

The role of serum IL-10 & IFN γ among women with infertility

Khalid Hassan Mazel¹, Maysaloon A.AL-Sadoon², Maysoon Sharif Flafil³

^{1,2} Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq

³ Department of Gynaecology, College of Medicine, University of Basrah, Basrah, Iraq
ph.randlaith@yahoo.com

Abstract

Infertility is one of the most important disorders in gynecology, the immune system can play a role in causing infertility in some couples. Various subsets of T cells secrete different cytokines, such as interferon (IFN)- γ which boost cell-mediated immunity. This study was aimed to assess serum level of IFN γ and IL-10, in women with infertility resulting from ovarian dysfunction and in normal matched control group. Blood samples were obtained from 75 infertile women and from 75 fertile as a control group. The serum level of IL-10 was decrease while IFN- γ evaluated using ELISA test. These finding showed that Pro& anti-inflammatory cytokines might be playing a role as a biomarker for female infertility. Additionally, it might be considered as a guide for treatment response.

Keywords: female infertility, IL10, IFN- γ , ovarian dysfunction.

1. Introduction

Infertility is one of the most important disorder in gynecology which explained as the inability to achieve pregnancy after one year of unprotected intercourse [1]. The World Health Organization (WHO) has identified infertility as a global public health problem [2, 3]. Infertility is classified as primary, which means that the woman has never conceived and Secondary infertility may occur at any time in a woman's life after the first pregnancy [4].

The endocrine system and the immune system can play a role in causing infertility in some couples. Some couples remain unexplained infertile since the reasons for their infertility are not known [5].

Some evidence has been found to indicate that the immune system is involved in unexplained infertility [6]. It has been reported that both humoral and cell-mediated immunity as potential mechanisms for implantation failure as well as for regulating maternal immunity for successful implantation and pregnancy [7].

Studies have been carried out on homeostasis between type 1 (Th1) and type 2 (Th2) helper T cells, as well as Th1/Th2 polarization in implantation, for many years [8]. Recent studies have found that fine-tuned balance and coordination among different subsets of Th1 and Th2 helps to support implantation efficacy. Various subsets of T cells secrete different cytokines, each of which plays a unique role in a complex regulatory pathway.

Th1 cells release inflammatory cytokines, such as interferon (IFN)- γ and IFN- α , which boost cell-mediated immunity. Th2 cells, in contrast, are involved in humoral immunity [7]. This study was aimed to assess serum level of IFN γ and IL-10, in women with infertility resulting from ovarian dysfunction and in normal matched control group. This study also assessed some of infertility risk factors including age, residence, body mass index and duration characteristics depend on type of infertility.

2. Materials and Methods

One hundred and fifty women who visited the Basra maternity hospital / infertility and IVF center between October 2020 and February 2021, aged 16 - 43. Seventy-five of them are infertile with ovarian dysfunction, while the other 75 are fertile, who had delivered a healthy baby within the last two years, taken as comparison group. The study excluded women with other types of infertility, such as tubal blockage and male infertility; endocrine problems (thyroid disorder, adrenal disorder, diabetes mellitus, pituitary disorder). Three ml of venous blood was collected in vacuum tubes (gel/clot activator) and left for one hour at room temperature for clot formation. The serum was aspirated and dispensed into sterile tubes after centrifugation for ten minutes at 2000rpm, and stored at deep freeze. Multiple freezing and thawing in serological study by ELISA was avoided. IL10 and IFN γ concentrations in serum were assessed by Sandwich Enzyme-Linked Immunosorbent Assay kits. (IL-10 and IFN γ ELISA Kit supplied by My BioSource.Inc. USA). The results were measured using an ELISA reader at 450nm.

Statistical Analysis

Statistical Package for Social Science (SPSS) was used to analyze the data. The results were expressed as numbers, percentages, and mean \pm S.D. (standard deviation). ANOVA was used to evaluate differences between groups using $p \leq 0.05$ lowest limit of significance.

3. Results

Demographic of the studied groups, listed according to age, residence and education levels are presented in Table 1. The mean age in infertile group was 30.16 ± 8.6 years while in the controls was 30.0 ± 7.5 years. (P . value > 0.05 not significant). Whereas, highly significant differences between infertile women in related to residence and education (69.3%) of them from urban area, (29.3%) low education levels.

Table (1): Age, Residence and Education level distribution of the studied groups

Parameters	Study Groups	Total
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		Infertility		Control			
		N	%	N	%	N	%
Age (years)	<20	12	16	14	18.7	26	17.3
	20-29	26	34.7	30	40	56	37.3
	30-39	23	30.7	20	26.6	43	28.7
	≥ 40	14	18.6	11	14.7	25	16.7
	Total	75	100.0	75	100.0	150	100.0
Mean age \pm SD* (year)		30.16 \pm 8.657		30.00 \pm 7.519			
Residence	Urban	52	69.3	33	44	85	56.7
	Rural	23	30.7	42	56	65	43.3
Mean residence \pm SD*							
Education	Pre-school/Primary Education	22	29.3	5	6.7	58	38.7
	Secondary/Higher Education	53	70.7	70	93.3	92	61.3

Sixty one of cases with Primary infertility and (14) with Secondary infertility the most time period of infertility within (1-5) years in both cases primary and secondary infertile women. Whereas, the number of cases in this

period with primary infertility 30 (49.2%) and the cases with secondary infertility 8 (57.2%), with a statistically non-significant differences ($p > 0.5$) that demonstrated in Table (2).

Table (2): Duration of infertility according to the type:

Type of infertility	Categorical duration of infertility (Years)							
	(1-5)		(6-10)		(11-15)		(16-20)	
	N	%	N	%	N	%	N	%
Primary infertility	30	49.2	20	33	6	9.8	5	8
Secondary infertility	8	57.2	3	21.4	2	14.3	1	7.1
Total	38	50.6%	23	30.7%	8	10.7%	6	8%

The highest concentration of serum IFN- γ was found in (G1 group) (2.34 \pm 1.22) and the lowest mean of

concentration were found in the control group (0.65 \pm 0.41) as shown in Table (3)

Table (3) Concentration of IFN- γ (Pg/ml) in serum among the studied groups.

infertile group	Type ovulation disorder	N	Mean \pm Std. Deviation	P-value
	G1	3	2.34 \pm 1.22	P > 0.05 (N.S)
G2	64	1.65 \pm 1.12		
G3	8	2.33 \pm 1.31		
infertile group		75	1.75 \pm 1.37	P \leq 0.00 (H.S)
Control group		75	0.65 \pm 0.41	

Table (4) Concentration of IL-10 (Pg/ml) from serum among the studied groups

infertile group	Type ovulation disorder	N	Mean \pm S.D	*P-value
	G1	3	1.83 \pm 1.55	P \leq 0.00 (H.S)
G2	64	0.50 \pm 0.24		
G3	8	0.45 \pm 0.05		
infertile group		75	0.43 \pm 0.109	P \leq 0.00 (H.S)
Control group		75	1.58 \pm 1.56	

The highest concentration of serum IL-10 was found in (G1 group) (1.83 \pm 1.55) and the lowest mean of concentration were found in the (G3group) (0.45 \pm 0.05) as shown in Table (4).

4. Discussion

Infertility is a second most common reason for women of childbearing age to visit their general practitioner; that affect around 3.5 million people [9]. The ages of all women involved in this study were below 43years. The

current results showed high percentage of infertility at women with the age groups 20-29 years which constitute about 34.7%. The results of our study are incompatible with some review that recognized infertility is positively correlated with advanced maternal age, which consider the women's age at marriage are factors that affect infertility [3].

Regarding the residency, the results appeared that the higher percentage of infertile women was living in the urban areas which are statistically significant and higher percentage among them with high education levels when

compared with women with low education levels. These results were agreed with a previous study which suggested that the increased in infertility is often associated with increasing Women's level of education which consider one of the factors affecting female infertility. Colleran et al. [10] reported that postponed marriage and delay in pregnancy may increase the risk of infertility in educated women.

The current study showed that the high percent 50.6% of infertility within duration (1-5) years, also this study clarified that primary infertility (81.3%) was higher than secondary infertility (18.7%) which similar to others studies were done among 250 infertile couples studied in Duhok, Iraq, 77.2% had primary infertility and 22.8% had secondary infertility [11]. In Turkey, 73.1% of women had primary infertility [12].

Cytokines are broadly categorized into pro-inflammatory and anti-inflammatory cytokines produced by Th1 and Th2 cells respectively [13]. This study showed a significant increase in the level of the pro-inflammatory cytokines serum IFN- γ had a value of (1.75 \pm 1.37) in the infertile women when comparing with the fertile group which have a value of (0.65 \pm 0.41). This finding is in agreement with the study by Raghupathy et al. [14] which also showed that raised IFN- γ levels are incompatible with successful pregnancy. Also a study by Wegmann et al. [15] reported that raised IFN- γ at implantation site is incompatible with a successful gestation.

For the anti-inflammatory cytokines (serum IL-10) this study showed significant decrease in their levels in the infertile women when compared with the fertile women. This supports a previous report that TH2 cytokines supports normal pregnancy by Dalton et al. [16]. The present study however differs from another study by Mahdi, which showed a rise in IL-10 in infertile women when compared with the fertile women [17]. It has been suggested that this increase may be caused by pregnancy-specific glycoproteins (PSG), which regulate maternal immunity.

References

- Montoya-Botero P, Polyzos NP. The endometrium during and after ovarian hyperstimulation and the role of segmentation of infertility treatment. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2019;33(1):61-75. <https://doi.org/10.1016/j.beem.2018.09.003>
- Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reproductive biology and endocrinology*. 2015;13(1):1-9. <https://doi.org/10.1186/s12958-015-0032-1>
- Cong J, Li P, Zheng L, Tan J. Prevalence and risk factors of infertility at a rural site of Northern China. *PLoS one*. 2016;11(5):e0155563. <https://doi.org/10.1371/journal.pone.0155563>
- Chamberlin G. *Gynecology by Ten Teachers*. 7th ed. Oxford University, New York, 2000.
- Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. *Reproductive biomedicine online*. 2012;24(6):591-602. <https://doi.org/10.1016/j.rbmo.2012.02.021>
- Carp HJ, Selmi C, Shoenfeld Y. The autoimmune bases of infertility and pregnancy loss. *Journal of autoimmunity*. 2012;38(2-3):J266-J74. <https://doi.org/10.1016/j.jaut.2011.11.016>
- Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *American journal of reproductive immunology*. 2010;63(6):601-10. <https://doi.org/10.1111/j.1600-0897.2010.00852.x>
- Al-Sadoon MA, Mahdi NK, Khosho EZ, Salman AA. The role of Th1 and Th2 cytokines among women with recurrent spontaneous miscarriage. *International Journal of Scientific & Engineering Research*. 2015;6(5):1846-55. Available from: <https://www.researchgate.net/publication/311353996>
- Raine-Fenning N. Subfertility. In: *Dewhurst's Textbook of Obstetrics & Gynaecology*. John Wiley & Sons Ltd, 2018. p. 689-703. <https://doi.org/10.1002/9781119211457.ch51>
- Colleran H, Jasienska G, Nenko I, Galbarczyk A, Mace R. Fertility decline and the changing dynamics of wealth, status and inequality. *Proceedings of the Royal Society B: Biological Sciences*. 2015;282(1806):20150287. <https://doi.org/10.1098/rspb.2015.0287>
- Razzak A, Wais S. The infertile couple: a cohort study in Duhok, Iraq. *EMHJ-Eastern Mediterranean Health Journal*, 8 (2-3), 234-238, 2002. Available from: <https://apps.who.int/iris/handle/10665/119155>
- Oskay UY, Beji NK, Serdaroglu H. The issue of infertility and sexual function in Turkish women. *Sexuality and Disability*. 2010;28(2):71-9. <https://doi.org/10.1007/s11195-010-9158-4>
- Moayeed S, Nazar W, ALAsadi SZ. Analysis of TNF, IL2 & IL6 in the serum and aqueous humour of patient with cataract in Basra/Iraq. *International Journal of Scientific & Engineering Research*. 2016;7(9):648-51. Available from: <https://www.ijser.org/onlineResearchPaperViewer.aspx?Analysis-of-TNF-IL2-IL6-in-the-serum-and-aqueous-humour-of-patient-with-cataract-in-Basra-Iraq.pdf>
- Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Human reproduction*. 2000;15(3):713-8. <https://doi.org/10.1093/humrep/15.3.713>
- Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunology today*. 1993;14(7):353-6. [https://doi.org/10.1016/0167-5699\(93\)90235-D](https://doi.org/10.1016/0167-5699(93)90235-D)
- Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon- γ genes. *Science*. 1993;259(5102):1739-42. <https://doi.org/10.1126/science.8456300>
- Mahdi BM. Role of some cytokines on reproduction. *Middle East Fertility Society Journal*. 2011;16(3):220-3. <https://doi.org/10.1016/j.mefs.2011.03.002>