The Effect of Interleukin-10 And Its Relationship with The Level of White Blood Cells in Women Spontaneous Miscarriage Undergoing Intracytoplasmic Sperm Injection (ICSI) technique

Dhirgam F. Hassan Al-Shimerty[1]; Alaauldeen S. M. AL-Sallami[2]

[1]Research Scholar, Faculty of Pharmacy/ University of Kufa; [2]Professor , Biology Department/ Faculty of Science/ University of Kufa , Iraq

Abstract

This study was conducted on 84 samples of women subject to the ICSI program . Blood sample was taken to measure WBC count , Interleukin-10 and β-hCG levels . Groups were divided based on the β-hCG level to two groups (Pregnant Women Group - Pregnancy Failure Group) and the total of pregnant women divided into (Pregnancy Group and spontaneous miscarriage) .

The results of the current research showed a significant increase in the level β-hCG and that the increase in the level of this hormone is evidence of the presence of high success rates for pregnancy in women who performed operations IVF, where the success rate at the beginning of the matter reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of spontaneous miscarriage of pregnant women due to various immunological and physiological reasons, as well as a positive correlation between the level of β-hCG and other parameters within the study (Interleukin-10 -WBC).

The results of the current research also showed a significant difference between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) in relation to the level of Interleukin-10, Also, The results of the current research showed a significant difference between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continuation of pregnancy) in relation to the level of WBC, and the present study found a positive relationship between the level of Interleukin-10 and WBC.

Keywords: *miscarriage , ICSI technique, Interleukin-10, white blood cells,*

Introduction

Infertility is a widespread disease worldwide and it means "the inability of the spouses to achieve pregnancy within one year of marriage" and the estimated rate of infertility in the world is around 15-20% [(1)] .

In vitro fertilization , intracytoplasmic sperm injection (ICSI) , and intrauterine insemination (IUI) be are the main methods of assisted reproductive technology (ART). Found several Various studies in recent years have indicated that occur risk factor for implantation failure of women after *In vitro* fertilization - ICSI which may be immunological parameters or biochemical parameters may be affected on *In vitro* fertilization - intracytoplasmic sperm injection results [(15)].

White blood cells (WBCs) They are considered an important part of the immune system in the body ,It should be noted that the increase in the number of white

Corresponding author
Dhirgam F;
Research Scholar**,** Faculty of Pharmacy/ University of Kufa; E-mail : dhurghamf.hassen@uokufa.edu.iqiq

blood cells or their lack of a normal limit indicates the presence of a health problem that afflicts the patient, he cause of white blood cells rise for a number of reasons, the most important of which are summarized below For pregnancy and childbirth or spontaneous miscarriage because increase Infection in Urinary tract with or immune system problem [2,16].

Interleukin -10 has several effects and has many roles in regulating the body's immunity as well as affecting inflammation and is involved in stimulating phagocytic cells as well as has a role in the survival of B cells and building antibodies, There are many studies that have demonstrated that interleukin-10a inhibits fatty polysaccharide and is also stimulated by the bacterial product of pro-inflammatory [3] .

Materials and Methods

This study was conducted in the laboratories of the Department of Biology, College of Science, University of Kufa, and in the Laboratory of the Fertility Center in Sadr City Medical City in Najaf Governorate / Najaf Health Directorate / Ministry of Health / Iraq.

Taked about five milliliters of intravenous blood samples were drawn in the morning from women which undergoing intracytoplasmic sperm injection (ICSI) technique during three parts , the first part after 14 day from injection , the second part after trimester spontaneous miscarriage while the third part for women which be continuous pregnant and using a needle and syringes which is used for one time from each patient and control. Four milliliters of the blood then it was left in a gel tube at room temperature for 10 minutes to complete the blood clotting, then centrifuged at 3000 rpm for 5 minutes, then the serum was separated for measurements of β-hCG level and Interleukin-10 level by ELISA method and one milliliter of the blood was put in EDTA-Na2 treated collection tubes for measurements of number of white blood cells count by Genex Hematology Analyzer.

Statistical Analysis

The popular statistical system (Graph Pad prism ver. 5) was adopted, and a one-way analysis of variance table - Anova method (by Tukey's multi-comparative test) was used to compare the groups divided into the measured parameters. The results are expressed as (Mean ± Stander Error). Correlation coefficients were calculated to estimate the correlation between tags and parameters. Descriptive statistics and correlation coefficients were performed using mega stat (V10.12 version) for excel 2010 [4].

Results

In this test showed found significant difference ($p<0.05$) between pregnant women which was 52 (4.24±0.25) and non- pregnant women (Implantation Failure) (2.56 ±0.10) which was 32 from women which undergoing intracytoplasmic sperm injection technique as shown in table (1)

Table (1): Result β- Human chorionic gonadotropin hormone test which differ between pregnant women which was 52 and non- pregnant women (Implantation Failure) which was 32 from women which undergoing intracytoplasmic sperm injection technique

Groups	Total Number	hcG (ml U/ml) Mean ± SEM	P value
Negative (Implantation Failure)	32	2.56 ± 0.1	0.001
Positive (Pregnant)	52	2.24 ± 0.25	

In this test showed found significant difference ($p<0.05$) between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and found non significant difference with spontaneous miscarriage (24)) as shown in table (2) .

Groups	Total Number	WBC Count (cell/ml) Mean ± SEM	P value
Pregnancy Failure)	32	9442 ± 473.8	0.001
Spontaneous Miscarriage	24	13860 ± 428.1	
Continued Pragnancy	28	7765 ± 331.8	

Table (2): Results of White blood cell Count test which differ between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24).

In this test showed found significant difference ($p<0.05$) between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24)) as shown in table (3).

Groups	Total Number	IL-10 (pg/ml) Mean ± SEM	P value
Pregnancy Failure	32	11.99 ± 0.4	0.001
Spontaneous Miscarriage	24	13.83 ± 0.75	
Continued Pragnancy	28	6.16 ± 0.72	

Table (3) : Results of Interleukin-10 test which differ between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24)).

The study showed the presence of a positive correlation between, Interleukin-10 with White blood cells count as in table (4).

Table (4): The correlation between Interleukin-10 with WBC Count.

Groups	IL-10	P value
WBC Count	r = 0.745	0.001

Discussion

The results of the current research showed a significant increase in the level of significance ($p<0.05$) in the level of the β- Human chorionic gonadotropin hormone, and the reason for this is that this hormone is evidence of the presence of pregnancy in women who underwent ICSI operations may be because it is excreted mainly from the placenta during the formation of the fetus and this study is consistent with what reached by some authors [5] in this regard and that the increase in the level of this hormone is evidence of high success rates of pregnancy for women who conducted ICSI operations, where the success rate initially reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of spontaneous abortions for women. Pregnant women for various immunological and physiological reasons, as this study indicated, and these results are consistent with what was reached [6-8].

The results of the current research showed a significant increase at the level of significance ($p<0.05$) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) in the concentration of interleukin-10 in When the differences were not significant between the group (pregnancy failure) and the group (spontaneous miscarriage may be because the pregnant women have a low white blood cells level compared to aborted women because white blood cells is evidence of infections in the genital and urinary tract as a result of the spontaneous miscarriage process and Interleukin-10 is one of the cytokines that stimulates the occurrence of infections and is responsible for the start of the inflammatory process and is called the inflammatory station The fourth of the cells devoured and through the diagnosis of the concentration of interleukins, the reaction of the immune system can be known as normal, high or low, and it is consistent with its findings [9,10] .

As it was explained by increasing the level of interleukin-10 in aborted women, it is an indication of the role of interleukins whose function is the communication between immune cells, whose action is directed at defending the body. .

The results of the current research showed a significant increase at the level of significance ($p<0.05$) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continuing pregnancy) with respect to white blood cells count while The differences were significant between the (pregnancy failure) group and the (spontaneous miscarriage) group, and this study is consistent with the findings of [11,12] and this result may be explained that the reason for this is that pregnant women have a low white blood cells count level compared to aborted women This may be because white blood cells count is evidence of the presence of infections in the genital and urinary tract as a result of the abortion process, where these results are consistent with what was reached [11,14] where he explained the presence of a significant increase in white blood cells women abortifacients and pregnancy after a period when the failure of vaccination compared to non- spontaneous miscarriage women. .

The current study also showed a positive correlation between the level of interleukin-10 and WBC and perhaps the reason for this is that the increase in the level of white blood cells during pregnancy or spontaneous miscarriage in women, which is often accompanied by an increase in urinary and genital tract infections which leads to an increase in the level of interleukin - 10 [15,16] .

Conclusions

The level of interleukin - 10, and increase in the level of significance ($p<0.05$) in the level of the β- Human chorionic gonadotropin hormone and The results of the current research showed a significant increase at the level of significance ($p<0.05$) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continuing pregnancy) with respect to white blood cells count.

Acknowledgements

This work was achieved at Kufa university, faculty of science, biology department, and al-Sajjad general hospital, in addition to workers and all participants. We would like to express our heartfelt thanks to those who helped us through this study.

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

References

[1] Poongothai S, Pradeepa R, Ganesan A, Mohan V. Prevalence of depression in a large urban South Indian population—The Chennai Urban Rural Epidemiology study (CURES–70). PLoS One. 2009 ; 28 : 4(9) : e7185.

[2] Glenn A, Armstrong CE. Physiology of red and white blood cells. Anaesthesia & Intensive Care Medicine. 2019 ; 20(3) : 170-4.

[3] Park SY, Karthivashan G, Ko HM, Cho DY, Kim J, Cho DJ, Ganesan P, Su-Kim I, Choi DK. Aqueous extract of Dendropanax morbiferus leaves effectively alleviated neuroinflammation and behavioral impediments in MPTP-induced Parkinson's mouse model. Oxidative medicine and cellular longevity. 2018 ; 15 : 422 .

[4] Hirsch AL. Industrial aspects of vitamin D. In Vitamin D . (pp. 73-93). Academic Press , UK , 2011 .

[5] Al-Fahham, A.A. Etiology of Antisperm Antibodies in the Serum of Virgins. Open Journal of Obstetrics and Gynecology, 2018 ; 8 : 236-245

[6] Gold RS, Azem F, Yovel I, Wagman I, Amit A, Lessing JB. Does ICSI affect early serum β-HCG in pregnancies achieved after IVF?. Human reproduction. 2000 ; 15(6) : 1221-1224.

[7] Yılmaz N, Ceran MU, Ugurlu EN, Gülerman HC, Engin Ustun Y. GnRH agonist versus HCG triggering in different IVF/ICSI cycles of same patients: a retrospective study. Journal of Obstetrics and Gynaecology. 2019 ; 29 : 1-6.

[8] Harun R, Ruban L, Matin M, Draper J, Jenkins NM, Liew GC, Andrews PW, Li TC, Laird SM, Moore HD. Cytotrophoblast stem cell lines derived from human embryonic stem cells and their capacity to mimic invasive implantation events. Human reproduction. 2006 21(6) : 1349-1358.

[9] Theilen LH, Mellnick VM, Shanks AL, Tuuli MG, Odibo AO, Macones GA, Cahill AG. Acute appendicitis in pregnancy: predictive clinical factors and pregnancy outcomes. American journal of perinatology. 2017 ; 34(06) : 523-528.

[10] Mukthayakka G, Sajjan AG, Kashid RA, Thejaswini HS, Vanitha S. Elevated Plasma Levels of TNF-Alpha, Inf-Gamma, Il-10 and TGF-Beta in Malaria Patients From Two Malaria Non-Endemic Regions In Karnataka, India. International Journal of Medical and Biomedical Studies. 2020 ; 4(2) : 29 .

[11] Sandi-Monroy NL, Musanovic S, Zhu D, Szabó Z, Vogl A, Reeka N, Eibner K, Bundschu K, Gagsteiger F. Use of dimethylxanthine theophylline (SpermMobil®) does not affect clinical, obstetric or perinatal outcomes. Archives of gynecology and obstetrics. 2019 ; 300(5) : 1435-1443.

[12] Dimitris MC, Perumal N, Craig-Barnes HA, Leadley M, Mahmud AA, Baqui AH, Roth DE. Effect of weekly high-dose vitamin D3 supplementation on serum cholecalciferol concentrations in pregnant women. The Journal of Steroid Biochemistry and Molecular Biology. 2016 ; 158 : 76-81.

[13] Sharef AA, Hussien SS, Noori FM. Vitamin D3 deficiency and early pregnancy loss. Middle East Journal of Family Medicine. 2020 ; 7(10) : 76.

[14] Funasaka N, Yoshioka M, Ueda K, Koga H, Yanagisawa M, Koga S, Tokutake K. Long-term monitoring of circulating progesterone and its relationship to peripheral white blood cells in female false killer whales Pseudorca crassidens. Journal of Veterinary Medical Science. 2018 ; 18 : 75 .

[15] AL-Msaid HL, AL-Sallami AS. Study the Level of Cytokine in Unexplained and Idiopathic Infertile Men. Journal of Pharmaceutical Sciences and Research. 2018 ; 10(4) : 808-811.

[16] Al-Msaid HL, AL-Sallami AS. Study of Catsper1 Protein Levels in Unexplained and Idiopathic Infertile Men. International Journal of Pharmaceutical Quality Assurance. 2018 ; 9(02) : 195-198.