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Evaluation of monocyte chemotactic protein-1 level in serum of fertile and infertile women

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***Abstract*---**Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse (Venkatesh et al., 2014). Female infertility, as a complex disorder, may be caused by medical conditions including pelvic inflammatory disease, endometriosis related infertility, ovulatory disorders, tubal factor infertility, and unexplained infertility(Gupta et al., 2014). Infertility affect approximately nine to fifteen percent of the childbearing population, and 55% of these influenced will seek medical help to achieve their desire to have children (Boivin et al., 2011).

***Keywords*---**evaluation, monocyte chemotactic protein, fertile, infertile women.

Introduction

Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse (Venkatesh *et al*., 2014). Female infertility, as a complex disorder, may be caused by medical conditions including pelvic inflammatory disease, endometriosis related infertility, ovulatory disorders, tubal factor infertility, and unexplained infertility(Gupta *et al*., 2014). Infertility affect approximately nine to fifteen percent of the childbearing population, and 55% of these influenced will seek medical help to achieve their desire to have children (Boivin et al., 2011). monocyte chemotactic protein-1 Cytokines act as mediators and modulators within highly localized environments and regulate immunological responses, hematopoietic development, and cell-to-cell communication as well as host responses to infectious agents and inflammatory stimuli(Arango and Descoteaux., 2014).

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MCP-1 interaction with CCR2 is crucial in inflammation and inflammation-related diseases, and CCR2 binding by MCP-1 has a role in the innate immunity response by recruiting monocytes into sites of inflammation. In addition, MCP-1 participates in adaptive immunity, controlling the preferential differentiation of T helper (Th) lymphocytes toward a Th1 or a Th2 phenotype(Daly and Rollins., 2003). Endometriosis is defined by presence of endometrial glands and stroma outside the uterine cavity and it affects approximately 5%–10% of women of reproductive age. Although endometriosis is usually considered to be due to retrograde menstruation, the true pathogenesis of this disease remains poorly understood. Endometriosis is associated with an inflammatory response and this inflammation leads to endothelial dysfunction and might even lead to carcinogenesis (Jiang, *et al*,. 2016). the relationship between inflammation and endometriosis was seen in infertile women, in whom intraperitoneal inflammation was observed. and was thought to be partially due to retrograde menstruation. These findings suggested that inflammation may be involved in endometriosis pathogenesis(Vercellini, *et al*,. 2014).

MCP-1 may influence both innate immunity, through its effects on monocytes, and adaptive immunity, through its control of T helper cell polarization. In human reproduction it may play a pivotal role in immune recognition, acceptance of the fetal allograft, maintenance of pregnancy, and parturition(Yadav, *et al*,. 2010). Some authors postulate that elevated MCP-1 expression in amniotic fluid can be a risk factor of pregnancy loss . In women with endometriosis, MCP-1 can be an attractive target to study both while the monocyte/macrophage system is considered to play a central role in the maintenance of humoral and cell-mediated immunity and while their activation status is increased. This could suggest that MCP-1 expression might be elevated in endometriosis-associated infertility patients (Gmyrek, *et al*,. 2005).

Material and Methods

the DEHA levels detected by ELISA sandwich method. The study included 125 subjects is divided into two groups: The first group is the patient group, which consists of 80 patients with infertility, which excludede 35 sample according exclusion criteria, And remained 45 patients with infertility was identified by a specialized gynaecologists of the "Fertility Center in AL-Sadder Teaching Hospital in Najaf Governorate/ Ministry of Health/Iraq, during the period from the 1st Nov. 2021 to 1st March. 2022 , which requires the presence of at least two of the following characteristics: polycystic ovaries on ultrasound scan, menstrual irregularities, Endometriosis and hyperandrogenism. The second group is the control group, which consists of 45 fertile women. The age group of the patients ranged from (17-44) years. The inclusion criteria for the patients are all women with infertility . The second group is the control group (fertile women), which consists of 45 fertile women, normal testosterone, a regular menstruation period, and normal ovulation are eligible, with normal ovaries as they were observed by the gynecologists which no take contraceptive, with no past of somewhat malady. The age group ranged from (17-42) years.

Exclusion criteria

Thirty fivet samples were excluded from the initial sample of Eighty due to these pathologies such as Diabetes mellitus, disorder of ovary, and ovarian tumor.

Blood Collection

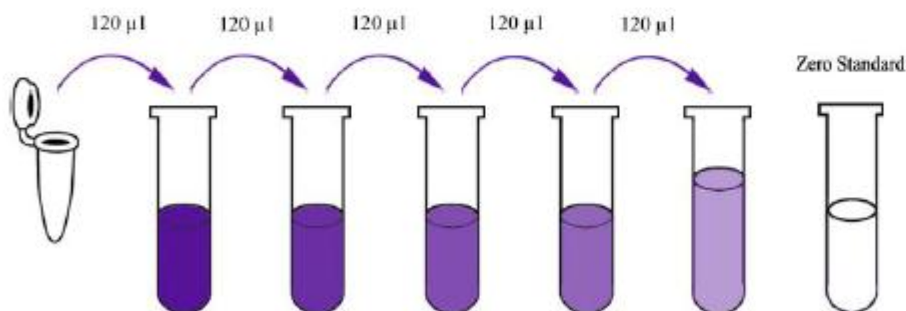
The blood samples of this study were obtained from female in 2nd of menstrual cycle through drag 5 ml of blood by using of medical sterile syringes from brachial vein, and placed in a gel tube. Then the gel tube were placed at room temperature for 30 minutes to coagulate the blood, and then samples were centrifuged (6000 rpm/min) for 5 minutes to separate the serum from other components of the blood. The serum was withdrawn by micro pipette and then placed in the Eppendorf tubes in two repeaters and kept frozen at -20 ◦C for the determination of Monocyte Chemotactic Protein-1 (Lesser *et al.*, 2020).

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human MCP-1/CCL2/MCAF antibody. MCP-1/CCL2/MCAF present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human MCP-1/CCL2/MCAF Antibody is added and binds to MCP-1/CCL2/MCAF in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated MCP-1/CCL2/MCAF antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human MCP-1/CCL2/MCAF. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

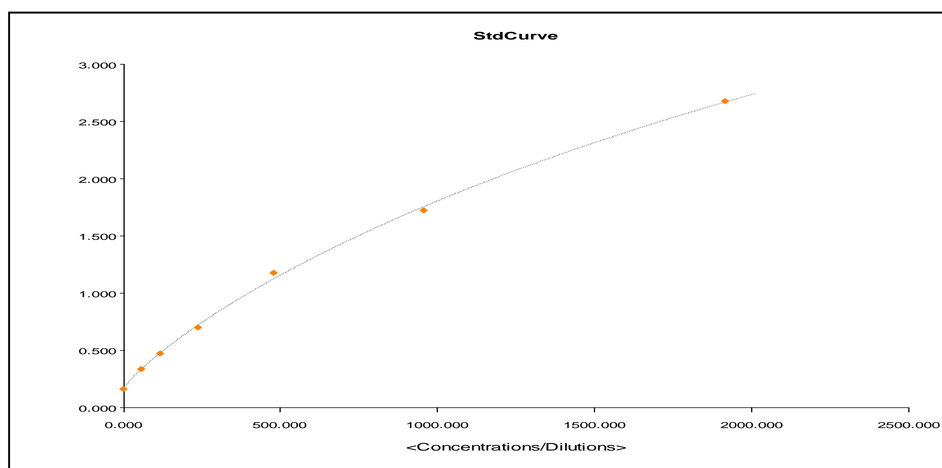
Assay Procedure

1. Prepared all reagents, standard solutions and samples as instructed. brought all reagents to room temperature before use. The assay is performed at room temperature.



2. Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Added 50µl standard to standard well. Note: Didn't added antibody to standard well because the standard solution contains biotinylated antibody.
4. Added 40µl sample to sample wells and then add 10µl anti-MCP-1/CCL2/MCAF antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.
5. Removed the sealer and washed the plate 5 times with wash buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, aspirated wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Added 50µl substrate solution A to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after added the stop solution.



Figure(3-2): Standard curve of MCP-1

Result and Discussion

The mean level of Monocyte Chemotactic Protein-1in infertile women group was (172.18 pg/ml) and was significantly lower than in fertile women (control) group that was (324.04 pg/ml), as shown in table (1) and figure (1).

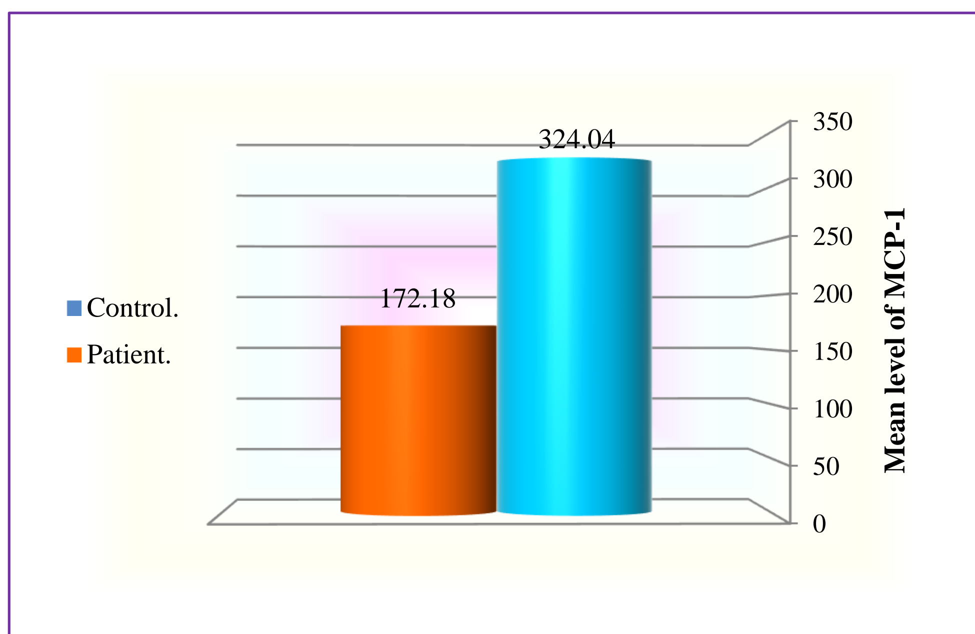


Figure (1): Comparison the mean levels of Monocyte Chemotactic Protein-1 in infertile women group and fertile women (control) groups

Table (2): Comparison the mean, standard error and p-value of Monocyte Chemotactic Protein-1 between infertile women group and fertile women (control) groups

Parameter		N	Mean	Std. E	P-value
MCP-1	Fertile women (Control)	45	324	15.84	0.0001**
	Infertile women	45	172.2	36.9	

P > 0.05 = Non-Significant (NS)
P < 0.05 = significant (*)
P < 0.01 = Highly significant (**)

Younis et al., (2014) found that ovarian stimulation causes significant increase in serum levels of IL-6, MCP-1 and PON-1 and decreased of TNF-α in infertile women with mild endometriosis, PCOS, or unexplained infertility. The infertility group had higher MCP-1 levels when compared with endometriosis and unexplained groups at peak E2. These data agree with the finding of several other studies that revealed association of PCOS as cause of infertility with increased pro-inflammatory cytokines and chemokines such as IL-6, TNF-α and MCP-1 (Gonzalez *et al.,* 2009).

Bouet *et al.,* (2020) showed that the MCP-1 (monocyte chemotactic protein-1), also referred to as CCL2, is produced by different cells, such as endothelial cells, fibroblasts, and immune cells. It plays an important role in the regulation of the migration and infiltration of monocytes, basophiles, lymphocytes-T, and natural killer cells in many tissues, including the ovary (Deshmane *et al.,* 2009). Studies have shown a transient increase in the level of MCP-1 in the FF and the ovarian

stroma at the time of ovulation (Dahm-Kahler *et al.,* 2009). MCP-1 could be implicated in follicular growth, ovulation, and the development as well as the regression of the corpus luteum (Dahm-Kahler et al., 2006).

These observations have led several researchers to investigate the role of MCP-1 in infertility. For instance, Younis et al., (2014) have shown an increase in the serum MCP-1 levels in infertile women with polycystic ovarian syndrome, but found no alteration in women with unexplained infertility or moderate endometriosis before or during ovarian stimulation. Other studies have shown an increase in MCP-1 levels in the peritoneal fluid of women with moderate and severe endometriosis (Jørgensen *et al.,* 2017). Buyuk *et al.,* (2017) found increased serum and FF levels of MCP-1 in obese women undergoing IVF that were negatively correlated with pregnancy rates. The authors postulated that the high levels of MCP-1 could negatively impact the follicular cells' function and the oocyte quality, by inducing an intracellular proinflammatory state via the activation of MCP-1 receptors or via an influx of monocytes towards the ovary.

Younis *et al.,* (2014) found that the cytokine profile was significantly altered in the MCP-1-high group compared to the MCP-1-low and the control group, but found no difference between the MCP-1-low group and the control group. Moreover, there was a significantly higher basal serum estradiol levels and a significantly lower number of oocytes retrieved in the MCP-1-high group compared to the MCP-1-low and the control groups. Even though some studies have shown an alteration of the estrogen metabolism in the endometriosis lesions (Zubrzycka *et al.,* 2015), serum estradiol levels are usually not modified in women with endometriosis (Coutinho *et al.,* 2019).

However, in that study, Younis et al., (2014) found an increase in the basal serum estradiol levels in the MCP-1-high subgroup, which could be due to an early follicular recruitment in these patients. Indeed, early recruitment is usually caused by a premature rise of FSH between two menstrual cycles, secondary to a decrease in the ovarian reserve, and is diagnosed by higher serum estradiol and FSH levels early in the cycle (Broekmans, 2009).

Therefore, the MCP-1-high subgroup of patients could have early signs of premature ovarian insufficiency, even though the classic markers of the ovarian reserve (Anti-Müllerian hormone, antral follicle count) are still within the normal range. This observation is further validated by the significantly lower number of oocytes retrieved in these patients despite the higher FSH doses used during controlled ovarian stimulation. In line with the report of Kitajima *et al.,* (2014), these findings suggest that there is a more advanced form, or a specific subtype of endometriosis, that is associated with a significant local inflammation causing focal depletion of primordial follicles. The dysregulation of the cytokine profile can lead to a significant alteration of oocyte microenvironment, causing this more advanced or specific from of endometriosis.

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