Evaluation Follicular Fluid Cytokines in Patients with Risk Factor for Development of Ovarian Hyper Stimulation Syndrome

Alaa K. Hameed[1], Alaa S. AL-Atabbi[2], Muna K. Mahmood[3]

[1]Masters Degree Student, College of Medicine, University of Kerbala, Kerbala, Iraq, [2]Ass. Prof. College of Medicine, University of Kerbala, Kerbala, Iraq, [3]Ass. Prof. College of Medicine, University of Kerbala, Kerbala, Iraq

Abstract

Aim of Study: Study the level of IL17 and IL23 in the follicular fluid and their relation with hyper stimulation syndrome.

Materials/Method: Eighty five who include (85 subject divided into 2 groups, control group whom included infertile female with male factor infertility (55) and case group whom infertility due to poly cystic ovarian syndrome (30) attended IVF centre.

Samples collected were follicular fluid, the follicular fluid has been collected in Eppendorf tube then stored at -20c to be used for ELISA test to determine concentration of IL 23 and IL 17 in follicular fluid. The results were analyzed using the IBM SPSS analytic software.

Results: the mean of interleukin (IL17) 96.11, Standard deviation 78.46 in the patients group. While the mean of interleukin (IL 17) for control 6.15, Standard deviation 14.33. There was a significant difference between polycystic and male factor infertility. P value < 0.005 both of them groups Poly cystic and male factor.

Conclusions: High concentration of follicular fluid IL -17 is positively associated with e disease of poly cystic ovary (POCS).

Keywords: *PCOS, IL17, IL23, fertility.*

Introduction

IVF stands for in vitro fertilization. It's one of the more widely known types of assisted reproductive technology (ART). IVF works by using a combination of medicines and surgical procedures to help sperm fertilize an egg. Where fertilization takes place outside the body. It's suitable for people with a wide range of fertility issues and is one of the most commonly used and successful treatments available for many people[1]. There are many types of fertility treatments available, ranging from simple interventions such as medication to help a woman ovulate through to more complicated procedures like IVF[2].

Ovarian hyperstimulation syndrome (OHSS) is considered the most serious complication of ovulation induction. It can vary from being a mild illness to a severe, life-threatening disease requiring hospitalization. OHSS can occur as soon as a few days after receiving HCG ('early OHSS') or later ('late OHSS'). Multiple pregnancy has been shown to be associated with a higher risk of late OHSS [3].

The incidence of severe OHSS has been reported to vary from 0.7 to 1.7% per initiated cycle[4-7]. Some

Corresponding Author:
Alaa S. AL-Atabbi
Ass. Prof. College of Medicine, University of Kerbala, Kerbala, Iraq
e-mail: alattabi486@gmail.com

case reports[8, 9], studies[5, 10], and reviews[11] describe some serious aspects of OHSS, such as thromboembolic events, pulmonary manifestations, and death, but the magnitude of the risk is unclear.

While there is robust evidence supporting the efficacy and safety of ART, it is important to be aware of the risks, the most serious of which is ovarian hyperstimulation syndrome (OHSS). OHSS is a rare, iatrogenic complication of controlled ovarian stimulation (COS). Severe OHSS occurs in approximately 1.4% of all cycles[12].

There are a number of well-established primary risk factors for the development of OHSS, including young age, polycystic ovary syndrome (PCOS) – characterized by ultrasound and the ratio of luteinizing hormone (LH) to follicle stimulating hormone (FSH) – and a history of an elevated response to gonadotropins, i.e. prior hyper-response/OHSS[13, 14].

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women in reproductive age which influences outcome and potential risks involved with controlled ovarian stimulation for artificial reproductive techniques (ART)[15]. It causes chronic oligo- or anovulation and often leads to infertility.

PCOS is mentioned as a common endocrinopathy in women who are at reproductive age and it is associated with metabolic disorder and reproductive dysfunction[16,17].

Ovarian dysfunction continues to be the main feature which makes this syndrome the major cause of an ovulatory associated with infertility[18]. Most say 5%-10% of reproductive-age women are affected[19, 20] but some say 6.6%-8%[21] and some others say PCOS is a disorder affecting up to 6%-10% of women in reproductive age[22].

This syndrome can be defined by specific clinical and bio-chemical criteria, and also using ultrasonography[23].

Environmental status and factors, such as obesity, appear to exacerbate the underlying genetic predisposition. PCOS is characterized by increased levels of circulating androgen, polycystic ovarian morphology (PCOM), arrested follicle development, and an ovulatory infertility[24].

Controlled ovarian hyperstimulation (COS) with gonadotropins for artificial reproductive techniques (ART) leads to a higher risk of ovarian hyperstimulation syndrome (OHSS) for patients affected by PCOS, because of a higher sensibility and exaggerated response to gonadotropins[26].

The Th17/IL-23 immune axis plays an important role in immuno-pathogenesis of some reproductive abnormalities such as polycystic ovary syndrome (PCOS) and endometriosis[27]. IL-23, a member of the IL-12 cytokine family, is a heterodimeric cytokine which is composed of the IL-12 p40 subunit and a novel p19 subunit. IL-23 is mainly secreted by activated macrophages and dendritic cells[28].

The significant higher levels of IL-6 and IL-10 and lower concentrations of IL-23 in FF from patients with PCOS indicated an important immunological microenvironment defect in the ovarian follicles[29]. The high level of IL-23 in the FF of women with endometriosis illustrated inappropriate oocyte quality in these subjects[30].

IL-23 amplifies and stabilizes the proliferation of IL-17 secreting CD4þ memory T cells which produce IL-17, a pro-inflammatory cytokine that stimulates the production of other pro-inflammatory cytokines and chemokines such as IL-1, IL-6, tumour necrosis factor-a and nitric oxide responsible for inflammation[31].

Also, the significantly higher IL-17 in the FF of women with endometriosis and PCOS is associated with immunological changes which may finally lead to infertility[32].

Some past examination exhibited that IL-10 was raised yet IL-23 was evoked in PCOS ladies which reflex sustain the neighbourhood enactment of Th17/Th1 cells by discharging a lot of IL-22, and IFN-γ-driven inflammation[33].

This study was aimed to investigate the concentration of level interleukin 17 and interleukin 23 and development of PCOS.

Materials and Method

A case control study was conducted in AL – Sader teaching medical city in Najef, AL Kadhimiya Teaching hospital, Umm AL Banin fertility centre and Nahrain Universility Higher institute in Baghdad to diagnose infertility and assist in childbearing randomly selected included a 85 patients, Who attended to consultant clinic for in Vitro Fertilization intracytoplasmic sperm

injection in the period between November 2019 to March 2020 under the supervision of fertility and in vitro fertilization specialists were included in this study.

Eighty five who include (85 subject divided into 2 groups, control group whom included infertile female with male factor infertility (55) and case group whom infertility due to poly cystic ovarian syndrome (30) attended IVF centre.

Samples collected were follicular fluid, Oocyte Pick up was done by a gynaecologist. Follicular fluid sample were collected by Needle size 16 Cage from ovary from 90 patients and their healthy controls and put in tube then, allowed to separate by centrifugation 3000 rpm for 5 minute. The follicular fluid has been collected in Eppendorf tube then stored at -20c to be used for ELISA test to determine concentration of IL 23 and IL 17 in follicular fluid.

Results

Table (1) shows that total of 85 groups: 30 patients groups which compared with control groups male factor (N=55). For both studied groups, age ranging from (25-45) polycystic and from male factor (32-42). The mean ages (mean-+SD) were 28.97±4.88 for poly cystic, 29.41±5.59 for male factor.

Body mass index in poly cystic ovary group (mean ±SD) were 26.58±2.52, minimum 22.66, maximum34.05. While control groups male factor (mean± SD) were 26.32±3.46, minimum 10,82 maximum 32.83

Infertility duration for polycystic ovary groups (mean± SD) were 8.20±2.28, minimum 6, maximum 11. While for control male factor groups (mean± SD) were 7.79±2.99, minimum 3, and maximum 13.

Right ovary follicle for poly cystic ovary groups (mean± SD) were 6.6±3.7, minimum 1,maximum 15.While for control male factor groups (mean± SD) were 6.1±3.6, minimum 1,maximum 17.

Left ovary follicle for polycystic ovary groups (mean± SD) were 6.10±3.39, minimum 1, and maximum 13. While for control male factor groups (mean± SD) were 5.47± 2.97, minimum 0.00, and maximum 13. The zero score with mean 00±0. P value is (>0.05) Non signification.

Table (1): Patient's Demographic characteristics.

	Polycystic ovary				Male factor (control)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Age P=0.708 NS	28.97	4.88	20.00	39.00	29.41	5.59	18.00	41.00
BMI P=0.721 NS	26.58	2.52	22.66	34.05	26.32	3.46	10.82	32.83
Infertility duration P=0.783 NS	8.20	2.28	6.00	11.00	7.79	2.99	3.00	13.00
Rt ovary follicle P=0.531 NS	6.6	3.7	1.0	15.0	6.1	3.6	1.0	17.0
Lt ovary follicle P=0.381 NS	6.10	3.39	1.00	13.00	5.47	2.97	0.00	13.00

Student's t-test was used.
NS: No significant difference (P > 0.05).

In table 2, a total of 85 groups: 30 patients poly cystic ovary group which compared with control group male factor 55. No significant correlation was found with any of the variables mentioned in the table and either of the studied groups.

Table (2): Demographic characteristics of the studied groups.

		Polycystic ovary		Male factor (control)	
		Count	%	Count	%
Medical history P=0.751 NS	No	23	76.7%	43	79.6%
	Yes	7	23.3%	11	20.4%

		Polycystic ovary		Male factor (control)	
		Count	%	Count	%
Infertility P=0.939 NS	Primary	23	76.7%	41	75.9%
	Secondary	7	23.3%	13	24.1%
	Yes	0	0.0%	0	0.0%
Miscarriage P=0.390 NS	0	27	90.0%	51	94.4%
	1.00	2	6.7%	3	5.6%
	2.00	1	3.3%	0	0.0%
History fertilization failure P=0.531 NS	No	27	90.0%	46	85.2%
	Yes	3	10.0%	8	14.8%

Discussion

Patients were asked about their medical history which included any past taken un-prescribed medications, psychological traumas, family history and any other causes of PCOS. There was no significant correlation found between the patients of PCOS and controls in regards to their medical history. Data collected from literature disagrees with our results in this regard, as a study by[41]found that there was a difference in prevalence of PCOS between urban and rural areas as a result of different effects of environmental or risk factors on the occurrence of the disease including the psychological trauma and physical activity.Another study by[42]had found an influence of the family history, physical activity and stress on the occurrence of PCOS patients in comparison to controls.

A study found that the uptake of hormonal un-prescribed medication had an effect on vitamin D deficiency witch in turns is a risk factor for PCOS. Another study found that the hormonal changes are a significant risk factor for PCOS patients[43]. Primary and secondary infertility were both found insignificant when correlated with PCOS patients and controls[44]. Primary infertility was defined as failure to become pregnant after at least one year of unprotected intercourse, while secondary infertility refers to women who have been pregnant at least once but failed to conceive after at least one year of unprotected intercourse[45]. A study by[46] found a significant correlation between primary and secondary infertility between patients and control groups. The same conclusions were made by[45]as a significant correlation was found between primary and secondary PCOS patients and their control group. also no significant relation was found by this study upon comparison of the number of miscarriages in PCOS patients and controls. In this study sample over 90% of both groups had no miscarriages, this could explain the difference between our results and those of other studies. The risk of miscarriage in PCOS patients is increased and amounts to 30–50%, which means that for these women, it is three times as high as for healthy women[47]. The results of prospective, randomized trials suggest that the miscarriage instances in PCOS patients occur only in 15–25% of cases, which is a percentage comparable with the frequency of miscarriage in the general population[48, 49]. Unlike this study,[50, 51]had both found a significant correlation between patients and controls in regards to miscarriages. Polycystic ovarian syndrome (PCOS) induces anovulation in women of reproductive age, and is one of the pathological factors involved in the failure of in vitro fertilization (IVF). Indeed, PCOS women are characterized by poor quality oocytes[52].

A valuable indicator of OHSS risk is estradiol serum level. It is recommended that E2 serum levels be determined during ovarian stimulation.[55]have investigated the estradiol serum level in PCOS patient and the predisposition of PCOS to OHSS and its relation to Triggering ovulation with gonadotropin-releasing hormone agonist in in vitro fertilization patients with PCOS. Several studies on the PCOS models of rats have effectively used estradiol in the induction of PCOS in rats proving the significance of the correlation between estradiol and PCOS[56, 57]. These results contradicts this study findings. A study by[58]have matched the characteristics of women with PCOS with their controls and found that estradiol serum level was found in a higher levels in women with PCOS than their controls. Study by[59]used multiple logistic regression and showed

that the number of oocytes retrieved, peak estradiol level, number of follicles at oocyte collection, and ovarian morphology (patient group) were all significantly correlated with regard to the patient developing severe OHSS on univariate analysis. Also, the number of follicles, oocytes retrieved and peak estradiol levels were all significantly increased in women with PCO and PCOS. There is a significant predictive association between E_2 levels measured on stimulation day 3 and 5 and both ovarian hyper-response and extreme-response in IVF. However, the clinical value of stimulated E_2 levels for the prediction of hyper-response is low because of the modest sensitivity and the high false positive rate. For the prediction of extreme-response the clinical value of stimulated E_2 levels is moderate[(60)].

On the contrary to our results, the studies done by (Tsikouras et al, 2015 and Ibanez et al, 2017) revealed a significant increase in the hormonal profile LH, LH/FSH, Testesterone and Progesterone compared to the control group. In women undergoing in vitro fertilization (IVF),[(61)] reported a negative association between FSH and AMH serum levels, concluding that the AMH level is highly predictive of the FSH level and can be used as an independent indicator of ovarian reserve. Recently,[(62)]also reported that intrafollicular AMH levels negatively correlated with FSH in follicles of normoandrogenic ovulatory women undergoing IVF, concluding that intrafollicular AMH levels reflect follicle sensitivity to FSH. In study by[(63)], we noted increased serum AMH levels in women with polycystic ovary syndrome (PCOS). Moreover, AMH levels were negatively correlated to body mass index (BMI) and were independently predicted by the levels of luteinizing hormone (LH) and testosterone and by BMI. We concluded that increased LH levels might be an independent link between PCOS-associated disorders of ovulation and increased serum AMH concentrations.

In conditions of increased LH and normal to low FSH levels (such as in PCOS), the AMH serum levels are increased and tend to be associated with serum LH levels; whereas in conditions of increased FSH levels (such as in premature ovarian failure), AMH serum levels are decreased and tend to be associated with serum FSH levels[(64)]. A neuro endocrine characteristic believed to be of PCOS is steadily rapid LH (GnRH) pulsatility, which favours pituitary synthesis of LH over that of FSH and contributes to the increased LH concentrations and thereby altered LH: FSH ratios typical of PCOS. Insufficient FSH levels contribute to impaired follicular development, while increased LH levels enhance ovarian androgen production[(65)]. A study found there was a persistent increase in LH level was noted among PCOS group starting from level of 5.04μlU/ml and reaching up to level of 22.06μlU/ml. Accordingly, LH: FSH ratio also showed a steady increase from 1 to 5.5 in these groups. There were no groups identified on the basis of LH: FSH ratios in the control as their LH: FSH ratios were within the same range[(66)].

Conclusion

High concentration of follicular fluid IL -17 is positively associated with e disease of poly cystic ovary (POCS).

Compliance with Ethical Standards: The authors declare that they have no conflict of interest.

The author declare that research involved human participants and consent was obtained.

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