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Evaluation of Maternal Serum Afamin and Vitamin E Levels in Pregnant Women with COVID-19 and Its Association with Composite Adverse Perinatal Outcomes

Running Head: COVID-19, Afamin and Vitamin E

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Abstract

Objective: To evaluate the maternal serum afamin and vitamin E levels in pregnant women with coronavirus disease 2019 (COVID-19) and to investigate their association with composite adverse perinatal outcomes.

Methods: This prospective, case-control study consisted of 60 pregnant women with COVID-19 infection and 36 age-matched pregnant women without any defined risk factors. Demographic features, laboratory test results, afamin and vitamin E levels were compared between the groups. Receiver operating characteristic (ROC) curve was used to assess the relationship of afamin and vitamin E levels in predicting composite adverse perinatal outcomes. A correlation analysis was performed between afamin and C-reactive protein (CRP) levels in pregnant women with COVID-19.

Results: Obstetric complication rate was higher in the COVID-19 group (13.3% vs 2.8%) ($p=0.01$). Afamin levels were higher and vitamin E levels were lower in the COVID-19 group ($p=0.02$, $p<0.001$, respectively). Vitamin E levels were lower in the COVID-19 group for the all trimesters ($p<0.001$, $p<0.001$, $p=0.004$, respectively). Afamin levels were higher in the COVID-19 group for the all trimesters without reaching statistical significance ($p>0.05$). The values in ROC curves with the best balance of sensitivity/specificity for afamin and vitamin E were 0.424 mg/l (70.6% sensitivity, 44.3% specificity) and 3.150 µg/ml (76.5% sensitivity, 58.2% specificity), respectively. A positive moderate statistically significant correlation was found between afamin and CRP levels ($r= 0.264$, $p=0.009$).

Conclusion: Higher afamin and lower vitamin E levels may support the elevated oxidative stress in the etiopathogenesis of COVID-19 and the relationship with composite adverse perinatal outcomes.

Keywords: COVID-19, SARS-CoV-2, pregnancy, adverse outcome, afamin, vitamin E

Introduction

Coronavirus disease 19 (COVID-19) is a novel viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The effect of COVID-19 in our lives has been maintaining its importance since the first days of the pandemic (1). The disease spread rapidly in a short time, resulting in a worldwide crisis. The world of science has devoted itself and developed various projects on this matter, which alerted the whole world. However, our knowledge is still limited, and further data are required on the pathophysiology and management of this unique disease (2).

Many adaptive physiological changes are observed during pregnancy particularly in hematological, immune, cardiovascular and respiratory systems (3). COVID-19 has significant effects on these systems. Therefore, clinicians are concerned about the course of COVID-19 during pregnancy. Pregnancy may worsen the clinical course of COVID-19. Moreover, increased obstetric complications such as miscarriage, preterm labor, prelabour rupture of membranes, preeclampsia, abnormal fetal heart rate patterns and fetal distress have been reported in pregnant women with COVID-19 (4). Increased systemic inflammatory response, hypercoagulability, decreased arterial oxygen saturation are associated with poor obstetric outcomes (5, 6).

Placental mitochondrial activity and reactive oxygen derivatives (reactive oxygen species = ROS) resulting from the production of free superoxide radicals increase, and antioxidant levels decrease during pregnancy. Poorly controlled oxidative stress (OS) causes the development of trophoblast dysregulation, which can lead to obstetric complications such as hypertensive disorders and fetal growth restriction (7).

Antioxidants are a line of defense against lipid peroxidation, stabilizing reactive free radicals. Vitamin E is a major protective lipophilic antioxidant protecting against OS during pregnancy and the postnatal period (8). Vitamin E amplifies the immune system and supplies the integrity of the T-cell membranes. Additionally, vitamin E reduces the duration of infection with the influenza virus (9). Afamin (also named alpha-albumin) is an alternative carrier plasma glycoprotein (vitamin E-binding protein) for vitamin E expressed from the liver. It plays a role in anti-apoptotic cellular processes related to OS (10). It is an indicator of oxidative stress (11). Higher serum afamin concentrations have been reported in hypertensive pregnancy complications such as preeclampsia and pregnancy-induced hypertension. Elevated afamin levels are associated with insulin resistance (IR) and metabolic syndrome components. It may act as a biomarker for pathological glucose metabolism during pregnancy (10). In insulin resistance, mediator release from the placenta, particularly tumor necrosis factor-alpha (TNF-α), increases in correlation with the severity of IR (12). Therefore, elevated afamin level is associated with poor obstetric outcomes (13). Thus, afamin and vitamin E levels may differ between infected pregnant women and healthy pregnancies. However, to the best of our knowledge, there is no study in the literature evaluating the afamin and vitamin E levels and their relationship with composite adverse perinatal outcomes in pregnant women with COVID-19 infection.

This study aims to compare afamin and vitamin E levels between pregnant women with COVID-19 and pregnant women without any defined risk factors. Moreover, their association with composite adverse perinatal outcomes were investigated.

Materials and Methods

This prospective case-control study was conducted on pregnant women, who were admitted to the Department of Obstetrics and Gynecology, Turkish Ministry of Health Ankara City Hospital between 26 June 2020 and 27 August 2020. Blood samples were obtained from the volunteering patients for the evaluation of afamin and vitamin E levels. The study protocol was approved by both Turkish Ministry of Health and the institutional ethics committee (E1-20-1148) and informed consent was obtained from all patients.

Turkish Ministry of Health Ankara City Hospital is a tertiary reference center and it has played a major role in the management of COVID-19 patients since the beginning of the pandemic (14). Special protocols have been prepared for pregnant women with COVID-19. The follow-up of the patients is carried out by a multidisciplinary team consisting of obstetricians, perinatologists, chest diseases specialists, infectious diseases specialists, anesthesiologists and neonatologists. Since it is a pandemic center, the follow-up of low-risk and high-risk pregnant women continues, with about 1100 deliveries per month (14).

In the present study, pregnant women with COVID-19 were compared with a control group of pregnant women without any defined risk factors in terms of demographic features, laboratory parameters, afamin and vitamin E levels. Maternal age, body-mass index (BMI) (kg/m^2), gravidity, parity, comorbid conditions, gestational age at diagnosis, pregnancy status, obstetric complications, white blood cell, neutrophil counts, neutrophilia rate (>$7500/mm^3$), initial lymphocyte counts, lymphopenia rate (<$1000/mm^3$), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), interleukin 6 (IL-6), procalcitonin, ferritin, hemoglobin (Hb), hematocrit (Hct),

platelet, blood urea nitrogen (BUN), creatinine, aspartate transferase (AST), alanine transferase (ALT), frequency of elevated liver enzymes (ALT or AST levels above the twice of rererance ranges), D-dimer, elevated troponin rate (>0.4 ng/ml), creatine kinase-MB (CK-MB), neutrophil-lymphocyte ratio, lactate dehydrogenase (LDH), afamin and vitamin E levels were compared between the groups.

Afamin and vitamin E levels were compared according to pregnancy trimesters in pregnant women with COVID-19 and the control group. Furthermore, cut-off values of afamin and vitamin E for the prediction of composite adverse perinatal outcomes were determined. Composite advers outcome was defined as the presence of an obstetric complication or modarate/severe COVID-19. Finally, a correlation analysis was performed between afamin and CRP levels in pregnant women with COVID-19.

COVID-19 was diagnosed by a Real-time polymerase chain reaction (RT-PCR) assay on the nasopharyngeal and oropharyngeal specimens (15). The severity of disease and management of the cases with COVID-19 were determined according to the national guidelines (16). Blood samples and related laboratory tests were obtained from the patients at the time of admission to the hospital.

Afamin and vitamin E levels were measured according to the instructions of commercial kits with the immun-based method (ELISA, Bioassay Technology Laboratory; Shanghai Coon Koon Biotech Co. Ltd, China) at 450 nm absorbance with a microplate spectrophotometer (Epoch Microplate Spectrophotometer, Biotek Company, USA) at Ankara University Faculty of Medicine, Pathophysiology Laboratories. Standart ranges of the kits were 5 mg/l - 600 mg/l (Afamin) and 0.5 µg/ml - 150 µg/ml (vitamin E).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS.22, IBM SPSS Statistics for Windows, Version 22.0 Armonk, NY: IBM Corp.). Descriptive analyses were presented as medians and the interquartile range since they were not normally distributed. The Mann-Whitney U test was performed to compare the median values between the groups. Categorical variables were presented by numbers and percentages. A Chi-square test was used to compare the categorical variables between the groups. Operating characteristic (ROC) curve was used to assess the performance of afamin and vitamin E levels in predicting composite advers outcomes. Youden index was applied to ROC curve to choose the best cut-off value (17). Correlation analysis was performed by Spearman test. A two-tailed P value < 0.05 was regarded as statistically significant.

Results

The total number of patients was 60 in pregnant women with COVID-19 infection and 36 in the control group. There were 20 and 12 patients for each pregnancy trimester in the study and control groups, respectively. In the COVID-19 group, 43 (71.7%), 16 (26.7%) and 1 (1.7%) patients had mild, moderate and severe disease, respectively. Computed tomography findings of 10 patients were compatible with COVID-19. The total number of patients under treatment was 18.

Comparison of demographic features and pregnancy complications between pregnant women with COVID-19 and control group was shown in Table 1. The two groups were similar except for type of pregnancy complications and composite adverse pregnancy outcomes (p>0.05). Composite adverse perinatal outcome rate was higher in the pregnant women with COVID-19 infection.

<Insert Table 1>

Comparison of laboratory parameters, serum afamin, and vitamin E levels between pregnant women with COVID-19 and the control group was shown in Table 2. Two groups were comparable in terms of neutrophilia rate, ESR, IL-6, procalcitonin, BUN, creatinine, elevated liver enzymes, elevated troponin rate, NLR and LDH. White blood cell, neutrophil, lymphocyte, Hb, Hct, platelet and vitamin E values were significantly lower in pregnant women with COVID-19 ($p<0.05$). However, lymphopenia rate ($<1000/mm^3$), CRP, ALT, AST, ferritin, CK-MB and afamin values were significantly lower in the control group ($p<0.05$).

<Insert Table 2>

Comparison of serum afamin and vitamin E levels of pregnant women with COVID-19 and healthy controls according to pregnancy trimesters was shown in Table 3. Afamin levels were higher in pregnant women with COVID-19 in the first, second, and third trimesters compared to the healthy controls; however, there was no statistical difference ($p>0.05$). Vitamin E levels were significantly lower in women with COVID-19 in the first, second and third trimesters compared to the control group ($p<0.05$).

<Insert Table 3>

ROC curve analysis for assessing the performance of afamin value in predicting composite advers outcomes in pregnant women with COVID 19 was shown in Table 4 and Figure 1. Area under the curve (AUC) was 0.67 (95% CI: 0.52-0.82) for composite adverse perinatal outcomes. The values in ROC curves with the best balance of sensitivity/specificity was 0.424 mg/l (70.6% sensitivity, 44.3% specificity).

<Insert Table 4>

<Insert Figure 1>

ROC curve analysis for assessing the performance of vitamin E value in predicting composite adverse perinatal outcomes in pregnant women with COVID 19 was shown in Table 5 and Figure 2. Area under the curve (AUC) was 0.66 (95% CI: 0.52-0.81) for composite advers outcomes. The values in ROC curves with the best balance of sensitivity/specificity was 3.150 µg/mL (76.5% sensitivity, 58.2% specificity).

<Insert Table 5>

<Insert Figure 2>

Correlation of afamin values with CRP in pregnant women with COVID-19 was shown in Table 6. A positive moderate statistically significant correlation was found between CRP and afamin values (r= 0.264, p=0.009).

<Insert Table 6>

Discussion

The available data on the pathophysiology of COVID-19 is still limited. Major pathological mechanisms such as cytokine storm, decreased lymphocyte count, excessive inflammation, hypercoagulopathy, the formation of microthrombi, and low blood oxygen levels determine the clinical course of COVID-19, (18-20) causing generalized endothelial vascular damage and hypoperfusion in severe cases (21). There may be severe complications such as acute respiratory distress syndrome (ARDS), acute cardiac complications, multiple organ dysfunction syndromes (MODS), septic shock and even mortality (22). During pregnancy, adaptive

physiological changes occur in respiratory, cardiovascular, hematological, and immune systems. Increased transverse diameter of the thorax, the elevation of the diaphragm and mucosal edema due to vasodilation reduce the tolerance of pregnant women to hypoxia. Adequate perfusion and oxygenation are required for fetal growth during pregnancy (23). Therefore, cardiac output increases, blood volume increases, and systemic vascular resistance decrease during pregnancy (3). COVID-19 is associated with adverse obstetric complications such as miscarriage, preterm labor, prelabour rupture of membranes, preeclampsia, and fetal distress (24). It has been stated that thrombus formation in placental vessels and impaired placentation may cause obstetric complications (25, 26). Additionally, SARS-CoV-2 virions have been observed in the placenta in the electron microscopic examination (27).

Oxidative stress (OS) is defined as the detoxification of reactive oxygen species (ROS) such as hydroxyl radical, superoxide radical, and hydrogen peroxide, which are formed during cellular metabolism, and the deterioration of oxidative balance resulting from the insufficiency of antioxidants. Harmful effects such as lipid peroxidation of free radicals, denaturation of proteins, DNA mutations, and increased inflammatory response can be observed in OS. The immune response is regulated by oxidative stress and inflammatory processes. OS is associated with various pathologies such as premature aging, diabetes mellitus, cancers, hypertension, and coronary heart diseases (28). Increased ROS has been reported due to the production of free superoxide radicals and mitochondrial activity of placental origin during pregnancy. It has been stated that poorly controlled OS may cause the development of trophoblast dysregulation, which may lead to obstetric complications such as hypertensive disorders and fetal growth restriction (7). An increase in free radical production and antioxidant consumption has been observed in RNA virus infections

including HIV 1, Hepatitis B, C, D, Herpes, respiratory viruses. SARS-CoV-2 may also trigger OS similar to other RNA viruses. Furthermore, OS caused by COVID-19 may be one of the reasons behind these complications (29).

Vitamin E is a powerful lipophilic antioxidant, which can neutralize free radicals and ROS by giving out a hydrogen ion from the chromanol ring. It acts as an immunomodulator through protein kinase C. Vitamin E deficiency is associated with high levels of lipid peroxidation in in vivo and in vitro models. An inverse ratio was reported between plasma lipoperoxidase and vitamin E in patients with ARDS (30). In critically ill patients, the level of thiobarbituric acid reactive substances (TBARS), which are the indicator of plasma lipid peroxidation, was found to be high, and α-tocopherol (vitamin E predominant form) levels were found to be low. Increased vascular permeability, gas exchange, and lung compliance were demonstrated in the α-tocopherol group within the albumin microembolization ARDS animal model. In another animal study, a decrease in OS and lipid peroxidation was observed with 60 mg/kg vitamin E supplement administered for 7 days in influenza infection (31). In addition, increased liver samples, reduced glutathione (GSH), and total antioxidant status (TAS) levels were reported in pregnant rats treated with vitamin E (32). It has been reported that supplementation of 200 IU vitamin E per day for a year leads to a decrease in the incidence of upper respiratory tract infections. Vitamin E supplementation has been shown to restore IL-2 production, improve T cell proliferation and immune system function (31). In another study, it was demonstrated that the 50mg/day supplementation of vitamin E to 2216 smoker patients for 5-8 years decreased the incidence of pneumonia by 69% in elderly men (33). In conclusion, vitamin E derivatives have been reported to increase the adaptive immune system response by causing an increase in T cell proliferation, a decrease in cyclooxygenase

(COX) activity, a decrease in ROS, and augmentation in cell membrane integration in ARDS due to COVID-19 (31). In our study, vitamin E levels were found to be significantly lower in pregnant women with COVID-19 in totally and in each trimesters. This suggests the increased oxidant status and consumption of antioxidants in the etiopathogenesis of COVID-19.

Afamin is a specific binding pleiotropic glycoprotein for vitamin E found in biological fluids such as plasma, cerebrospinal fluid, ovarian follicular, and seminal fluids. It is synthesized from the liver and released into the bloodstream. It is a potential chemokine involved in osteoblast metabolism and bone formation (13). It plays a role in cases where the lipoprotein system is insufficient to carry the vitamin E. In vitro studies have demonstrated that vitamin E facilitates blood-brain barrier transport and may have a neuroprotective function. Afamin concentrations were found to be lower in diseases such as ovarian cancer, heart failure, pneumonia, and sepsis when compared to healthy controls. On the other hand, there was no difference in plasma afamin levels in patients with chronic renal failure and chronic obstructive pulmonary disease compared to the healthy controls (13).

Afamin is an indicator of OS, involved in anti-apoptotic cellular processes associated with OS. Elevated afamin concentrations are associated with pathological glucose metabolism, insulin resistance (IR), and metabolic syndrome components (e.g. obesity, type 2 diabetes, hypertension, dyslipidemia, polycystic ovary syndrome, pregnancy complications) (10). In a large-scale, population-based study, it was stated that every 10 mg/l increase in afamin levels caused a 19% increase in metabolic syndrome components (11). Afamin levels increase due to hormonal regulations and increased expression of genes in the liver during pregnancy. It has been emphasized that afamin could a potential predictive marker due to its elevation in pregnancy-

related disorders (e.g. preeclampsia, gestational diabetes mellitus) (10). Hubalek et al. found higher levels of afamin in patients with preeclampsia (PE) (70 mg/l vs 55.4 mg/l, respectively, p=0.007). This supports the increased OS in the tissues with impaired placental development in preeclampsia (34). The relationship between inflammatory markers and afamin levels is controversial. While a strong negative correlation has been reported between plasma afamin and CRP levels in a small number of hospitalized patients (r_s −0.463, $p < 0.001$) (35) a moderate and positive significant correlation was reported in large-population based studies (r_s +0.108, r_s +0.222, r_s +0.213 and $p < 0.01$, $p < 0.001$, $p < 0.001$, respectively) (11). It is stated that this contradiction may be due to the more acute and higher grade inflammation in hospitalized patients (13). In our study, a moderate and positive correlation was observed between plasma afamin levels and CRP. This can be explained by the fact that the clinical course was mostly mild and the number of severe cases hospitalized in the group with COVID-19 was low in our study.

The findings of the present study indicated that serum afamin values were higher and vitamin E levels were lower in pregnant women with COVID-19 compared to the healthy controls. No significant correlation was found for afamin and vitamin E levels with inflammatory markers and clinical characteristics in pregnant women with COVID-19, except for a positive moderate statistically significant correlation with CRP. Additionally, the results of the present study indicated that higher afamin and lower vitamin E values were associated with increased rates of composite adverse perinatal outcomes in pregnant women with COVID-19. However, this is a preliminary study and further data are required to make more precise inferences.

It has been reported that COVID-19 infection may have an effect on iron metabolism (36). Lower hemoglobin and higher ferritin levels were observed in patients with

COVID-19 infection indicating the possible impact of altered iron metabolism in the pathogenesis of this novel disease. Increased ferritin levels were found to be associated with poor prognosis in various studies (37, 38). Iron is an essential micronutrient for pathogens and for this reason the host's immune system generally restricts the availability of iron during infections as a defensive mechanism leading to increased ferritin levels (36). Iron takes part in various vital physiologic processes including transport of oxygen in tissues, electron transfer reactions and repair of DNA. On the other hand, excessive cumilation of iron in cells and tissues may lead to altered oxidative stress processes (39). Thus, in the present study impaired iron metabolism may also have an effect on the levels of afamin and vitamin E. However, in our institution iron levels are not routinely measured. Only ferritin level is evaluated in infected individuals due to its association with disease prognosis. Significantly higher ferritin levels in the present study may reflect the potential impact of impaired iron metabolism in patients with COVID-19. Ferroptosis is a process characterized by non-apoptotic regulated cell death dependent on iron and ROS. Altered antioxidant status may trigger ferroptosis and may cause damage in the cellular membranes. Vitamin E is one of the most potent antioxidants in the human body which may inhibit ferroptosis (40). Therefore, low vitamin E levels observed in the present study may be involved in ferroptosis resulting in impaired oxidative stress.

The strengths of the present study were a novelty, prospective design including each one of all trimesters, and a high number of study parameters. However, the limited number of pregnant women with COVID-19 and healthy controls, absence of serum iron levels, the low number of severe cases and lack of neonatal outcomes were the main limitations.

In conclusion, to the best of our knowledge, this is the first investigation of maternal serum afamin and vitamin E levels in pregnant women with COVID-19. Higher afamin and lower vitamin E levels may support the elevated oxidative stress in the etiopathogenesis of COVID-19 and the relationship with composite advers outcomes.

Authors' Contributions

Seyit Ahmet Erol: study design, statistical analysis, manuscript writing.

Atakan Tanacan: study design, statistical analysis, manuscript writing.

Ali Taner Anuk: data collection, manuscript writing.

Eda Ozden Tokalioglu: data collection, manuscript writing.

Derya Biriken: statistical analysis, manuscript writing.

Huseyin Levent Keskin: critical review, study design.

Ozlem Moraloglu Tekin: supervision, manuscript writing.

Nuray Yazihan: study design, supervision, manuscript writing.

Dilek Sahin: study design, supervision, manuscript writing.

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Conflict of interest statement

The authors state that they have no conflict of interest in this study.

Data Availability Statement

Author elects to not share data.

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Tables

Table 1: Comparision of demographic features, clinical characteristics and obstetric complications between pregnant women with COVID-19 and healthy controls

Variables	Pregnant women with COVID-19 (n=60)	Healthy controls (n=36)	p value
Maternal age (years)(median, IQR)[a]	28.5 (6)	27 (8.5)	0.24
BMI (kg/m^2)(median, IQR)[a]	26.48 (5.84)	25.63 (4.77)	0.72
Gravidity (median, IQR)[a]	2 (2)	2 (2)	0.60

Parity (median, IQR)[a]	1 (2)	1 (2)	0.53
Comorbidity (n, %)[b]	16 (26.7%)	5 (13.9%)	0.14
Comorbidity type (n,%)[b]			
Obesity (n, %)	3 (5%)	4 (11.1%)	
Hypothyroidism (n,%)	4 (6.7%)	1 (2.8%)	
Hypertension (n,%)	2 (3.3%)	0 (0%)	
Asthma (n,%)	1 (1.7%)	0 (0%)	
Epilepsy (n, %)	1 (1.7%)	0 (0%)	
Diabetes mellitus type 2 (n, %)	1 (1.7%)	0 (0%)	
Ulcerative colitis (n, %)	1 (1.7%)	0 (0%)	
Ankylosing spondylitis (n, %)	1 (1.7%)	0 (0%)	
Immune thrombocytopenic purpura (n, %)	1 (1.7%)	0 (0%)	
Mitral valve stenosis (n, %)	1 (1.7%)	0 (0%)	
Gestational age (weeks)(median, IQR)[a]	25 (24.25)	19 (22,75)	0.63
Pregnancy status (n,%)[b]			
On-going pregnancy (n, %)	49 (81.7%)	32 (88.9%)	0.34
Delivered (n, %)	11 (18.3%)	4 (11.1%)	

Composite advers outcome	17 (28.3%)	1 (2.8%)	
Obstetric complication (n, %)[b]	8 (13.3%)	1 (2.8%)	0.01
Obstetric complication type (n, %)[b]			
Preeclampsia (n, %)	2 (3.3%)	0 (0%)	
Preterm delivery (n, %)	1 (1.7%)	0 (0%)	
Miscarriage (n, %)	1 (1,7%)	0 (0%)	0.17
GDM (n, %)	1 (1.7%)	1 (2.8%)	
ICHP (n, %)	1 (1.7%)	0 (0%)	
GHT (n, %)	1 (1.7%)	0 (0%)	
DVT (n, %)	1 (1.7%)	0 (0%)	

BMI: Body-mass index, COVID-19: Coronavirus disease 2019, DVT: Deep vein thrombosis, GDM: Gestational diabetes mellitus, GHT: Gestational hypertension, ICHP: Intrahepatic cholestasis of pregnancy, IQR: Interquartile range

[a] Statistical analysis was performed by Mann-Whitney U test

[b] Statistical analysis was performed by chi-square test

Table 2: Comparision of laboratory parameters, afamin and vitamin E levels between pregnant women with COVID-19 and healthy controls

Variables	Pregnant women with COVID-19 (n=60)	Healthy controls (n=36)	p value
WBC (10^3/ml) (median, IQR)[a]	6205 (3802)	8480 (2167)	**0.001**

Neutrophil (10^3/ml) (median, IQR)[a]	4620 (3360)	6100 (2145)	**0.007**
Neutrophilia (n, %)[a]	17 (28%)	6 (16%)	0.19
Lymphocyte (10^3/ml) (median, IQR)[a]	1230 (645)	1745 (692)	**<0.001**
Lymphopenia (n, %)[a]	18 (30%)	2 (5.1%)	**0.004**
CRP (mg/dl) (median, IQR)[a]	10.5 (15.2)	5 (5.6)	**0.002**
ESR (mm/hr) (median, IQR)[a]	31 (14)	23 (27.2)	0.12
IL-6 (pg/ml) (median, IQR)[a]	6.6 (7.5)	3.5 (1.2)	0.14
Procalcitonin (ng/ml) (median, IQR)[a]	0.03 (0.02)	0.03 (0.00)	0.27
Ferritin (ng/ml) (median, IQR)[a]	18.5 (27)	11 (10)	**0.03**
Hb (g/dl) (median, IQR)[a]	11.3 (1.8)	11.9 (1.7)	**0.01**
Hct (%) (median, IQR)[a]	35.6 (5.25)	36.6 (4.3)	**0.01**
Platelet (10^3/ml) (median, IQR)[a]	228 (92.2)	257 (64.0)	**0.001**
BUN (mg/dl) (median, IQR)[a]	17 (6)	17 (5)	0.43
Creatinine (mg/dl) (median, IQR)[a]	0.49 (0.13)	0.49 (0.13)	0.68
ALT (U/l) (median, IQR)[a]	18 (12)	14 (9)	**0.03**
AST (U/l) (median, IQR)[a]	19 (11)	13 (5)	**0.04**

Elevated liver enzymes (n, %)[a]	11 (18.3%)	2 (5.6%)	0.07
D-dimer (mcg/ml) (median, IQR)	1.2 (1.3)	0.6 (0.5)	**<0.001**
Elevated troponin (n, %)[a]	5 (8.3%)	0 (0%)	0.07
CK-MB (U/l) (median, IQR)[a]	0.4 (0.7)	0.3 (0.3)	**0.04**
NLR (%) (median, IQR)	3.6 (2.9)	3.3 (1.5)	0.21
LDH (U/l) (median, IQR)[a]	201 (50.7)	179 (56.5)	0.06
Afamin (mg/l) (mean±sd) (median, IQR)[a]	**0.91±0.10 (0.20-3.44)**	0.47±0.58 (0.05-1.19)	**0.002**
Vitamin E (µg/ml) (mean±sd) (median, IQR)[a]	**2.57±0.27 (0.67-8.31)**	6.73±0.56 (2.91-16.17)	**<0.001**

ALT: Alanine transferase, AST: Aspartate transferase, BUN: Blood urea nitrogen, CK-MB: Creatine Kinase-MB, COVID-19: Coronavirus disease 2019, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, Hct: Hematocrit, IL-6: Interleukin 6, IQR: Interquartile range, LDH: Lactate dehydrogenase, NLR: Neutrophil to lymphocyte ratio, Sd: Standard deviation, WBC: White blood cell

[a] Statistical analysis was performed by Mann-Whitney U test

Table 3: Comparision of afamin and vitamin E values between pregnant women with COVID-19 and healthy controls according to pregnancy trimesters[a]

Parameters	COVID-19 Groups (1^{th} trimester, n=20) (2^{th} trimester, n=20) (3^{th} trimester, n=20)	Control Groups (1^{th} trimester, n=12) (2^{th} trimester, n=12) (3^{th} trimester, n=12)	p value[b]
Afamin (mg/l)	0.90±0.17 (0.20-2.59)	0.49±0.10 (0.05-	>0.05

		1.08)	
	1.12±0.21 (0.21-3.44)	0.51±0.85 (0.17-0.95)	>0.05
	0.72±0.13 (0.22-2.39)	0.42±0.11 (0.12-1.19)	>0.05
Vitamin E (µg/ml)	**3.53±0.49 (0.70-7.70)**	8.00±0.95 (4.44-13.62)	**<0.001**
	2.26±0.51 (0.67-8.31)	6.71±0.84 (3.18-13.64)	**<0.001**
	1.93±0.34 (0.76-6.94)	5.49±1.04 (2.91-16.17)	**0.004**

COVID-19: coronavirus disease 2019

[a] Values are given as mean ± standard deviation (minimum-maximum)

[b] Statistical analysis was performed by Independent samples test (t-test)

Table 4: ROC curve analysis for assessing the performance of afamin in predicting composite advers outcomes in pregnant women with COVID-19

Composite advers outcomes (AUC: 0.67, 95% CI: 0.52-0.82)	Cut-off value for Afamin	Sensitivity	Specifity	p value
	0.424	70.6%	44.3%	**0.024**

AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic

Table 5: ROC curve analysis for assessing the performance of vitamin E in predicting composite advers outcomes in pregnant women with COVID-19

Composite advers outcomes (AUC: 0.66, 95% CI: 0.52-0.81)	Cut-off value for Vitamin E	Sensitivity	Specifity	p value
	3.150	76.5%	58.2%	**0.029**

AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic

Table 6: Correlation of afamin values with C-reactive protein (CRP) in pregnant women with COVID-19

Parameter	r value[a]	p value[a]
CRP	0.264	**0.009**

CRP: C-reactive protein, COVID-19: Coronavirus disease 2019

[a] Correlation analysis was performed by Spearman test

Figures

Figure 1: Receiver operating characteristic (ROC) curve of afamin value in predicting composite advers outcomes in pregnant women with COVID-19

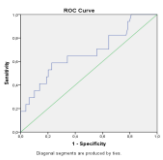

Figure 2: Receiver operating characteristic (ROC) curve of vitamin E value in predicting composite advers outcomes in pregnant women with COVID-19