infertility center (Baghdad Specialist Center) all subjected to a controlled ovarian stimulation for IVF /ICSI treatment were either by antagonist protocol or agonist protocol according to their clinical findings.

Hormonal analysis was performed at day two of the menstrual cycle, included serum follicle stimulation hormone, luteinizing hormone, Estradiol (E_2), Prolactin hormone, Testosterone, and Thyroid stimulating hormone.

All ultrasound scanning in assisted conception was performed transvaginally, while the Hysterosalpingography (HSG) was performed to exclude tubal blockage and to check for hydrosalpinges as it has a negative impact on the success rate, also to assess the integrity of the uterine cavity.

While a full history and physical examination for male partner were done by the urologist, a thorough semen analysis was carried out for all males referred for assisted conception to assure the most convenient technique that would be proper for the patient after a period where a (3-5) day of abstinence[11].

Transvaginal ultrasound guided oocyte retrieval was done 34 -36 hours following the hCG injection under general anesthesia.

Blood samples were collected from each woman enrolled in ICSI procedure. The blood was allowed to coagulate for 30 minutes then centrifuged at 1500 rpm for

15 minutes to separate the serum. The serum was quickly frozen and stored at -20C° until hormonal assays were performed. For Inhibin-B assay ,the serum and follicular fluid were collected on the ova pick up day(OPU) then centrifuged ,frozen and stored at -20 C° until the analysis or assay process.

Results

Statistical analysis in the current study was based on the inclusion of 60 infertile women and at the end of the study, positive clinical pregnancy was achieved in 28 ladies accounting for a rate of 46.7 %, as shown in **figure-1**. From now and then, results will be outlined according to pregnancy outcome so that characteristics of pregnant women will be contrasted with those women who failed to get clinical pregnancy.

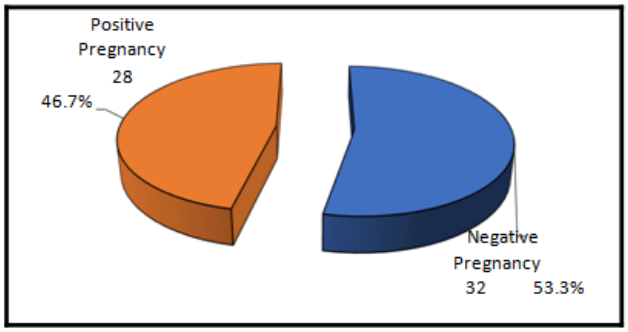

Figure 1: Pie chart showing the pregnancy rate in sub-fertile women included in the present study

Serum and follicular fluid marker, Inhibin-B, of infertile women participating in the current study was described as median and inter-quartile range (IQR) due to non-normality distribution of these quantitative variables following performance of Kolmogorov-Smirnov test.

Results shows there was no significant difference in the serum Inhibin-B between pregnant and non-pregnant ladies, 68.24 (101.77) versus 65.72 (19.63),

respectively (P = 0.480). Added to that, there was no significant difference in follicular fluid Inhibin-B between pregnant and non-pregnant ladies, 68.45 (63.09) versus 60.80 (20.02), respectively (P = 0.174), (**Table-1**), (**Figure 2**)

Table 1: Serum and follicular fluid Inhibin-B in association with clinical pregnancy outcome

Characteristic	Total $n = 60$	Positive pregnancy $n = 28$	Negative pregnancy $n = 32$	*P*
Serum Inhibin-B				0.480 † NS
Median (IQR)	67.10 (32.03)	68.24 (101.77)	65.72 (19.63)	
Range	7.57 - 876.56	40.22 - 876.56	7.57 - 752.39	
FF Inhibin-B				0.174 † NS
Median (IQR)	64.20 (32.86)	68.45 (63.09)	60.80 (20.02)	
Range	9.27 - 736.05	9.27 - 736.05	31.89 - 575.02	

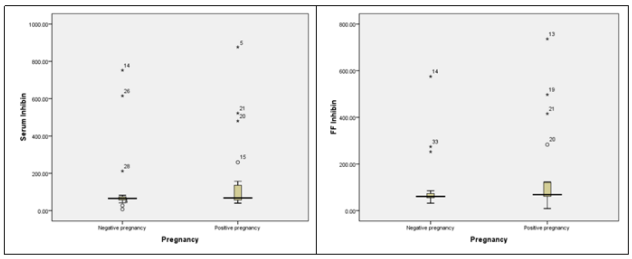

Figure 2: Median serum and follicular fluid Inhibin-B in pregnant and non-pregnant ladies.

The serum and follicular fluid Inhibin-B was further analyzed aiming at finding the best cutoff value that can predict positive clinical pregnancy outcome. The cutoff value of serum Inhibin-B was > 82.84 with poor accuracy of 56.5 %, poor sensitivity of 36.8 % and high specificity of 86.6 %. The cutoff value of follicular fluid Inhibin-B was > 59.8 with poor accuracy of 62.4 %, good sensitivity of 79.0 % and poor specificity of 50 %. (**Table-2**), (**Figure 3**).

Table-2: Characteristics of ROC curve concerning serum and follicular fluid Inhibin-B.

Characteristic	Inhibin-B	
	Serum	Follicular fluid
Cutoff	> 82.84	> 59.8
AUC	0.565	0.624
95%CI	0.401 - 0.719	0.460 - 0.771
Accuracy	56.5	62.4
P	0.491 NS	0.171 NS
Sensitivity	36.8	79.0
Specificity	86.6	50.0

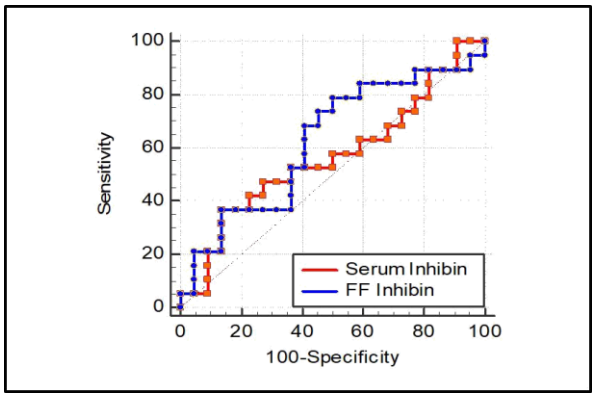

Figure 3: Receiver operator characteristic (ROC) curve analysis to find the best serum and follicular fluid Inhibin-B cutoff values that predict positive clinical pregnancy outcome.

Discussion

In the present study, positive clinical pregnancy was achieved in 19 ladies accounting for a rate of 46.3 %. In one Iraqi study carried out at Fertility Center in An-Najaf governorate at Al-Sadr Medical City, the successful clinical pregnancy rate following ICSI was 28.9 % [(12)], which is less than that of the present study. In another Iraqi study included 54 couples and conducted in Baghdad IVF infertility center, the clinical pregnancy outcome following ICSI was 20.4 % [(13)], which is lower than that achieved in the current study.

However, a pregnancy rate of 51 % was recorded by some Iraqi authors in a study carried out at out at the High institute for infertility diagnosis and Assisted Reproductive Technologies/AL-Nahrain University and Specialist Fertility Department/ Al-Bonook hospital [(14)].

In nearby countries, the successful clinical pregnancy rate following ART's has ranged from 21.5 to 33.9 % [(15,16)], indicating that the rate of positive clinical

pregnancy in the current study is one of the best achieved rates.

However, in a number of fertility centers in the United States, the rate of positive clinical pregnancy has reached relatively high figures between 43.9 % and 54.2 % [17]. Therefore, the conductance of the current study and other studies in our fertility center is aiming an increasing the rate of successful clinical pregnancy following ART's procedures.

In the current study in addition, there was no significant difference in the serum Inhibin-B when comparing between pregnant and non-pregnant ladies. Added to that, there was no significant difference in follicular fluid Inhibin-B between pregnant and non-pregnant ladies.

The growing ovarian follicles with its granulosa cells produce inhibins; as follicles develops toward the stage of antral formation, the majority of inhibin-producing granulosa cells proliferates and increases. For this reason, inhibin levels can be used as a tool utilized for providing a good surrogate measuring the health and the viability of the follicle. In women with issue of declining ovarian reserve, reduced serum Inhibin-B levels at third day of the menstrual cycle are observed in conjunction with a low FSH concentration[18].

Conversely, high levels of serum and follicular fluid Inhibin-B levels get together with increased pregnancy rate and good ovarian response in women applied to hormonal stimulation for oocyte pick up in IVF. These results are consistent with observation of other works which has been documented that women shows low serum inhibin when the pool of recruitable follicles become reduced [10].

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