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FOLLICULAR MYOINOSITOL IS PREDICTOR OF OOCYTE MATURATION AND BEST QUALITY EMBRYOS IN POLYCYSTIC AND NON-POLYCYSTIC PATIENT IN ICSI CYCLES

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ABSTRACT

Background: Inositol are distributed in mammalian tissues and cells where they achieve important biologic functions, which have a role in regulating hormones activities like follicle stimulating hormone FSH, thyroid stimulating hormone TSH and insulin as a second messenger. Myoinositol has a positive effect on fertility and a role in reproduction and actively imported into mammalian cells, including oocytes and pre implantation embryos. Poly cystic ovary syndrome (PCOS) is a common endocrine disorder and usually associated with insulin resistance. Myoinositol used as insulin sensitizer drug in PCOS patients with insulin resistance. ***Objective:*** To study the effect of myoinositol in the follicular fluid of PCOS patients with insulin resistance on the number of retrieved oocytes, oocytes maturity and number of embryos and to find if there is any correlation between myoinositol levels in the serum and in follicular fluid. ***Patients and methods:*** This prospective study was undertaken in the High Institute of Infertility diagnosis and Assisted Reproductive Technologies / Al-Nahrain University / Baghdad/Iraq, during the period from July 2016 to April 2017. A total of 58 infertile women forty women with Polycystic ovary syndrome (twenty of them with insulin resistance (IR) and the other twenty were non-insulin resistance (NIR)) and eighteen non PCOS women as a control. All of them were underwent controlled ovarian hyperstimulation for intracytoplasmic sperm injection cycle. Long agonist protocol was used as ovulation induction protocol in all the cases. Serum and follicular myoinositol levels were measured on the day of oocyte

retrieval by using Enzyme linked immune sorbent assay (ELISA) for all cases. Comparison in serum and follicular myoinositol level between the groups and their correlation to oocyte maturation and embryo quality is done to all cases. ***Results:*** There was no significant difference among the three groups (PCOS (IR), PCOS (NIR), control) in the serum and follicular myoinositol level. In all the cases there were significant positive correlation between the follicular myoinositol level and the total number of oocytes, total number of MII, number of fertilized MII, number of grade I embryos and serum myoinositol level. Serum myoinositol levels showed strong positive correlation *p*-value (0.044) to the number of grade I embryos, in PCOS patients with (IR), regarding other ICSI parameters as total number of retrieved oocytes, number of mature oocytes (metaphase II), number of fertilized metaphase II; there were positive correlation between these parameter and serum myoinositol levels even the correlation was statistically not significant. ***Conclusion:*** Follicular myoinositol can be assumed as a good indicator for oocytes maturity, and predictor of higher number of fertilized MII and good quality embryos in stimulated ICSI cycles in PCOS and non PCOS patient.

KEYWORD: IVF, myoinositol, follicular fluid, oocyte & embryo quality.

INTRODUCTION

Infertility is a complex disorder with significant medical, psychosocial and economic problems and defined as failure to achieve a successful pregnancy within 12 months or more of regular unprotected sexual intercourse.[1] Polycystic Ovary Syndrome (PCOS) is one of the important causes of female infertility; The syndrome acquired its widely used name from the common sign on ultrasound examination of multiple (poly) ovarian cysts, these “cysts” actually are immature follicles not cysts, the follicles have developed from primordial follicles, but the development was arrested at the antral stage due to disturbed ovarian function. These follicles may be located along the periphery of the ovaries, giving the look 'string of pearls’ on ultrasound examination.[2] PCOS is multifactorial in origin[3], the pathogenesis of PCOS has been linked to the development of insulin resistance and hyperinsulinemia, which is commonly observed in these patients.[4] It has been assumed that an altered insulin signal transduction in PCOS patients may cause insulin resistance, which in turn induces abnormal ovarian steroidogenesis.[5] IVF has been proven to be highly effective therapy for treating infertility and childless couples with a variety of etiologic causes (female or male factor infertility, or combined). Intracytoplasmic sperm injection (ICSI) is expensive,

time consuming, therefore efforts have been made to set the factors which predict a successful outcome in a given patient or couple.

Myoinositol has nine stereoisomers, all belong to the family of inositols with formula $C_6H_{12}O_6$[6], Inositols are largely circulated in mammalian tissues and cells where they achieve important biologic functions.[7] Within the cell, Myoinositol is not only present as free form, but also as a part of membrane phosphoinositides and as inositoltrisphosphate (InsP3), which have a role in regulating hormones activities that include Follicle-Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH) and insulin as a second messenger[8], myoinositol has a positive influence on fertility and a role in reproduction.[4]

PATIENTS, MATERIALS AND METHODS

Patients

Fifty eight infertile couples underwent ICSI cycles forty women with Polycystic ovary syndrome (twenty of them with insulin resistance (IR) and the other twenty were non-insulin resistance (NIR)) and eighteen non PCOS women as a control were enrolled in a prospective case control study. The study was approved by Local Medical Ethical Committee of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University; Patients with thyroid dysfunction, cushing's syndrome, hyperprolactinaemia, late onset congenital adrenal hyperplasia, hypogonadotropic hypogonadism, uterine pathology and patients on Insulin sensitizing drugs or inositol supplements were excluded from the study. All patients were subjected to full history taking, complete physical examination, measurement of BMI, basal hormone level (FSH, LH, LH/FSH ratio , prolactin, and estradiol) in cycle day 2 and estradiol at the day of hCG injection.

All patients were treated with a similar long agonist protocol of GnRH analogue (Decapeptyl® 0.1 mg, Ferring Co., Germany) administered from day 21 of the preceding period and continued until the day of HCG injection in a daily dose of 0.1 mg subcutaneous injection. Ovarian stimulation started day 2 of menstrual cycle after confirmation of pituitary desensitization with a daily dose of 150–450 IU rFSH (follitropin alfa, Gonal F Merck Serono) depending on patient age, BMI, and antral follicles count. Later in the stimulation period daily dose of rFSH was adjusted individually. Patients monitored by serial trans-vaginal sonography (TVS), serum estradiol level. Subcutaneous recombinant HCG (Ovitrelle 250 microgram, Merck Serono) was administered when 3 or more follicles reached a diameter of 18 mm for final oocytes maturation.

IVF procedures and Follicular Fluid collection

Transvaginal oocytes retrieval was performed 34-36 hours after HCG injection with ultrasound guidance. A clearly visible follicle, preferred as start of ova retrieval procedure, individually aspirated, the aspirated follicular fluid (FF) should be clear, centrifugation of FF was performed at 600 *g* for 10 min to remove debris. The clear FF samples were then stored at –20°C until assayed. Meanwhile, once the oocyte-cumulus complexes were collected they are rinsed with flushing media For removing any remaining blood from the follicular aspirate, transferred into drops of Ferticult Flushing media then washed by Gain medium and overlaid by paraffin/ mineral oil in an incubator at 37°C with 5-6% CO2 and at 95% humidity.

The ICSI procedure was performed (4–6) hours after oocyte retrieval to all patients. In preparation for intracytoplasmic sperm injection, the cumulus corona cells are removed by a combined enzymatic and mechanical treatment to denude the oocytes from the cumulus cells. Each oocyte is carefully assessed, noting the presence or absence of germinal vesicle or the first polar body. Only those ova that have been extrude the first polar body (metaphase II) and morphologically intact were suitable for microinjection. Around 12-17 hours after ICSI procedure, fertilization was assessed for evidence of normal fertilization which was defined as the existence of two pronuclei (2PN).

Prior to embryo transfer, the developed embryos were graded in accordance with embryo grading system.[9] According to this system the embryo graded as grade1, 2 and 3. Grade 1 embryo with less than 10% fragmentation, stage-specific cell size, no multinucleation. Grade 2 embryo with 10–25% fragmentation, stage-specific cell size for majority of cells, no evidence of multinucleation. Grade 3 embryo with severe fragmentation (>25%), cell size not stage specific, evidence of multinucleation.

Measurement of myoinositol

Follicular fluid and serum obtained on the day of oocyte retrieval were estimated for myoinositol levels by enzyme-linked immunosorbent assay (ELISA), technique using diagnostic kit (Myoinositol oxygenase, Mybiosource, USA) that provides quantitative determination of endogenic human myoinositol concentration in serum and follicular fluid.

Measurement of fasting blood glucose and fasting insulin level

Blood samples (5ml) were taken, on the day of oocyte retrieval, immediately before the procedure, from the median cubital vein by disposable syringe, into serum separating tube,

and allowed to clot for 30 minutes. Serum were obtained after centrifugation at a rate of 3500 rpm for 10 minutes, then the clear serum are stored at -20°C until assayed.

Blood sugar measured using a spectrophotometer after 8-12 hours fasting; the test is usually done in the morning, using diagnostic kit (fasting blood sugar kit, spin react, spain). Determination of fasting serum insulin levels by ELISA technique using diagnostic kit (Human Insulin ELISA Kit, Accu Bind, USA). Insulin resistance identified using the homeostatic model assessment HOMA, which is a method used to quantify insulin resistance and beta-cell function. HOMA can be measured by using the following equation[10]:

HOMA-IR= fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5.

Normal value in adults is < 2.[11]

Statistical analyses

Analysis of data was carried out using the statistical package SPSS-24 (Statistical Packages for Social Sciences- version 24); data were presented in simple measures of frequency, percentage, mean, standard deviation and range. The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means. Categorical variables were assessed by Fisher's exact test. A p-value <0.05 was considered statistically significant. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation).

RESULTS

Fifty eight patients enrolled in ICSI cycles in this prospective study, the demographic data of the PCOS patients with insulin resistance (PCOS IR), PCOS patients with non-insulin resistance (PCOS NIR) group and non-PCOS patients (control) group are shown in (Table1). The statistical analysis showed no significant difference among the three groups concerning the age, BMI and duration of infertility.

Table 1: Demographic information, hormonal and myoinositol values for the patients.

	PCOS IR		PCOS Non-IR		Controls		*P* value
	No.	Mean±SD (Range)	No.	Mean±SD (Range)	No.	Mean±SD (Range)	
Age (years)	20	29.95±4.62 (20-37)	20	27.55±5.46 (18-41)	18	29.61±6.93 (18-41)	0.365
BMI (Kg/m^2)	20	28.08±5.71 (19.14-39.78)	20	27.38±3.96 (20.00-36.03)	18	27.58±4.90 (19.38-37.80)	0.899

Duration of infertility (years)	20	6.88±3.75 (1.5-15.0)	20	6.73±3.05 (1.5-12.0)	18	4.96±2.69 (1.5-12.0)	0.137
FSH (mg/dL)	20	6.48±2.03 (2.50-10.40)	20	5.73±1.96 (1.90-9.50)	18	6.08±1.70 (3.10-9.43)	0.466
LH (mg/dL)	20	6.57±5.17 (1.04-19.36)	20	6.29±3.20 (1.10-14.79)	18	5.02±1.91 (1.30-9.21)	0.407
LH/FSH Ratio	20	1.019±0.72 (0.201-2.616)	20	1.249±0.922 (0.397-4.429)	18	0.814±0.17 (0.342-1.123)	0.165
Basal E2 (pg/ml)	20	47.29±27.8 (17.4-123.0)	20	37.57±19.13 (10.8-98.93)	18	38.21±14.0 (11.50-67.0)	0.285
Prolactin (mg/dL)	20	23.48±9.19 (12.80-40.80)	20	20.78±7.02 (7.90-34.50)	18	19.79±6.10 (9.21-30.40)	0.305
E2 at hCG day (mg/dl)	20	1410.46±1232.15 (134-5718)	20	1374.28±776.79 (396-3100)	18	1480.23±739.31 (403-3000)	0.305
Follicular Myoinositol level (ng/ml)	20	3.02±1.18 (1.193-7.019)	20	3.31±1.10 (1.651-4.90)	18	2.97±0.69 (1.798-4.32)	0.532
Serum Myoinositol level (ng/ml)	10	3.84±1.32 (2.06-6.621)	10	3.48±0.86 (1.91-4.79)	10	3.82±1.12 (2.37-5.81)	0.726

*Significant different at 0.05 level, PCOS = polycystic ovary syndrome, IR=insulin resistance, NIR= non- insulin resistance, BMI=body mass index. FSH=follicular stimulating hormone, LH = luteinizing hormone, E2 = estradiol, HCG=human chorionic gonadotropin.

In the present study, it was found that forty two of patients (72.40%) were presented with primary infertility and sixteen patients (27.60%) were presented with secondary infertility, from the twenty PCOS (IR) patients it was found that thirteen of them were with primary infertility and seven of them were with secondary infertility. Eighteen of PCOS(NIR) patients were with primary infertility and two were with secondary infertility, while in control group eleven were with primary infertility and seven were with secondary infertility and it was found there is no significant difference among the three groups.

Hormonal profile for all patients are shown in table (1), the mean of basal FSH and LH and LH/FSH ratio, prolactin, basal estradiol (E2) and estradiol (E2) at the day of hCG show no significant difference among the three groups.

Even the difference was not significant but it was noticed that basal LH hormone level higher in PCOS (IR) and (NIR) groups than in control group.

With regard to Follicular myoinositol and serum myoinositol levels at the day of oocyte retrieval among PCOS patients with (IR), PCOS patients with (NIR) and control groups show non significant difference in between the groups as seen in table (1). Statistical analysis showed there were positive significant correlation between the follicular myoinositol level

and total number of oocytes, number of MII oocytes, number of fertilized MII and number of grade I embryos as shown in table (2) also there was significant positive correlation between the follicular myoinositol level and serum myoinositol level as shown in table (2).

Table (2): Correlation of follicular myoinositol level with ICSI parameters and serum myoinositol level.

		Follicular Myoinositol level (ng/ml)
Oocyte number	r	0.272*
	P	0.039
	N	58
MII (Best one)	r	0.321*
	P	0.015
	N	57
Fertilized MII	r	0.389*
	P	0.004
	N	54
Number of grade I embryos	r	0.358*
	P	0.008
	N	53
Serum Myoinositol level (ng/ml)	R	0.5*
	P	0.005
	N	30

Significant different at 0.05 level, r =correlation, p=significance, n =number, MII=metaphase.

While serum myoinositol levels showed strong positive correlation *p*-value (0.044) to the number of grade I embryos, in PCOS patients with (IR), regarding other ICSI parameters as total number of retrieved oocytes, number of mature oocytes (metaphase II), number of fertilized metaphase II; there were positive correlation between these parameter and serum myoinositol levels among PCOS patients with (IR), PCOS patients with (NIR) and control groups even the correlation was statistically not significant. With regard to the BMI, statistical analysis show that there is negative correlation between the BMI and serum myoinositol and follicular myoinositol levels among PCOS patients with (IR), PCOS patients with (NIR) and control groups even the correlation was statistically not significant.

DISCUSSION

Myoinositol has many functions at the ovarian level, it is essential for proper oocyte maturation; the action of this molecule is related to the role played by inositol triphosphate (InsP3) on the modulation of intracellular calcium ion concentration in response to the action of the hormones LH and FSH.[12] In oocytes, this mechanism involves specific receptors,

inositol triphosphate receptors (InsP3-R1) which seems to play a key role in the maturation process[13], When the culture medium supplemented with Myoinositol this will increase meiotic progression of mouse oocytes with the production of fertile eggs, while the depletion of intracellular stores of Myoinositol desensitizes inositol-dependent transductions pathways, this will cause decrease in the levels of InsP3 and the proper release of calcium and reduces oocyte maturation, when oocytes matured in the presence of Myoinositol and then fertilized in vitro and transferred to foster mothers, this will increase the implantation rate and post implantation viability of the resulting embryos.[14]

Our data have shown that there is no significant difference present between the concentrations of myoinositol in the serum and in follicular fluid in the three groups PCOS (IR), PCOS (NIR) and control as seen in table (1). These results are in agreement with Tony T.Y. Chiu *et al*,[4] who revealed in his study that there is no statistical difference present between serum myoinositol levels in the group which had matured and fertilized oocytes and group which had immature and unfertilized oocytes.

While Vittorio Unfer *et al*,[15], found that the myoinositol content was significantly lower in the follicular fluid of PCOS patients with hyperinsulinemia than that in healthy women, but they took two groups only PCOS patients with hyperinsulinemia and healthy women groups.

In this study there was negative correlation between myoinositol levels in serum and follicular fluid and BMI, even though the correlation was statistically not significant. The obesity is highly linked to insulin resistance whether in PCOS or in normal ovaries patient, indeed, hyperinsulinemia due to insulin resistance occurs in approximately 80% of women with PCOS which presented with central obesity, as well as in 30% to 40% of lean women diagnosed with PCOS[5] and the follicular and serum myoinositol will be altered in obese PCOS patients whose presented with hyperinsulinemia as presumed by Vittorio Unfer *et al*. study.[15]

In addition, when we assess the correlation of follicular myoinositol in all patient without dividing them in to groups with total number of oocytes, number of MII, number of fertilized MII and total number of grade I embryos, the correlation was significantly positive as seen in table (2), this result concur of Tony T.Y. Chiu *et al*,[4], who revealed that there was a positive correlation between the amount of myoinositol in follicular fluid and both the cell number and the morphological score of the developed embryos and he concluded that follicles

containing good quality oocytes have higher concentrations of myoinositol in their follicular fluid, probably due to the intricate relationship between myoinositol and inositol phosphates in the phosphatidylinositol (PtdIns) cycle activation for oocyte maturation.

Concerning the number of embryos, the data of the present study showed that there was positive direct correlation between the follicular myoinositol level and number of embryos in the three groups. This is in agreement with the results obtained by T.Y. Chiu *et al*,[4], who found the mean concentration of myoinositol significantly higher in follicular fluid containing oocytes that developed into 4-cell embryos with good morphology. A positive correlation was found between the concentrations of myoinositol and the cleavage rate of fertilized oocytes harvested from the respective follicles.

The present study has been assessing the correlation between serum myoinositol and numbers of embryos, the results demonstrated that in PCOS (IR) group there was significant positive correlation, while positive non significant correlation between serum myoinositol and number of grade I embryos in control and PCOS (NIR) groups.

The current study assess the correlation between follicular and serum myoinositol in the whole patient without dividing them in to groups, the analysis show significant positive correlation as noticed in table (2), so level of serum Myoinositol can reflect direct relation with follicular Myoinositol.

CONCLUSION

Follicular myoinositol can be assumed as a good indicator for oocytes maturity and predictor of higher number of fertilized MII and good quality embryos in stimulated ICSI cycles in PCOS and non PCOS patient.

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