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Original Research Paper

Evaluating the Effect of IL35 and IL38 on Fertility in Women Previously Infected with Coronavirus

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Abstract: Novel infectious pathogens have the potential to seriously threaten both international security and health. Infectious disease surveillance continues to be based on monitoring the location, population, and possible mode of transmission of these pathogens using a standardized case definition. The aim of this research Detect anti-inflammatory cytokine levels and Provide realistic guidelines for cytokine level determination for female fertility with COVID 19 infection diagnosis. Blood samples were obtained from a total of 180 volunteers who volunteered between April 2022 and November 2022 at the Fatima Al-Zahraa and Kamal al-Samarrai hospitals in Baghdad. Each patient and control participants got 6 mL of blood via vein puncture with disposable syringes. the blood samples were stored in clear test tubes and allowed to clot at room temperature before being separated by centrifugation; to extract serum, all serum samples were instantly frozen at (-20C) until used and estimated of cytokine agents by ELISA. Our results revealed statistical significance, as evidenced by $p < .001$. These results indicate that there are substantial differences in Interleukin-35 (IL35) levels among the various categories of Fertility and COVID_19 Moreover, the interaction effect between Fertility and COVID_19 was also found to be significant, with $p = .008$. This suggests that there are meaningful variations in IL35 levels for each combination of Fertility and COVID_19, as influenced by their interaction while analysis yielded highly significant results, with $p < .001$, indicating the presence of substantial differences in Interleukin-38 (IL38) levels among the different categories of Fertility and COVID_19. However, it's noteworthy that the interaction effect between Fertility and COVID_19 was not statistically significant, with $p = .658$ This suggests that there were no meaningful variations in IL38 levels for each combination of Fertility and COVID_19, as indicated by the interaction term. Conclusion of research showed statistically significant differences in IL 35 were observed, and it can be used to control the cytokine storm in COVID-19 patients as well as an immunological treatment.

Keywords: ssRNA; COVID19; Fertile Female; Interleukin 35; Interleukin 38

1.Introduction

A class of RNA viruses called coronaviruses (CoVs) infects humans and other animals [1]. The SARS-CoV pandemic started in southern China and quickly

expanded to neighboring nations The enclosed viruses known as coronaviruses, with their massive single-strand positive sense RNA genomes, belong to the family Coronaviridae [2]. Human coronavirus receptors The heart, lungs, kidneys, and digestive system all contain Angiotensin-converting enzyme 2 (ACE2) receptors, which have complementary shapes to the spikes. This allows for efficient attachment and facilitates the virus's entry into target cells [3]. They can infect the respiratory, gastrointestinal, hepatic, and central nervous systems of humans, pets, birds, mice, bats and a range of other wild species [4]. Like any other membrane, the viral envelope was composed of a lipid bilayer and several structurally important proteins, such as membrane (M), envelope (E), and spike (S) In February 2020, the Iraqi city of Najaf, which is south of Baghdad, reported the first COVID-19 cases [5]. the disease quickly spread throughout the nation, accounting for nearly 40% of those cases in Baghdad [6]. The rare intense acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the COVID-19 outbreak [7]. Clinical signs of the COVID-19 infection resemble those of the SARS-CoV infection, with fever, dry cough, dyspnea, chest pain, exhaustion, and myalgia being the most typical symptoms [8]. Headache, lightheadedness, stomach discomfort, diarrhea, nausea, and vomiting are less frequent symptoms [9].

The airborne virus known as SARS-CoV-2 spreads similarly to how the flu and common cold do. The virus is spread through feces or by coughing or sneezing, which releases microscopic droplets into the air [9]. Therefore, inhaling such droplets or coming into contact with contaminated surfaces increases the risk of infection [10]. Recent studies have already discovered a live SARS-CoV-2 in patient feces [11]. the virus remained active in the gastrointestinal system and changed the symptoms associated with the gastrointestinal system [12], Immunization against COVID-19 induces both innate and adaptive immunity via a number of mechanisms. Adaptive immunity can result in the development of antibodies that are specific for a number of SARS-CoV-2 antigens and involves both the cellular (T cells) and antibody (B cells) responses [16]. The majority of COVID-19 patients experience mild to moderate symptoms, but some may experience hyper-inflammation brought on by a high production of cytokines and chemokines [17]. While an imbalance can result in tissue damage, equilibrium between the various arms of the immune system can lead to disease clearance with little to no side effects [18].

Anti-inflammatory cytokine, interleukin-35 (IL-35) belongs to the IL-12 family of cytokines, which additionally includes interleukin-12, 23, 27, 35, and IL-39 [19]. The surface of macrophages, monocytes, and DCs frequently express IL-35, which is only known to be produced by regulatory T cells. CD8+ T cells may expressIL-35, which contributes to immune suppression. By reducing the proliferation of early T cells, IL-35 can prevent the development of Th1 and Th17 cells [20]. IL38 is a crucial component of host defense and inflammation. It belongs to the interleukin-1 (IL-1) family [21]. In humans, this cytokine is known as IL-1F10. Within chromosome 2q13, the organization of IL-1F10 gene is conserved with other members of the IL-1 family [21]. Mammalian cells can bind the type I IL-1 receptor and produce IL-38. It is expressed in the spleen, other tissues, and the basal epithelia of the skin as well as in the tonsil and proliferating B cells [18]. This cytokine is crucial for the control of both adaptive and innate immunity [22].IL-38 have anti-inflammatory effects by lowering the amount of pro-inflammatory cytokines secreted by macrophages [23]. Furthermore, it might prevent T-cell cytokines from being produced [24]. Commercial ELISA is a reliable method for detecting IL-38 protein in human serum, plasma, or cell culture supernatants [25]. IL-38 might attach to plasma factors. In cases of sepsis and other acute inflammatory events [26]. In addition to its potential roles in immune cell balance and cytokine regulation, IL-38 has been shown to reduce fever and systemic inflammation following infection [27]. One possible therapeutic cytokine that reduces inflammation in viral infections is IL-38 [28].

2.Methodology

Samples collection

This study was a hospital-based cross sectional, case-control study which was done on Iraqi population from Baghdad city. Blood samples were collected from a total of) No.180(volunteers in a period from April 2022 till November 2022 from Kamal al-Samarrai hospital, and Fatima Al-Zahraa hospital in Baghdad city. The (No.180) volunteers were classified to patients group (No.110) which include (No.40) fertile infected with COVID-19, (No.40) infertile infected with COVID-19 (No.40), and infertile uninfected with COVID-19 (No.30). The control group (No.70) apparently healthy individuals without symptoms or chronic disease and fertile female and uninfected with COVID-19 at the time of collected sample. Serum sample into aliquots was stored at (-20°C) until needed for studied parameters investigation.

Criteria for inclusion

All patients between the ages of 18 and 40 were included in the study. Two groups of women: those with

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children born within the last five years, and those with secondary infertility. She didn't use artificial insemination or IVF for her prior natural birth. You have never had any prior fertility treatments. For the past six months, you have not received the Covid-19 vaccination. While the excluding standards primarily infertile females. Women who did not receive excessive exposure to any of the physical, biological, chemical, or environmental factors such as pesticides, benzene, toluene, lead, cadmium, or mercury that compromise female fertility. You don't have any gonadal injuries from radiation therapy to the abdomen or pelvis or from high-dose chemotherapy with alkylating agents. Will not be eligible for other conditions that impact female fertility, such as severe thyroid dysfunction. Her previous miscarriages are history. Her inability to conceive has run in her family. Morbid obesity. those taking birth control pills or who are pregnant. The individuals who declined to partake in the research.

Statistical analysis

The statistical analysis for the study was done using the social sciences statistics program. (SPPS Version 21). The statistical test used ANOVA for the quantitative variable, data were shown as Mean±SD so that study groups' means could be compared to one another. ROC Curve was used to study relations.

3.Results

Serum estimation of IL35 and IL38 levels

Table 1 shows that the Mean± SD of IL35 level for the Infected COVID-19 (fertile and infertile) were (13.01± 0.98 and 15.38± 2.00, respectively) was significantly lower than for uninfected COVID-19 (Fertile and Healthy control) were (10.63± 1.70 and 9.77± 2.08, respectively) ($P < 0.001$).

According to IL-38 level the Mean± SD of IL38 level for the Infected COVID-19 (fertile and infertile) were (131.79± 6.55 and 151.46±10.09, respectively) was significantly higher than for uninfected COVID-19 (Fertile and Healthy control) were (109.59± 7.02 and 91.02± 7.54, respectively) ($P < 0.001$).

Markers	Groups	Variable	Mean± SD	P value
IL-35	Infected COVID-19	Fertile	13.01± 0.98	<0.001
		Infertile	15.38± 2.00	
	uninfected COVID-19	Infertile	10.63± 1.70	.035
		control	9.77± 2.08	
IL-38	Infected COVID-19	Fertile	131.79± 6.55	<0.001
		Infertile	151.46±10.09	
	uninfected COVID-19	Infertile	109.59± 7.02	<0.001
		control	91.02± 7.54	

Table 1: - Serum level of interleukins (IL35and IL38)

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ROC curve analysis for IL35 and IL38

In the fertile group, the AUC for IL35 and IL38 were (0.891 and 1.000, respectively) with sensitivity 100%, specificity 74.29 %, and sensitivity and specificity 100% for IL38 with a standard error of 0.000 and a 95% CI ranging from 0.967 to 1.000 as showed in table 4.6.1 and figure 4.2. Moreover, In the infertile group, IL35 exhibited an AUC of 0.965 with a standard error of 0.0179 and a 95% CI from 0.890 to 0.994. The optimal cutoff value of >12.34 resulted in a sensitivity of 92.50% and a specificity of 86.67%. For IL38, the AUC was again a perfect 1.000, with a standard error of 0.00 and a 95% CI ranging from 0.949 to 1.000. A cutoff value of >119.44 yielded both 100.00% sensitivity and specificity table 2.

Both IL35 and IL38 appear to be highly effective markers for differentiating between COVID-19 infected and uninfected individuals, irrespective of their fertility status. The AUCs suggest may be excellent diagnostic accuracy, with IL38 showing flawless performance in both fertility groups. The sensitivity and specificity values further bolster the case for the utility of these markers, particularly IL38, in COVID-19 diagnosis. However, it is noteworthy that the cutoff values and the specificity of IL35 differ between the fertile and infertile groups, suggesting that fertility status may modulate the expression or diagnostic threshold of this marker.

Table 2: - Estimation of IL35, IL38 cut-off values, ROC curve, Sensitivity, Specificity and AUROC in studied group

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Fertile	**Variable**	**AUC**	**Sens**	**Spc**
COVID - 19 infected vs uninfected	IL35	0.891	100.00	74.29
	IL38	1.000	100.00	100.00
Infertile	**Variable**	**AUC**	**Sens**	**Spec**
COVID - 19 infected vs uninfected	IL35	0.965	92.50	86.67
	IL38	1.000	100.00	100.00

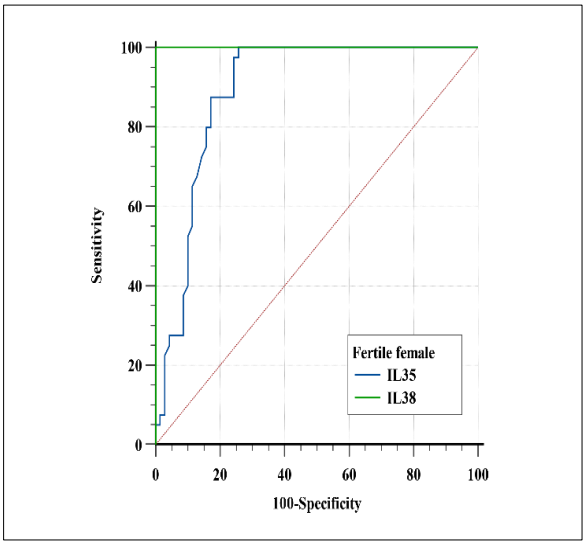

Figure 1: - ROC curve of IL35, IL38 in fertile female.

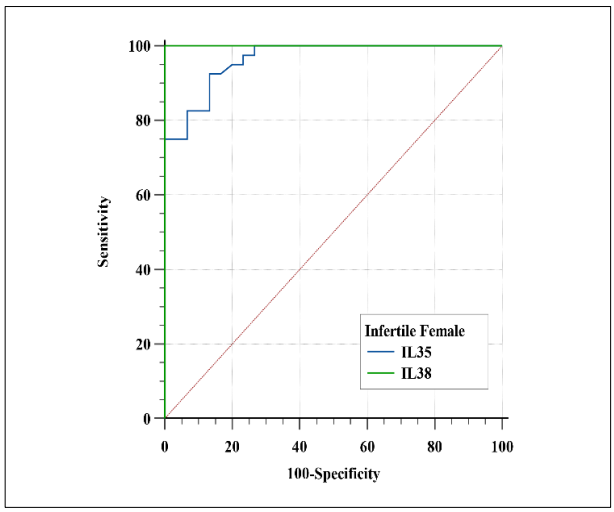

Figure 2: - ROC curve of IL35, IL38 in infertile female.

4. Discussion

The immune system's reaction to an infection may be more dangerous than the infection itself [18]. Therefore, the role that adaptive immunity plays in comprising COVID 19, immunopathogenesis must also be taken in to consideration [28]. Cytokines and their receptors have a major impact on the pathophysiology of viral infections [29].

The study compared the serum levels of anti-inflammatory cytokines (IL35, IL38) in a group of females who tested positive for COVID-19 and were infertile and fertile. The functions of IL 35 in preventing viral infection by suppressed RNA replication and viral protein synthesis via induction of type I and III interferons (IFN) [30]. In the current study, the main effect of infection of COVID-19 was statistically significant, with p <.001 indicating significant differences in IL35 levels, there were a noticeable increase in people infected with COVID-19 compared to healthy people. Additional research revealed the same thing, there were statistically significant differences between the control group and the patient group in the serum level of IL 35 during a viral infection [31].

Another study in Turkya demonstrated that unexplained infertile females had lower IL-35 level in comparison with fertile females [32]. While in case of infected persons with COVID-19 other investigation demonstrated a substantial difference in IL-35 levels between infected people and the control group, this outcome was comparable to the immunological function of IL-35 in previous investigations [33]. This study was in agreement with another study showed IL‑35 statically

significant differences between patients and control group [34].

It is believed that IL-35 is an essential modulator of the host's immune response and specialized immunosuppressive actions via influencing local inflammatory responses [35]. In addition to its suppressive role, IL-35 is also implicated in tumor growth and immune surveillance, and several human cancers, have been reported to have elevated amounts of circulating IL-35 [36]. According to some immunological investigations, T-cells that release IL-35 and have a protective role have an immunosuppressive effect on inflammations induced by T-cells and protect Th1 and Th17 cells [37]. Previous studies demonstrated that IL-35 was the inhibitor cytokine when it appeared in various chronic inflammatory illnesses and viral infections [38].

In contrast to healthy individuals, patients infected with the seasonal influenza A virus (IAV) had higher levels of IL 35 in their throat swabs and peripheral blood mononuclear cells. In human lung epithelial and primary cells, IAV infection also increased the amounts of IL 35 mRNA and protein [34]. In addition, infection causes the expression of pro inflammatory and anti-inflammatory cytokines, both of which reduce female fertility [39].

The majority of immune cells and tissues that form epithelial barriers contain IL-38, one of the most important anti-inflammatory cytokines, which may have an effect on protecting these barriers from infection [40]. Another study found that the main effect of fertility was significantly different in women with irregular menstrual cycles compared to healthy women, indicating significant differences in IL38 levels based on fertility categories [41]. The effects of IL-38 as anti-inflammatory cytokines extend to menstruation, implantation, pregnancy, and parturition [41].

This results were in line with a previous investigation that found serum levels of IL-38 was increased in SARSCoV-2 infected individuals and dropped when the infection cleared [42]. According to another study conducted on female patients, the relationship between IL38 and COVID-19 infection does not appear to have an effect on the patients' fertility [43] and suggests that there were no significant variations in IL38 levels for infected with COVID-19.

Conclusion

The immune system and immunopathology during SARS-CoV-2 infection are significantly impacted by cytokines. By boosting the immune system, they also have an impact on uterine cell line, which in turn impacts women's fertility. Between the patients and the control group, there were statistically significant differences in IL 35. As a new biomarker, it can be used with COVID 19 patients. Controlling the cytokine storm that arises in female COVID patients may be crucial when using IL 35 as an immunological therapy. The current study found that serum IL-38 levels were unaffected by fertility of females infected with COVID-19.

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Ethical Approval

The specimens of this study took the patient's approval for adult patients and the consent of the irrigation for young people as the law and directives of the human rights organizations with adequate information in an ethical manner.

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