

Original Research Article

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# Biochemical Study to Evaluate Tumor Necrosis Factor and Ferritin in the Blood Serum of Pregnant Women Infected with Anemia

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## ABSTRACT

### Keywords

Pregnant, anemia, Tumor necrosis factor, Ferritin

### Article Info

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The current research was conducted on 50 blood samples, 30 of which were from women pregnant with anemia caused by iron deficiency and healthy pregnant women, whose ages were between (20-40) years. The blood samples were collected from Samarra General Hospital and outpatient medical clinics, a period between 1 / 11 / 2023 – 15/1 / 2024. after which samples were collected from patients and healthy women and were separated by a centrifuge. Then biochemical variables were measured, which included (Packed cell volume - PCV%, Hemoglobin -Hb, Iron, Ferritin, Tumor necrosis factor-TNF- $\alpha$ ). The results of the current study showed a significant decrease in each of the (PCV%, Hb, Iron, and Ferritin) concentrations in the groups of patients compared to healthy women, also the result showed a significant increase in (TNF- $\alpha$ ) in patient groups compared to healthy women.

## Introduction

Anemia is a common condition among women and is often caused by iron deficiency, pregnancy, or blood loss during the menstrual cycle (Shahen Mohammed Faraj, 2024). Pregnancy is a natural physiological and functional phenomenon that is accompanied by an increase in vital processes within the body with the activity of structural processes and a change in hormonal secretions in a manner aimed at stabilizing the fetus inside the uterus and preparing the pregnant woman's organs to perform their functions as efficiently as possible (Najib *et al.*). Signs of pregnancy include an increase in blood volume, fluctuating mood, increased

heart rate, and feelings of anxiety and fever. This is the stage in which the female feels the need for frequent retrieval. The duration of pregnancy is 9 months, which is equivalent to 40 weeks and equivalent to 280 days (Uyar, Seyit, *et al.*, 2018).

The pregnant woman goes through changes during pregnancy, which are natural adaptation mechanisms that the female goes through during pregnancy to better accommodate the fetus. Including changes in the cardiovascular, blood, metabolism, kidney, and respiratory systems, and are of great importance in the event of complications (Chauhan, Sunanda *et al.*, 2021; Favaloro, Emmanuel, 2019). Iron deficiency is the most

common nutrient deficiency and the leading cause of anemia worldwide, as it is a comorbidity in multiple medical conditions. The causes are variable and are due to many risk factors that reduce iron intake and absorption or increase iron demand and loss (Cappellini *et al.*, 2020). It was also found that inflammatory cytokines have a relationship with pregnant women suffering from anemia, as tumor necrosis factor is one of the cytokines that increase with the disease.

Therefore, tumor necrosis factor- $\alpha$  is considered one of the cytokines that are secreted by macrophages, and killer cells, and this cytokine works to enhance inflammation. And activating the endothelium of blood vessels (Al-dulaimy *et al.*, 2016), Tumor necrosis factor has been linked to many diseases, including heart disease (Entedhar R. Sarhat and Ayhan R. Mahmood, 2018).

It is one of the cytokines that are important in stimulating reactions in the advanced stages of inflammation (Shuey and Brant, 2004). The current research aimed to know the relationship of TNF- $\alpha$  to pregnant women suffering from anemia caused by iron deficiency, as well as the diagnostic importance of inflammatory cytokines and the extent of their relationship to low iron, which causes anemia in pregnant women.

## **Subject and Method**

### **Sample collection**

The study was conducted on 50 blood samples, 30 samples from women pregnant with anemia caused by iron deficiency, and 20 samples of a control group (Pregnant women without anemia), the age range (20 - 40) year. Patient samples were collected from Samarra General Hospital and outpatient medical clinics, during the period between 1\11\2023 -15\1\2024 The blood was separated by a centrifuge to obtain the serum and then stored until the study tests were conducted.

### **Methods**

The present study includes the determination of:

#### **Determination of Packed cell volume (P.C.V) ratio**

It represents the percentage of packed blood cells to the total blood volume, as it was measured using the

capillary tube method (P.C.V.), a capillary tube with open ends and free of heparin. After filling it with an amount of blood reaching two-thirds of the tube, one end of it is closed with a clay sealant, then it is placed in Microcentrifuge hematocrit for five minutes at a speed of 5000 rpm. The capillary tube is read on the Haematocrite reader, which represents the percentage of the volume of compressed cells (Hillman and Ault, 1976).

#### **Determination of Hemoglobin concentration**

The Cyanomethemoglobin method was used to estimate the concentration of hemoglobin in the blood using Drabkin's solution. The hemoglobin concentration in the blood sample was estimated using the Cyanomethemoglobin method in which Drabkin's solution is used as a dilution solution. Iron potassium cyanide works to oxidize the iron present in the hemoglobin from Ferrous converts to ferric acid and then to methemoglobin, which gives a brown color as a result of the reaction (Brown, 1976).

#### **Determination of biochemical concentration**

The concentration of iron, ferritin, and tumor necrosis factor in sera was estimated using a kit Cloud-clone Corp prepared by the company American (Nicolas *et al.*, 2002).

#### **Statistical analysis**

The SPSS statistical program was used and the mean  $\pm$  S.D was determined for the two groups (patients and control), using the F. Test and at the probability level of  $P \geq 0.01$ .

The area under the curve analysis of the variables studied for the two groups was also studied, as well as a correlation study. Tumor necrosis factor with the variables studied.

#### **Results and Discussion**

$P \geq 0.01$

The results showed a significant decrease in the levels of (PCV%, Hb, Iron, Ferritin) and a significant increase in tumor necrosis factor concentration in the blood serum of patients compared to the control group. As fig (1, 2, 3, 4, 5).

## **ROC curve calculation**

The ROC curve was calculated for the diagnostic and biochemical variables for patients with pregnant women with anemia, represented by (PCV%, Hb, Iron, Ferritin, and Tumor necrosis factor) in addition to the healthy group.

### **ROC curve for PCV% in blood of all groups**

The findings revealed that the area under the curve's (AUC) value was (1), and the sensitivity was (100), while the specificity was (100) at the probability concentration ( $P < 0.01$ ) for the two groups of patients and healthy ones, as in the figure (Cappellini *et al.*, 2020). The results showed that the sensitivity and specificity were excellent for the percentage of volume of packed blood cells. Therefore, the diagnosis of pregnant women with anemia depends mainly on measuring the extent of the decrease in the percentage of volume of packed blood cells in pregnant women with anemia.

### **ROC curve for Haemoglobin in the blood of all groups**

According to the findings, the (AUC) value was (1), and the sensitivity was (100), while the specificity was (100) at the probability level ( $< 0.01$ ) for the two groups of patients and healthy ones, as in the figure (7) (Al-dulaimy *et al.*, 2016).

The results of (13) indicated that the sensitivity rate for hemoglobin in women with anemia was 60.82%, with a specificity of 53.33%, while the area under the curve was 0.656. This does not agree with the results of the current research, as the results showed that the rate of sensitivity and specificity was excellent, as this is due to the choice of the type and the sample size is selected selectively, as low hemoglobin levels are one of the diagnostic variables for patients with anemia.

### **ROC curve for Iron in sera of all groups**

The results showed that the value of the area under the curve (AUC) was (0.992) and the sensitivity was (96.7), while the specificity was (100) at the probability level ( $< 0.0001$ ) for the two groups of patients and healthy ones, as in the figure (8). The results showed that the AUC value was high, in addition to the high sensitivity and specificity. Therefore, iron deficiency may be one of

the diagnostic features in pregnant women suffering from anemia, as its decrease is an independent factor in predicting the risk of anemia.

Based on these results, it can be concluded that iron deficiency could be a distinctive diagnostic feature in pregnant women with anemia. Low iron level is an independent factor that can be used to predict high risk of anemia because it consistently achieves high AUC values and high sensitivity and specificity.

### **ROC curve for ferritin in sera of all groups**

The results showed that the value of the (AUC) was (0.992) and the sensitivity was (96.7), while the specificity was (100) at the probability level ( $< 0.01$ ) for the two groups of patients and healthy ones, as in the figure(9) (Shuey and Brant, 2004).

The results showed that the sensitivity and specificity rates are high for these groups, as low iron stores are one of the important diagnostic variables for patients with anemia.

### **ROC curve for Tumor necrosis factor in sera of all groups**

The results showed that the value of the (AUC) was (0.996) and the sensitivity was (100), while the specificity was (94.4) at the probability concentration ( $P < 0.001$ ) for the two groups of patients and healthy ones, as in figure(10) (Hillman and Ault, 1976).

Sensitivity rate: 100%, which means that TNF- $\alpha$  is able to identify all cases of anemia in the first trimester of pregnancy. Specificity rate: 94.4%, which means there is a small chance of misdiagnosing unaffected people. Based on these results, it can be said that TNF- $\alpha$  could be an excellent diagnostic tool for anemia in pregnant women during the first trimester of pregnancy. The high AUC value and complete sensitivity provide high confidence in the ability of TNF- $\alpha$  to identify affected cases, given the small chance of misdiagnosis in non-anemic individuals.

### **Correlations between Tumor necrosis factor with biochemical variables**

It was found from the table (2), that there is a high positive significant correlation between the concentration

of tumor necrosis factor and the levels of iron and ferritin in the group of pregnant women suffering from anemia, as the value of the correlation coefficient was (0.446, 0.393) respectively in the group of patients, and there was no significant correlation Negative or positive between the level of tumor necrosis factor and the variables studied in the healthy group.

There are many factors that can lead to anemia as a result of iron deficiency, including insufficient iron, and increased physiological demand for nutrients (pregnancy).

Iron losses increase during pregnancy, so iron requirements increase approximately tenfold. Consequently, pregnant women are mainly at risk of emergency iron deficiency, which is accompanied by anemia (Sawada *et al.*, 2014; Klajnbard *et al.*, 2010). Therefore, a decrease in iron levels is accompanied by a decrease in the rate of blood levels in pregnant women suffering from anemia.

However, this discrepancy does not cause a significant decrease unless it is accompanied by a real deficiency in the basic requirements for building red blood cells. Which then leads to the occurrence of anemia (Amit Kumar Mani Tiwari *et al.*, 2010), and this is consistent with the results of the current study, as pregnant women suffer from anemia. This study's results also agree with Al-Ani, Mohammed Qais (2007), who indicated a low percentage of P.C.V. In pregnant women, and the results of Mohammed, Hanan Naif (2020). On the other hand, it was found that pregnant women with anemia are more susceptible to a decrease in hemoglobin concentration (Andra H. James, 2021).

The results of the current study agreed with the results of the study of Sabah *et al.*, (2018) and the results of Mohammed, Hanan Naif (2020) who indicated in their study a reduction in the rate of Hb concentration in pregnant women suffering from anemia, as the rate of hemoglobin concentration in pregnant women suffering from anemia is lower compared to its concentration.

In healthy pregnant women, this reflects that anemia leads to a decrease in hemoglobin values in pregnant women, which leads to an increase in blood plasma and a decrease in RBC, which occurs as a result of physiological changes in the second and third trimester of pregnancy, including the increased needs for iron during pregnancy due to the rise in blood plasma, which is

estimated at 50%, with the expansion of red blood cell mass by about 25% (American College of Obstetricians and Gynecologists, 2008). Therefore, patients with low hemoglobin concentrations in the blood suffer from pathological conditions (Christof *et al.*, 2024).

It was also found that the decrease in blood components during pregnancy is due to an elevated in the volume of blood plasma, and to hormonal changes that increase fluid retention, and this is what was indicated by the study of Patrick (Ichipi-Ifukor *et al.*, 2013), which showed that the decrease in hemoglobin concentration is due to an increase in the demand for iron with As the duration of pregnancy passes, in order to meet the expansion in the mother's hemoglobin mass and the needs of the fetus' growth, the elevated in serum volume is relatively larger than the increase in red cell mass, which leads to a decrease in maternal hemoglobin concentration, and thus physiological anemia occurs that occurs during the duration of pregnancy. On the other hand, a study (Mahmoud *et al.*, 2022) found that hemoglobin and ferritin levels increased in patients suffering from anemia after receiving treatment for four weeks, which caused an increase in the hemoglobin concentration rate.

On the other hand, the current study found a decrease in the iron level in pregnant women suffering from anemia compared to pregnant without anemia. This result was consistent with many studies that indicated that anemia occurs due to iron deficiency and is one of the most common diseases in pregnant women, as 40 women are affected. -60% of pregnant women suffer from this disease (Shu and Ogbodo, 2005), and anemia occurs in the third trimester of pregnancy as a result of a lack of iron in the body below the normal concentration (Sukrat *et al.*, 2010).

The results of the current study agreed with Madhavan *et al.*, (2004), as they explained that the decrease in iron and ferritin in pregnant women who suffer from anemia may be due to iron deficiency. This study also agreed with the results of Nasser's study (Nasser, Rula Sabah, 2017), as it showed that the percentage of decrease in iron and ferritin is linearly proportional. With the severity of anemia, that is, their levels decreased for the affected group and their percentage increased for the control group. Hussein (Hussein *et al.*, 2018) also indicated that the levels of RBC, iron, and ferritin are the main stores of iron and a new sign of the maturation of iron in the body.

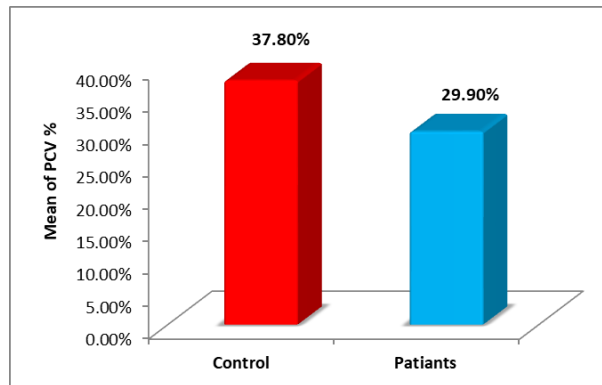
**Table.1** Mean ± S.D of PCV %, Hb, Iron, ferritin, TNF- α in groups under investigation

Groups Parameters	Mean ± SD		P-Value
	Control	Patients	
PCV %	37.80±1.936	29.90±0.712	<b>0.0001**</b>
Hb (g/L)	12.575±0.642	9.940±0.254	<b>0.0001**</b>
Iron(mg/L)	128.444±18.460	83.378±9.465	<b>0.0001**</b>
Ferritin(ng/ml)	26.556±5.643	10.549±3.272	<b>0.0001**</b>
TNF α(pg/ml)	134.150±55.556	664.192±202.122	<b>0.0001**</b>

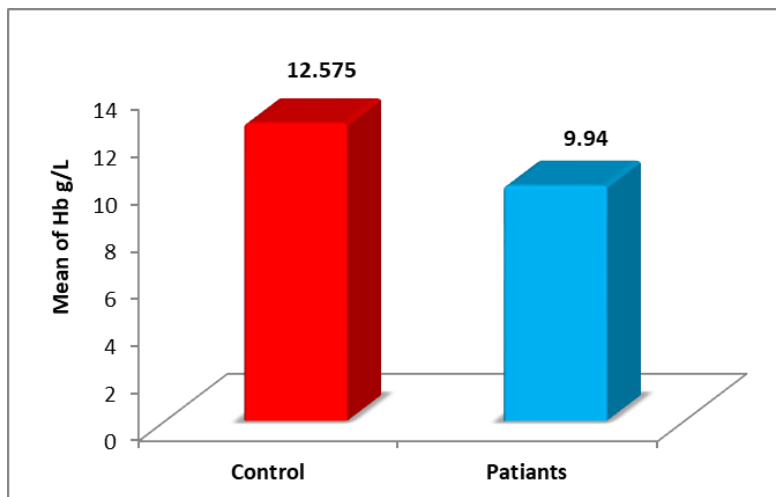
**Table.2** Shows correlations for the variables studied in the group of patients and healthy people

Parameters	TNF-α	
	Control	Patients
PCV%	-0.167	0.117
Hb	-0.160	0.138
Iron	0.293	0.446*
Ferritin	0.263	0.393*

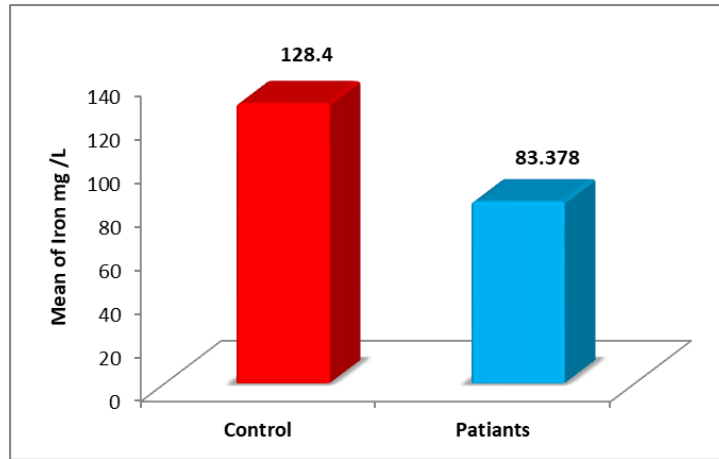
**Figure.1** Average of PCV% in all groups



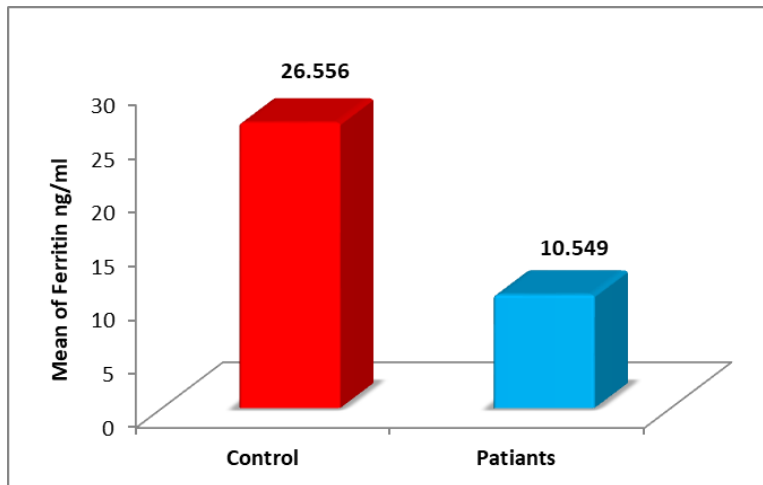
**Figure.2** Average of Hb in all groups



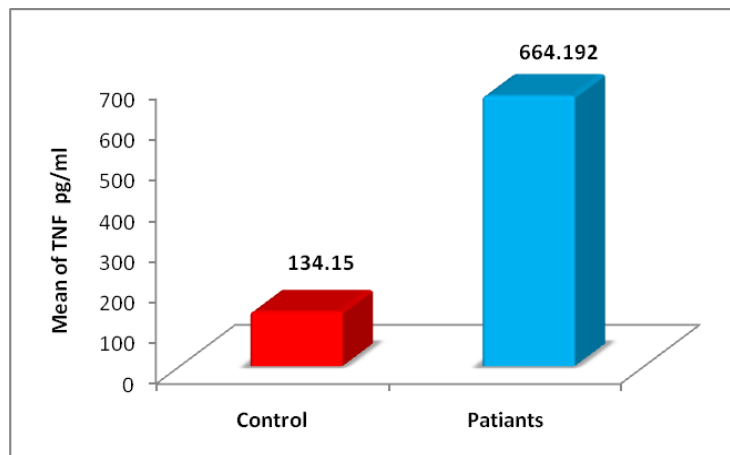
**Figure.3** Concentration of Iron in all groups



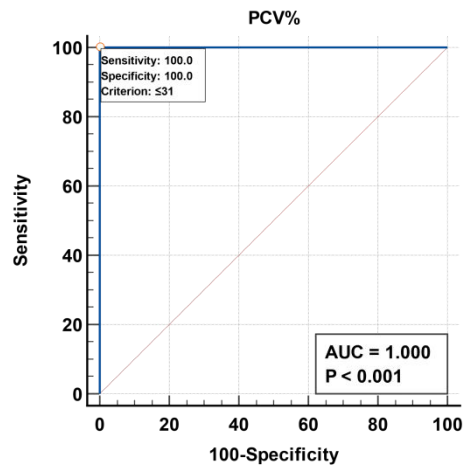
**Figure.4** Concentration of Ferritin in all groups



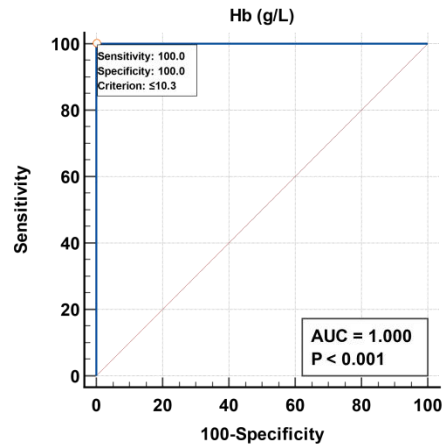
**Figure.5** Concentration of Tumor necrosis factor in all groups



**Figure.6** Analysis of the ROC curve for PCV in the study group



**Figure.7** Analysis of the ROC curve for Hb in the study group



**Figure.8** Analysis of the ROC curve for Iron in the study group

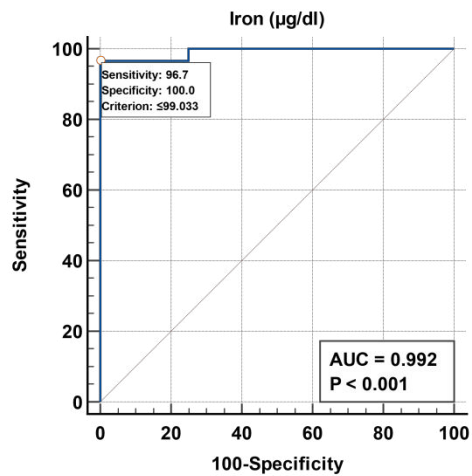


Figure.9 Analysis of the ROC curve for ferritin in the study group

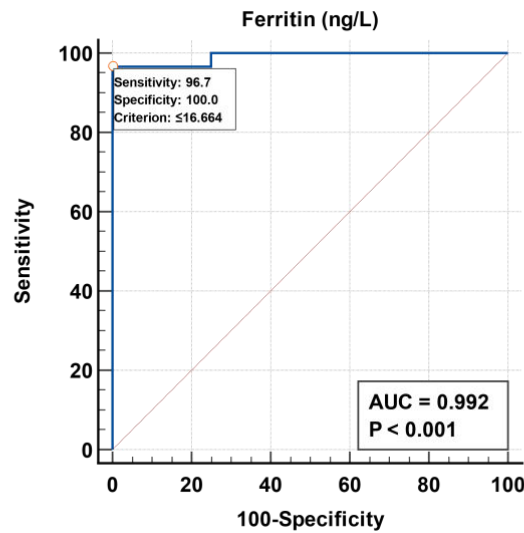
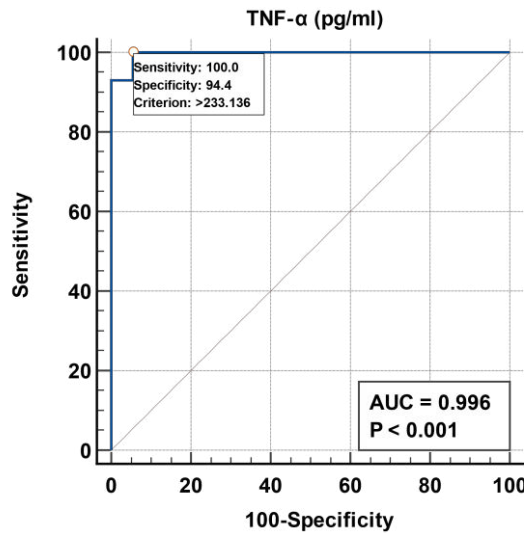


Figure.10 Analysis of the ROC curve for TNF- $\alpha$  in the study group



The results also agreed with (Aras *et al.*, 2022), which indicated a reduce in ferritin levels in pregnant women suffering from anemia. The reason for the decrease in ferritin in pregnant women suffering from anemia may be the increased need for iron (Amadi *et al.*, 2020). Therefore, a decrease in the ferritin in the blood of pregnant women with anemia has been observed, indicating a decrease in iron stores, which inhibits one of the hormones that regulate the level of iron, including the

hormone hepcidin, as it low levels of hepcidin in the blood activate ferroportin to rise iron absorption from the intestine. This explains the significant low in sera ferritin concentration in pregnant women with anemia (Hussein and Hussein, 2018; Jolanta Artym *et al.*, 2021).

The current research results indicated an increase in the level of inflammatory factors, including tumor necrosis factor, in the sera of pregnant women with anemia



compared to women without the infection. In a study conducted on mice, iron deficiency was found to increase the concentration of (TNF- $\alpha$ ) only in the blastula cells of the placenta. Data therefore suggest that iron deficiency has direct effects not only on iron concentration and metabolism, but also on other growth and development regulators, such as placental cytokines, and that these changes may explain, at least in part, the deleterious consequences of maternal iron deficiency [Gambling et al., \(2002\)](#). Studies show that increased levels of (TNF- $\alpha$ ) are associated with pregnancy loss and preeclampsia ([Trisnawati and Nontji, 2020](#)). In a study conducted by ([Gawel and Krolak-Olejnik, 2023](#)), it was found that the concentration of TNF- $\alpha$  in normal pregnant women was 24.47 pg/ml, while its concentration increased to about 57.052 pg/ml in pregnant women at risk of preeclampsia.

It is concluded from the results of the research that inflammatory factors, including tumor necrosis factor, are increased in pregnant women with anemia and are highly sensitive in diagnosing the disease, and thus their levels higher than the normal rate play a role in the future in predicting the occurrence of preeclampsia, while iron storage protein plays a role in diagnosing the disease.

### Author Contributions

Naghham Imad Abdul Hameed: Investigation, formal analysis, writing—original draft.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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