The Role Of IGF-I in Females Selected for Assisted Reproductive Technique (Art)

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Abstract

Objective: To assess the role of Insulin growth factor-I (IGF-I) in females selected for assisted reproductive technique (ART).

Methods: In this cross-sectional study 133 infertile females were recruited for Intracytoplasmic sperm injection (ICSI). The antral follicle count (AFC), pre-ovulatory follicle count (PFC), total doses of follicle stimulating hormone (FSH) were estimated. IGF was measured. Successful pregnancy conception after ICSI was confirmed by an intrauterine gestational sac with cardiac activity after embryo transfer. Receiver Operating Curve (ROC) analysis was also done to find out the area under the curve for clinically pregnant samples using IGF-I. Binary logistic regression analysis was done to estimate the odds ratio with 95% confidence interval for clinical pregnancy using IGF-I; all p-values less than 0.05 were considered significant.

Results: Study results revealed that IGF-I gave positive association with clinical pregnancy outcome, and is a more reliable predictor of clinical pregnancy. IGF-I gives 39.8% positive correlation with number of oocytes fertilized. These correlations were statistically significant with p-values less than 0.05. Area under the curve for clinical pregnancy using IGF-I, was 72.4% which reveals the importance of IGF-1 in predicting the outcome of ART.

Conclusion: Based on the results of the present study we came to the conclusion that IGF-I is a better and cheaper option and an important tool in the field of infertility.

Keywords: Insulin like growth factor-1, intracytoplasmic sperm injection, assisted reproductive technique, infertility.

IRB: Approved by the Institutional Review Board Committee of Jinnah Postgraduate Medical Centre, Ref# F02081/2019-GENL/34896/JPMC. Dated: 5th October 2019.

Citation: Salahuddin H, Anjum A, Rehman R, Rehman S, Kumar W, Jan NA. The Role Of IGF-I In in Females Selected for Assisted Reproductive Technique (Art) [Online]. Annals ASH KMDC 2020;25:

Introduction

Infertility is considered as a major health care problem of different societies. Assisted reproductive technologies (ART) including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are globally utilized to resolve human infertility issues. IGFs play an important role in initiation and maintenance of follicle growth[1].

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Date of Submission: 2nd December 2019
Date of Acceptance: 12th March 2020

Infertility is a biological inability of a women to conceive a baby naturally within or after a year of consistently having regular unprotected intercourse[1]. Infertility affects around 8-12%[2], couples worldwide approximately while the prevalence of infertility in Pakistan is about 21.9%[3]. Of the two main type of infertility, primary infertility is the one in which couples have not been able to conceive a baby after one year or more of consistent regular unprotected intercourse[4], whereas if couples have conceived once previously but are unable to conceive again is considered as secondary infertility[4].

Female factor is the most common cause of infertility (40-55%)[5], followed by the male factor (30-40%), cause in both partners 10%, and unexplained (10%) factor in the remainder[6]. While male causes

of infertility include alteration in the sperm parameters, namely the motility, the count or the morphology[7], the female causes of infertility include multiple causes like pelvic inflammatory disease (PID), endometriosis, polycystic ovarian syndrome (PCOS), premature ovarian failure, uterine fibroids, ovulation problems, tubal blockage, age-related factors, uterine problems, previous tubal ligation and hormone disturbances[8].

There are number of conditions which result in infertility in males. Some important are production of abnormal sperms because of undescended testicles, genetic deficiencies, health issues for instance, diabetes or diseases like chlamydia, gonorrhoea, mumps or HIV. Further the quality of the sperm can also get affected by the enlarged veins in the testes (varicocele)[9]. Difficulty in delivery of sperms is another cause of infertility. It occurs because of sexual problems like early ejaculation. It may also be due to genetic diseases like cystic fibrosis, physical complications like obstruction in the testicle or impairment or damage to the reproductive organs[9]. Moreover, environmental factors like excessive exposure to insecticides and other harmful chemicals along with marijuana or certain medications or drugs like antibiotics, antihypertensives[10] and anabolic steroids are also harmful and may affect potency. Also, recurrent contact to heat like taking hot baths or saunas result in increase in the core temperature of the body and eventually affect sperm production. Cancer and its treatment can cause severe damage in males by impairing the production of sperms. Other than that, there are lots of female related causes like menstrual disturbance often referred to as ovulation disorder is leading cause of infertility[11]. This affects the ovulation. These include hormonal conditions like polycystic ovarian syndrome and hyper prolactinemia, a disorder in which one has over production of prolactin, the hormone that activates production of breast milk and possibly disturbs ovulation. Both hyperthyroidism and hypo thyroidism can cause disturbances in menstrual cycle[12]. Moreover, intense physical activities or exercises, abnormal eating habits, injuries or tumours may also affect fertility. There are uterine or cervical anomalies leading to infertility like abnormalities related to the cervical opening, growth within or outside the uterus. The presence of non cancerous (benign) tumours in the uterine wall (uterine fibroids) can block the fallopian tubes which results infertility. Further, implantation process of the fertilized egg may get inhibited by fibroids[13].

Blocked or damaged fallopian tubes are most commonly caused by the inflammatory disease of the pelvis which is a result of sexually transmitted infections or endometriosis. It happens when the lining grows beyond the uterus and disturbs the functioning of the ovaries, uterus and fallopian tubes[14].

Premature ovarian failure is commonly referred to as early menopause. This occurs when the ovaries fail to work normally before the age of 40[15]. There are certain factors related to early end of menstruation such as diseases related to immune system, inherited illnesses, radiation or chemotherapy and drug usage[16].

Pelvic adhesions are basically the growth of scar tissues between organs in pelvic area after appendicitis, pelvic or abdominal surgery and any related surgeries[17].

There are certain risk factors for infertility which include, age factor as with passage of time the health of human body declines and requires extra effort to fulfil certain tasks[18]. Particularly in women, fertility starts declining after 30s and swiftly drops after 37. The quality and quantity of eggs produced by an older woman is immensely affected by health issues and ultimately results in infertility[19]. However, men over 40 tend to have less infertility issues as compared to other medical problems like cancer or psychiatric disorders[20]. Other than age, either partner smoking tobacco or marijuana can decrease the couple's chance of conceiving a baby. Further, smoking also interferes in treating infertility[21]. As per the researches, miscarriages are often observed among women who are into smoking. On the other side, men who smoke have higher risk of erectile dysfunction and low sperm count. Also, use

of alcohol is a complete no for women during conception or pregnancy. Infertility and birth defect risks are higher in alcoholic women[22]. Meanwhile, in men it affects the sperm count and motility. Moreover, overweight women are more prone to infertility. In overweight men, sperm count may get affected. Underweight women are also likely to have fertility issues particularly the ones having eating disorders (anorexia or bulimia) and those who follow strict low calories diet[22]. Lack of physical movement and routine exercise can lead to obesity and eventually increases infertility possibilities. On the other side, intense exercise regime is often associated with ovulation problems in women who have normal weight.

Infertility is treatable in many or almost all cases if the cause is properly diagnosed. Assisted reproductive techniques (ART) refer to different methods that are used to achieve pregnancy by artificial means. The simplest ART procedure is in vitro fertilization (IVF), which has been around for over 20 years and is the most commonly recognized and widely practiced ART of all procedures. ICSI is microscopic procedure that facilitates fertilization of an oocyte by directly injecting a single spermatozoon[22]. Insulin like growth factor-1 (IGF-1), a member of the ovarian IGF system, has shown to exert direct effects on human granulosa cell function. A major role of IGF-I in regulation of human follicular and embryonic development through regulation of the cell cycle has been reported[22]. It has also been implicated in mediating aromatase activity and oestrogen production by the developing follicle[22]. IGF-1 has been suggested as a potential biochemical marker of embryo quality and is also found to be responsible for successful implantation rates in IVF cycles. It has been established and proven in some researches that during ART cycles the process of ovulation is controlled by insulin growth factor (IGF-1).The action of IGF-I system as an intra-ovarian regulator of folliculogenesis has been intensely studied in a number of mammal species. Multiple researches have shown the importance of IGF-I in the promotion of FSH action and follicular development and survival[23]. IGF-I is formed

by granulosa cells and the increased levels can rectify the regulatory system of ovarian folliculogenesis to facilitate the activation and growth of more pre-antral follicles, preservation of larger pools of small antral follicles, enrolment of more follicles within the series of developing follicles and then the selection of two or more leading follicles within follicular panel[23].

The entire treatment process of assisted reproductive technique (ART) accompanies challenges with it, most importantly the retrieval of healthy egg from a woman receiving infertility treatment in order to achieve the desired outcome[24]. Miscarriages and difficulty in conception in majority of cases happen due to poor quality of eggs. Hence, it is highly necessary to predict the condition of oocyte and embryo before implantation. As ART is an expensive procedure and requires expertise, it's better to assess the outcome to save the patient from undue efforts and exposure, this study will be of importance since it will give an idea of outcome of ART and can save the cost of treatment for the ease of patients and the risk of unnecessary interventions.

The reason we assessed the role of IGF-I is that IGF-I has the potential to assess and impact the pregnancy outcome in females who are selected for ART. This study aims to assess the role of insulin growth factor I, in prediction of pregnancy outcome in infertile females selected for intracytoplasmic sperm injection (ICSI).

Subjects and Methods

It was a cross-sectional study in Australian Concept Infertility Clinic in collaboration with Basic Health Sciences Institute (JPMC). Taking the sensitivity of IGF-I at 80% and specificity at 80%, margin of error at 5% and a confidence interval of 95%, the calculated sample size was 133. This sample size was calculated using the online available software open epi.com using the following formula.

$$n = \frac{Z^2_{\alpha/2}pq}{e^2} = \frac{(1.96)^2(0.3)(0.7)}{(0.05)^2} = 323 \quad where\ \alpha = Confidence\ interval, p = Probability\ of\ incidence\ and\ e = error\ Margin$$

The study subjects were selected via convenient sampling technique. The patients were grouped on the types and causes of infertility. A total of 133 infertile females were enrolled during the study period and informed written consent was signed by both the partners at time of enrollment. Cases of both primary and secondary infertility were included, other inclusion criteria included: duration of infertility more than 2 years, female aged 20-45 years, normal ovulatory cycles (25-35) days, no morphological abnormalities in any of the two ovaries. Females more than 45 years of age, women with previously failed IVF/ICSI, detection of ovarian cysts, and endometriosis with ovarian pathologies were excluded from the study. Detailed history was taken, and general physical examination was performed to assess potential causes of infertility in both the partners.

IGF-I Elisa kit was used for research purpose only. The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IGF-I. The kit number was SEA050Hu. The detection range was (0.19-12 ng/ml).

The microplate provided in this kit was precoated with an antibody specific to IGF1. Standards or samples were then added to the appropriate microplate wells with a biotin-conjugated antibody specific to IGF1. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contained IGF1, biotin-conjugated antibody and enzyme-conjugated Avidin showed a change in colour. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of IGF1 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

All women went through the usual IVF/ICSI procedure explained above, our aim was to observe the antral follicle count, the pre-ovulatory count, the total dose of FSH and calculate the FSI then correlating this with pregnancy.

Results were analysed as non-pregnant (beta hCG 5-25m IU/ml) and clinical pregnancy with (beta hCG more than 25m IU/ml) with cardiac activity.

Data was analyzed using IBM SPSS version 23.0. Mean and standard deviation were reported for quantitative variables. Count and percentages were reported for qualitative variables. Non-parametric Mann Whitney U test was used to compare the mean values between primary and secondary type of infertile samples. Pearson coefficient of correlation was used to measure the strength of relationship between IGF-I, number of oocytes fertilized, number of oocytes retrieved, number of oocytes in Metaphase II and number of cleaved embryos. Binary logistic regression analysis was done to estimate the odds ratio with 95% confidence interval for clinical pregnancy using IGF-I. All p-values less than or equal to 0.05 were considered significant.

Results

The following bar chart (Fig.1) gives the comparison of IGF-I with the types of infertility, showing that the couples in which there is male factor infertility their females show higher values of IGF-I as compared to couples in which there is female factor infertility or unexplained infertility. Thus, proving the importance of IGF-I in fertility treatment process.
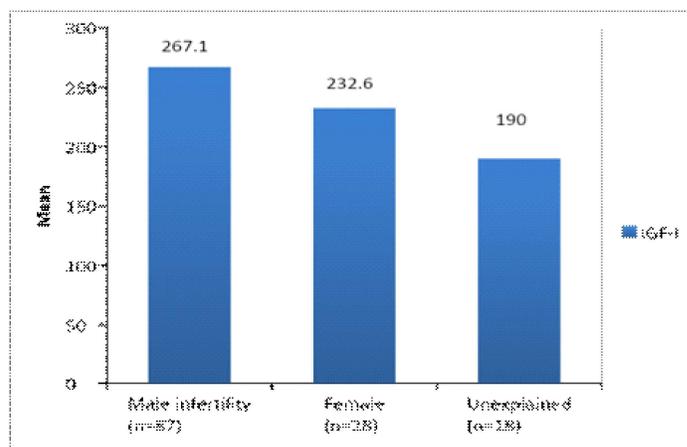

Fig 1. Comparison of IGF-I with types of infertility

Table 1 gives the multiple comparisons between groups for significant parameters, results showed that mean BMI of male infertile samples was significantly higher as compared to female infertile samples and mean IGF-I of male infertile samples was significantly higher as compared to unexplained infertility, with p-values less than 0.05.

Table 1. Mean comparison of characteristics across causes of infertility.

Parameters	Cause of infertility						p-value
	Male infertility (n=87)		Female (n=28)		Unexplained (n=18)		
	Mean	SD	Mean	SD	Mean	SD	
Female age(yrs)	31.59	4.00	32.11	6.15	32.22	3.52	0.38
BMI(kg/m^2)	26.08	2.99	24.50	1.90	26.06	2.84	<0.01*
IGF-I	267.1	77.6	232.6	46.5	190.0	95.2	<0.01*

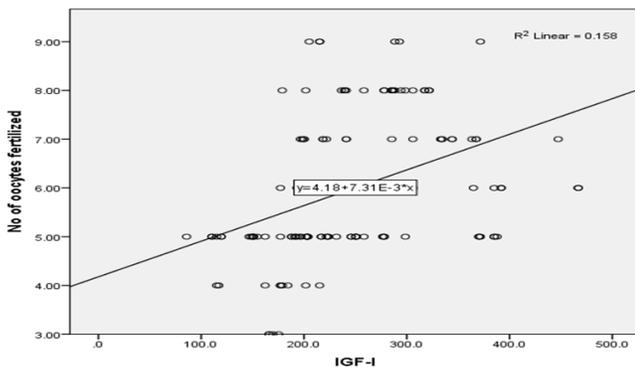*p-value <0.05 was considered significant using Kruskal Wallis Test

Fig.2 IGF-I showing positive corellation with number of Oocyted fertilized

Discussion

Infertility is frustrating both emotionally and psychologically, the causes of infertility are manifold but the advancements in assisted reproductive techniques most commonly IVF/ICSI (intra cytoplasmic sperm insemination), have opened the doors of hopes for couples seeking help.

Being an expensive procedure requiring a lot of precision, it is extremely important to examine the condition of oocyte and embryo before implantation to save the patient from undue efforts and expenses. In past many researches have been done to identify different kinds of markers to predict ovarian and follicular response to exogenous stimulation that can guide treatment protocol for each patient and help in reaching better results with less complications.

In this study we have used IGF-I (a biochemical marker) which works as an intra-ovarian regulator of follicle function as a potential marker of embryos quality and predictors of the successful outcome of ART. We compared the female age, BMI, duration of infertility in years and IGF-1 values between the primary and secondary infertile women. The difference in the IGF-1 values between primary and secondary infertile women was significant with IGF-1 values higher in the women with secondary infertility as compared to primary infertility (p=0.02). The differences in all the other parameters were statistically insignificant between primary and secondary infertile women. Similarly, in another study it was seen that the difference in the age of the females and the BMI between primary and secondary infertility was statistically insignificant (p value= 0.08 and p value= 0.14 respectively)[16]. The same study however had differing results from our study for the duration of infertility, where the difference of the duration of infertility in years between primary and secondary infertility was found to be significant (p<0.001)[16]. The difference in the age and the duration of infertility between women with primary and secondary infertility was found to be significant (p<0.001 for both) in another study while the difference in the BMI between the two was insignificant (p=0.39)[17]. According to the findings of our study we found that higher IGF-I values predicts higher pregnancy rates in normo-ovulatory women undergoing IVF cycles. Increase in IGF-I levels were associated with clinical pregnancy, it is thus a good predictor of viable pregnancy outcome. Women with higher IGF-I values had higher oocytes retrieved and a progressive increase in clinical pregnancy rates. Another advantage of IGF-I is that it can guide in cases of failed ART cycles, such that females with low IGF-I values should receive higher doses of FSH in future[18], while those with higher levels of IGF-I

should receive same doses, this makes IGF-I more reliable predictor of viable pregnancy outcome.

On assessing the results of IGF-I, we found it to be a better predictor of follicular response to hormones and thus a better guide for the management of future IVF/ICSI cycles[19]. To our knowledge it is the first novel study of its kind that is done locally. The results of this study will help the infertile couples seek help through ART. It is an important tool that is not yet used much locally. Since we also proved the importance of IGF-I levels in the positive outcome of pregnancy therefore it can also be used as potential biomarker to assess the outcome of fertility treatment. The limitation of this study is its small sample size. Therefore, study with a larger sample size may be useful in the future.

Conclusion

Considering the researches available and after the results of present study we came to the conclusion that IGF-I is a better and cheaper option and is also an important tool in the field of infertility. Insulin-like growth factor-1 (IGF-1), a member of ovarian IGF system, has been believed to work as an inter-ovarian regulator of follicle function which exerts its direct effects on human granulosa cell function. IGF-1 has been proposed to be a probable and potential biochemical marker of embryo quality and was also found to be responsible for successful implantation rates in IVF cycles. Some researchers have reported a major role of IGF-1 in regulation of human follicular and embryonic development through regulation of the cell cycle.

Conflict of Interests

Authors have no conflict of interests and received no grant/funding from any organization.

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