

Maternal Serum Tumor Necrosis Factor Level in Women with Hyperemesis Gravidarum

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Abstract:

Background: Between 0.3% and 2% of pregnant women experience hyperemesis gravidarum. It is recognized by persistent and unexplained vomiting and dehydration, and the diagnosis is confirmed by significant loss of weight, usually more than 5% of the pre-pregnancy weight. Tumor necrosis factor- α is a multipotent cytokine that affects a vast array of cells. It is now well accepted that the tumor necrosis factor- α is crucial in the very early stages of pregnancy.

Objectives: To assess the relationship between the tumor necrosis factor- α and hyperemesis gravidarum.

Methods: This case-control study was carried out at the Baghdad Teaching Hospital, Obstetrics and Gynecology Department, from 1 January to 1 November 2022. The study involved 50 pregnant women, who suffered from hyperemesis gravidarum (mild or severe) as the case group, and another 50 healthy pregnant women as the control group, in the first trimester. The (Pregnancy-Unique Quantification of Emesis and Nausea) Index was used to evaluate the severity of the symptoms. Five milliliters of venous blood were taken to measure the tumor necrosis factor- α .

Results: In contrast to the women in the control group, pregnant women with hyperemesis gravidarum had a mean hemoglobin that was significantly lower (10.8) g/dL compared to the control group (11.5) g/dL and their liver enzymes were significantly higher except for alkaline phosphatase, (294.4) IU/L compared to the control group (49.5) IU/L. Their mean tumor necrosis factor- α level was also significantly higher, (200.6) pg/ml versus (96.34) pg/ml in the control group.

Conclusion: The mean tumor necrosis factor- α level was higher in patients with hyperemesis gravidarum than in women with normal pregnancy.

Keywords: Assessment; hyperemesis; tumor necrosis factor- α ; women.

Introduction:

Hyperemesis gravidarum (HG) affects 0.3% to 2% of pregnant women, while reports of populations with noticeably higher incidence have been made. (1). Hyperemesis gravidarum is variably demarcated as severe unrelenting nausea and vomiting that occurs in the fourth to the seventh week of gestation and produces loss of weight of more than 5%, dehydration, ketosis, alkalosis because of the loss of hydrochloric acid, and hypokalemia. It tends to be relieved by 20 weeks of gestation (2).

HG is believed to be a consequence of placental metabolism because it does not require the fetus to occur. It occurs commonly in multiple gestations and advanced molar gestation. During pregnancy, there is a strong temporal relation between human chorionic gonadotropin (hCG) concentrations and the time at which the symptoms of nausea and vomiting are at their peak (3). The hCG is a thyroid stimulator of pregnancies and biochemical hyperthyroidism is seen commonly in HG (4). However, because the concentrations of total hCG vary greatly in healthy and sick individuals, it has proven challenging to

establish a direct correlation between the degree of nausea and vomiting during pregnancy and hCG (3). The Pregnancy-Unique Quantification of Emesis and Nausea (PUQE) score is a validated means used to evaluate the severity of HG in pregnant women (5). It evaluates the degree of nausea, the frequency of vomiting, and the extent to which daily activities are affected. The PUQE score is reliable and useful in clinical practice, helping the healthcare providers to monitor the progress of HG and adjust the treatment accordingly. The score can also be used to stratify women, based on their risk for adverse outcomes and to guide decisions regarding hospitalisation and nutritional support. Overall, the PUQE score is a valuable tool in the management of HG, providing a standardised approach to evaluate and monitor symptoms and improve the outcomes for pregnant women (6).

The cytokine tumor necrosis factor- α (TNF- α) is essential for inflammation and the immune system. Its structure is that of a homotrimer, meaning it is made up of three identical subunits, each consisting of 157 amino acids. The three subunits are linked by disulfide bonds, forming a stable complex (7).

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It has been demonstrated that (TNF- α) affects uterine cyclicity, placental differentiation, steroidogenesis, embryonic and follicular development, hormone production, and parturition. (8) The levels of TNF- α are found to be significantly high in patients with hyperemesis and could be involved in the etiology (9).

The study aims to assess the level of the tumor necrosis factor in hyperemesis gravidarum.

Patients and methods:

This case control study was carried out at Baghdad Teaching Hospital, Obstetrics and Gynecology Department, during the period from 1 January to 1 November 2022. One hundred women were recruited and divided into two groups: 50 pregnant women who suffered from HG (mild or severe, according to the PUQE score) as the case group, and another 50 healthy pregnant women as the control group.

Inclusion criteria: First-trimester pregnant woman with hyperemesis gravidarum

Exclusion criteria: Chronic medical conditions (like liver or renal disease), smoker, chronic drug users, twin pregnancy, hydatidiform mole pregnancy, patients with peptic ulcer and active infection.

The PUQE Index was used to evaluate the severity of the symptoms. At least two of the following factors necessitated the hospitalization of women experiencing hyperemesis: Hypokalemia (serum potassium level < 3.0 mEq/dL) or hyponatremia (serum sodium level < 134 mEq/dL) requiring intravenous replacement, vomiting at least twice a day or nausea so severe that they were unable to eat or drink, weight loss exceeding 5% of pre-pregnancy weight, ketonuria > 80 mg/dL in a random urine specimen, or a positive test result for serum acetone (5)

All the participants were informed about the nature of the study and their verbal consent was taken. The data was collected through the distribution of a well-designed questionnaire including general and clinical information such as maternal age, educational level, occupation, residency, parity, and body mass index (BMI), which was calculated by using weight in kilograms (kg) divided by the square of height in meters (m²). The same scale was used to measure each subject's height and weight.

General examination, vital signs, systolic and diastolic blood pressure, abdominal and obstetric examinations, laboratory testing, and sonographic examinations were all conducted on all participants.

The investigation results, which were β -HCG, general urine exam, complete blood count, renal function tests, liver function tests, serum electrolytes, and serum TNF- α level, were shared with all included participants. For the laboratory analysis of TNF- α levels, five millilitres of venous blood were collected from the participants.

The blood then underwent centrifugation (3000 RPM for 15 minutes), and the plasma was separated and either measured directly or frozen at -2°C at the time of measurement. Thawing of the frozen samples was done and mixed with the reagent of the ELISA kit

(RayBio® Human TNF-alpha ELISA Kit, Catalog No: ELH-TNFa) and kept for a few minutes. Following this it was introduced into the ELISA reader (BioTek ELx800, USA), the standard range of TNF- α provided by the manufacturer was 82-103 pg/ml.

Statistical analysis:

IBM-SPSS (USA Chicago) was used for statistical analysis once all data had been imported into Microsoft Excel 16. The information was displayed using tables, charts, or graphs and included counts, percentages, mean, standard deviation (SD), minimum (Min), and maximum (Max).

Although continuous variables were examined using the student t-test or the Mann Whitney u test when applicable, the Chi-square or Fisher exact tests were used to determine the level of significance of the categorical data.

The optimal cutoff points were estimated using the receiver operator characteristics curve,

(after running of Yoden j index test) at which estimation of the area under the curve (AUC), sensitivity (SN), specificity (SP) positive predictive value (PPV), negative predictive value (NPV), accuracy of the test (Acc) and relative risk of each variable were identified. A p-value of < 0.05 was considered statistically significant.

Results:

Table 1 shows that there were no significant differences between the cases and controls or significant associations between the demographical variables.

Table 1: Description of the study groups by demographic characteristics

Variables		Cases		Controls		P value
		Mean	SD	Mean	SD	
Maternal age (years)		28.7	5.51	27.7	5.92	0.394
Gestational age (weeks)		7.6	2.79	8.7	2.88	0.065
Gravidity		3.9	2.05	4.2	1.93	0.396
Parity		2.2	1.8	2.5	2.01	0.497
Miscarriage		0.7	0.76	0.8	0.84	0.619
BMI		28.7	3.73	28.4	3.73	0.781
Educational level	No.		%	No.	%	0.973
	Illiterate	5	10	4	8	
	Primary	11	22	10	20	
	Secondary	24	48	25	50	
Residency	University	10	20	11	22	0.137
	Urban	41	82	46	92	
	Rural	9	18	4	8	

Regarding severity of HG, seven cases had severe symptoms (as shown in Figure 1) and were admitted to hospital as their serum electrolytes showed a picture consistent with HG (dehydration, hyponatremia, hypokalemia, and hypochloremia). They have all responded well to fluid therapy.

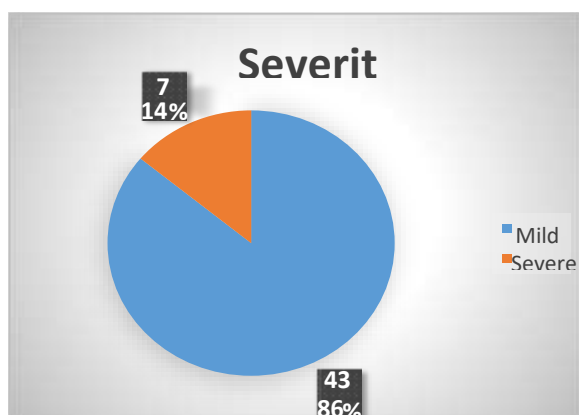


Figure 1: Distribution of HG cases according to severity
TNF- α level was not different between cases of mild and severe HG, and was elevated in both mild and severe cases, as shown in Figure 2.

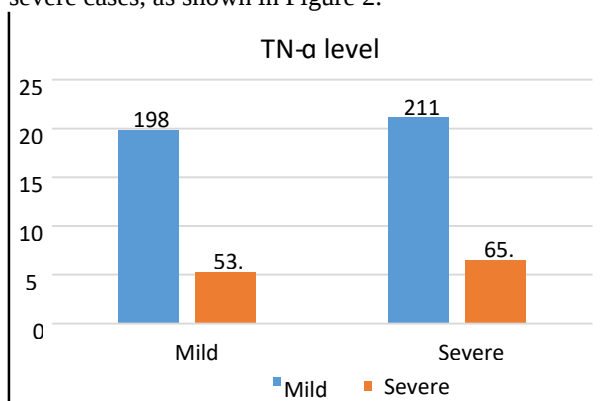


Figure 2: Distribution of TNF- α according to severity

Patients with HG had a considerably lower mean hemoglobin than the control group. With the exception of ALP, which did not differ between the two groups, liver enzyme levels were considerably greater in HG patients than in controls. Serum electrolytes, blood urea, and serum creatinine did not differ between the two groups. The case group's TNF- α level was noticeably higher than the control groups, table 2.

Table 2: Mean $\pm$ SD of the lab investigations for the study groups

Investigations	Cases		Controls		P value
	Mean	SD	Mean	SD	
Hb	10.8	1.27	11.5	1.23	0.012
AST	294.4	117.54	49.5	22.22	<0.0001
ALT	269.4	138.22	59.1	20.84	<0.0001
ALP	127.8	34.65	127.6	32.78	0.970
Urea	45.7	15.57	41.1	15.28	0.14
Creatinine	1.2	0.35	1.1	0.29	0.072
Na	139.3	2.98	138.7	3.21	0.386
K	3.7	0.33	3.7	0.37	0.669
Cl	102.3	5.77	100.5	6.01	0.122
TNF- α	200.6	54.61	96.3	16.99	<0.0001

For estimation of the predictive ability of TNF- α we applied ROC curve analysis and showed that the area under curve to be 0.975 with accuracy of 88%. The level of TNF- α equal to or higher than 115.5 pg/ml

(cutoff point) is associated with 92% sensitivity and 84% specificity. TNF- α level equal to or higher than this cutoff point would be 60.38 times common in cases of HG than normal pregnancy, figure 3 and table 3.

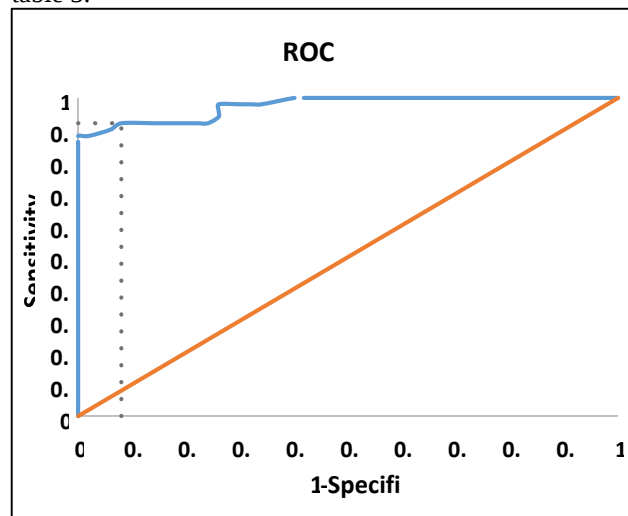


Figure 3: ROC curve analysis.

Table 3: Predictive ability of TNF- α in marking hyperemesis gravidarum

Predictor	Value
Area under the curve	0.975 $\pm$ 0.013
s95% confidence interval	0.950-1.000
Cutoff point (pg/ml)	≥ 115.5
Sensitivity (%)	92
Specificity (%)	84
Positive predictive value (%)	85.2
Negative predictive value (%)	91.3
Accuracy (%)	88
Odd ratio	60.38

Discussion:

Pregnancy-related nausea and vomiting impacts most pregnancies, although only a significantly lower percentage of women experience HG, the most severe kind. Despite the serious effects of HG and the ubiquity of nausea and vomiting during pregnancy, the etiology of these diseases is not entirely understood. (1). The absence of a significant difference in the mean age of cases and controls in the present is not in agreement with the findings of Nurmi *et al.*, (10) who found that the younger the maternal age, the higher the rate of HG. The mean gestational age, gravidity and parity being not significantly different between the two groups, disagrees with the findings of Alimah *et al.*, (11) that primiparous patients were more prone to develop HG. The finding that the rate of miscarriages was not significantly different between the two groups disagrees with that of Nurmi *et al.*, (10) who observed that women with a higher rate of previous miscarriages experienced a lower incidence of HG. Unlike the finding of Thakur *et al* (12) that underweight women were more likely to develop HG, the current study found no significant difference in the mean body mass index between the two groups. While Türkmen *et al.*, (13) found that

low educational level was associated with HG, the current study found no such association.

The lower mean hemoglobin level in HG cases than the controls in the current study is comparable to the findings of Febrianti *et al* (14) and Salah *et al* (15). Anaemia causes decreased oxygen delivery which may contribute to developing HG, as suggested by Michihata *et al* (16). Our HG cases had considerably higher mean levels of AST and ALT than the controls. Similarly, Gaba *et al.*, (17) found that both were elevated, but ALT elevation was significantly higher than AST elevation. This may be the cause or the consequence of HG, as a pre-existing liver disease could become more evident in early pregnancy, or it may result from prolonged vomiting, mainly occurring one week after the start of repeated vomiting, as stated by Sasamori *et al* (18).

In the current study, the mean alkaline phosphatase levels among HG cases were not different from controls. Guarino *et al.*, (19) described the liver function tests in normal pregnancy and stated that the ALP level would be elevated in normal pregnancy, and all cases of HG had a similar level of ALP. Similarly, Florentin *et al.*, (20) found no significant difference between patients with HG and normal pregnancy regarding ALP.

Blood urea and serum creatinine mean levels were not different in the HG cases from the control. The renal functions of severe HG cases could develop a varying degree of abnormality depending on the level of dehydration, electrolyte disturbance, and deteriorating liver function, as was found by Weiss *et al* (21). In the current study, only seven cases had electrolyte disturbance having severe forms of HG. Both Popa *et al* (22) and Fejzo *et al.*, (23) found that cases of severe HG were associated with life-threatening electrolyte disturbance.

In the current study, the mean TNF- α level was significantly higher in the HG group than in the control group, in agreement with Chuan *et al.*, (9). This may be the cause or the effect of HG, as the initiation of the process of HG could be attributed to a placental abnormality that might be evident due to two points: The elevation of β -hCG in cases of HG and the other placental diseases associated with elevated TNF- α , such as, in placental infection, Fedorka *et al.*; (24) placenta accreta and previa, Özgökçe *et al.*; (25) and placental insufficiency, Berbets *et al* (26). This elevation of TNF- α may be a consequence of repeated vomiting and hypovolemia as part of the cytokines released, Gulati *et al* (27). As for the estimation of the marking for TNF- α in cases of HG, we found that it was associated with 88% accuracy, 92% sensitivity, and 84% specificity. Cases of HG had 60.38 times the risk of having elevated levels of TNF- α . This result gave an idea of the strong relationship between HG and inflammation.

Conclusion:

The mean TNF- α level was higher in cases of hyperemesis gravidarum than in a normal pregnancy. Thus, TNF- α may be involved in the etiology of hyperemesis gravidarum.

Limitation:

1. Small sample size.
2. Short data collection time.
3. Relatively high investigation cost

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, had been given permission for republication attached to the manuscript. Authors sign on ethical considerations.

Approval-Ethical Clearance: The local ethical committee in (Place where the research was conducted or samples collected and treated) approved the project according to the code number (41) on (20/10/2021).

Conflict of Interest: None.

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Author Contribution:

Study conception & design: (Dr. Najmah M. Meran, Rifka Mohammed). Literature search: (Dr. Rifka Mohammed). Data acquisition: (Dr. Rifka Mohammed). Data analysis & interpretation: (Dr. Najmah M. Meran Dr. Rifka Mohammed). Manuscript preparation: (Dr. Rifka Mohammed). Manuscript editing & review: (Dr. Najmah M. Meran, Rifka Mohammed).

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مستوى عامل نخر الورم في مصل الأم لدى النساء المصابات بفرط القيء الحملي

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الخلاصة

خلفية البحث: يحدث القيء الحملي لدى 0.3% إلى 2% من النساء الحوامل. ويشخص بالقيء المستمر وغير المبرر والجفاف، ويؤكد التشخيص بفقدان الوزن بشكل ملحوظ، عادة أكثر من 5% من وزن ما قبل الحمل. يعد عامل نخر الورم- ألفا سايتوكينا قويا متعدد القدرات، يتمتع بنطاق واسع من التأثيرات على مجموعة كبيرة ومتنوعة من الخلايا. وقد تراكمت أدلة كثيرة تدعم فكرة أن عامل نخر الورم- ألفا يلعب دورا أساسيا في المراحل المبكرة جدا من الحمل.

هدف الدراسة: تقييم العلاقة بين القيء الحملي وعامل نخر الورم- ألفا.

المنهجية: هذه دراسة حالة وشاهد أجريت في قسم التوليد وأمراض النساء في مستشفى بغداد التعليمي خلال الفترة من 1 كانون الثاني إلى 1 تشرين الثاني 2022. شملت الدراسة 50 امرأة حاملًا يعانين من فرط القيء الحملي (خفيف أو شديد) كمجموعة حالة. و50 امرأة حامل أخرى سليمة كمجموعة ضابطة في الأشهر الثلاثة الأولى من الحمل. تم استخدام مؤشر PUQE (القياس الكمي الفريد للقيء والغثيان أثناء الحمل) لتقييم شدة الأعراض. تم أخذ خمسة مل من الدم الوريدي لقياس عامل نخر الورم- ألفا.

النتائج:- في النساء الحوامل المصابات بفرط القيء الحملي، كان متوسط الهيموغلوبين أقل بشكل ملحوظ، (10.8) غم/دل مقارنة بالمجموعة الضابطة (11.5) غم/دل، وكانت إنزيمات الكبد أعلى بشكل ملحوظ في النساء الحوامل المصابات بفرط القيء الحملي باستثناء الفوسفاتيز القلوي (294.4) وحدة دولية/ل مقارنة بالمجموعة الضابطة (49.5) (وحدة دولية/ل، وكان مستوى عامل نخر الورم- ألفا أعلى بشكل ملحوظ (200.6) pg/ml مقابل (96.34) pg/ml في المجموعة الضابطة.

الإستنتاج: كان متوسط مستوى عامل نخر الورم- ألفا أعلى لدى المريضات المصابات بفرط القيء الحملي مقارنة بالحمل الطبيعي.

الكلمات المفتاحية: التقييم، فرط القيء، عامل نخر الورم- ألفا، نساء