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WORD COUNT 4443
TIME SUBMITTED 13-MAY-2024 12:08PM
PAPER ID 108927610

Evaluation of genotoxicity and some biochemical parameters in preeclampsia patients with SARS-CoV-2

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ABSTRACT

Background and objectives. Preeclampsia is accompanied by biochemical changes, including hepatic activity, increased blood glucose, thrombocytopenia, urea, creatinine, uric acid, lipid profile changes, hypoalbuminemia, demand for electrolytes, and C-reactive protein. Objective of study: Our research aims to explore the correlation of one of the well-known biochemical profiles with genotoxicity in preeclampsia COVID-19 patient infection in Mosul, Iraq.

Material and Methods. A cross-sectional study in a cohort of 178 as 58 preeclampsia patients with COVID-19 infection, 60 healthy Pregnant women, and 60 healthy controls attending private clinics were enrolled for this study based on the following inclusion and exclusion criteria. The preeclampsia patients with COVID-19 infection have already been diagnosed with COVID-19 and they have symptoms and signs. Each of the patients, healthy pregnant, and healthy controls had the exfoliated cells from scraping the oral mucosa gathered for micronucleus test, and their whole blood samples were collected to be analyzed for; serum hepcidin, apelin, and galectin-3. Also

analyze other biochemical parameters such as iron, ferritin, TIBC, UIBC, transferrin, and TSAT.

Results. The results show that the highest MN were in preeclampsia patients with SARS-CoV-2 at 3.51± 0.471% than in Healthy pregnant as 2.96± 0.109%. The results revealed that hepcidin and galectin-3 levels of the preeclampsia patients with SARS-CoV-2 were higher 152.82±14.18 ng\ml, 22.76±3.39ng\ml respectively than healthy pregnant 109.67±10.59 ng\ml, 20.43±3.17ng\ml respectively. Also, the results show that apelin level was lower in preeclampsia patients with SARS-CoV-2 0.47±0.16 ng\ml than healthy pregnant 0.52±0.14 ng\ml.

Conclusion. The preeclampsia patients with Covid-19 infection may have increased in MN and elevated the levels of serum hepcidin, and galectin-3. Therefore, monitoring these genotoxicity markers is important, especially in pregnant with severe Covid-19 infection.

Key words: Covid-19, biochemical parameters, hepcidin,, apelin,, galectin-3

introduction

A condition known as preeclampsia, which affects multiple systems during pregnancy, typically presents itself in women who previously had normal blood pressure. Symptoms of preeclampsia include the onset of high blood pressure (with readings of 140/90 mmHg or higher on two separate occasions, at least 6 hours apart) and the presence of protein in the urine (with a protein excretion of 300 mg in a 24-hour urine collection, or a dipstick reading of 2+) [1]. Preeclampsia, also known as toxemia, is a significant contributor to adverse outcomes in underdeveloped countries, causing neonatal death, intrauterine growth restriction (IUGR), preterm birth, maternal mortality, and morbidity, as stated by Uzan *et al.* [2]. This condition affects approximately 4 million women globally each year,

resulting in the loss of around 70,000 mothers and 500,000 infants, according to Magee et al. [3]. Infants born to preeclamptic mothers are at a higher risk of preterm birth, perinatal death, neurodevelopmental delay, and future cardiovascular and metabolic diseases [4]. Furthermore, women who survive preeclampsia face reduced life expectancy and increased chances of stroke, cardiovascular disease, and diabetes [5]. The long-term health implications of preeclampsia are estimated to affect over 300 million mothers and children worldwide, as estimated by Davis and colleagues [6]. It is a serious health condition defined by the American College of Obstetrics and Gynecology (ACOG) as blood pressure and proteinuria after 20 weeks of pregnancy in patients previously normotensive [7]. But how exactly does stress cause organ damage in patients with pre-eclampsia, Studies show that people with pre-eclampsia have high levels of stress, which leads to oxygen-free radicals in the body.When oxygen free radicals cause the body to be sad or anxious, or as a result of diseases like pre-eclampsia, more oxygen free radicals form. DNA damage by oxygen-free radicals has been incriminated in the causation of maladaptive processes such as inflammation, cancer, heart and brain diseases, infections, and gerontological disorders [8]. Several studies have given priority to the evaluation of genotoxicity and genetic adversity as a marker of toxicity. Some are as under: Comet assay. Sister-chromatid exchange (SCE) test. Micronucleus (MN) test. Chromosomal aberration analysis. Among these, the well-established and most frequently used genotoxicity test is single-cell gel electrophoresis, known as the comet assay. It is simple, rapid, and highly sensitive. This test is frequently used for the determination of endogenous DNA damage [9]. Preeclampsia is linked to alterations in biochemical markers such as hepatic dysfunction, elevated blood glucose,

thrombocytopenia, urea, creatinine, and uric acid, as well as abnormalities in lipid profiles, hypoalbuminemia, electrolytes, and C-reactive protein. The viewpoint of different researchers about changes in biochemical parameters varies, as we have seen from reading the literature [10]. According to Karar et al.'s findings, increased levels of serum creatinine, urea, urine protein, sodium, potassium, and plasma glucose prevent them from being considered reliable predictors of pre-eclampsia or pregnancy-related hypertension [11]. Maged and colleagues [12] found that preeclampsia is associated with increased activity of blood cells, in the bloodstream and higher concentrations of C reactive protein (CRP). Ekun *et al*., [13] discovered that preeclampsia negatively impacts kidney and liver function as indicated by alterations, in these measures. According to Quan *et al*.'s examination of many variables, pregnancies with a history of gestational diabetes, high blood lipids, advanced age, and a history of hypertension are all significant risk factors for preeclampsia [14]. Pregnant women are more susceptible to SARS-Cov2 because of their immunocompromised state [15]. Premature birth, spontaneous abortion, endotracheal intubation, intrauterine growth restriction, critical care unit hospitalization, renal failure, intravascular coagulopathy, and transmission to the fetus or kid are all possible outcomes of immune system alterations that take place during pregnancy [16]. The majority of SARS-Cov2-infected pregnant women have been shown to have symptoms, pregnancy problems, CT manifestations, and maternal vertical transmission [17]. Since parturients with SARS-Cov2 may have a higher risk of miscarriage, early birth, and baby mortality, the necessity for cesarean delivery was questioned at the beginning of the SARS-Cov2 epidemic [18]. SARS-Cov2's underlying causes and side effects have been extensively researched, however, it is unknown how to treat it when pregnant. Previous

studies have demonstrated that the clinical features of SARS-CoV-2 in pregnant and non-pregnant individuals are the same [16]. Conflicting findings have been found in the few studies on this subject. As a result, the researchers aimed to evaluate the results of several serum biochemical tests and alterations in micronuclei between preeclampsia pregnant women who were SARS-CoV-2 infected, healthy pregnant women, and the control group.

Materials and Method

Declaration of ethics

On a September, 2023, the Scientific and Ethical Committee at the Medical Technical Institute/Mosul - Northern Technical University reviewed and approved the research protocol, the subject information, and the permission form, as per document number ……. Patients also gave their verbal informed permission prior to the collection of specimens.

Study design

58 preeclampsia patients with positive SARS-CoV-2 quick molecular test findings were included in this study, along with 60 healthy pregnant women and healthy non-pregnant women who attended a private clinic in Mosul, Iraq, from March 15 to October 15, 2022, as controls. The mean age was 31.27 years, with the range of ages being 18 to 42. All patients had SARS-CoV-2 symptoms and signs, and they all gave informed permission on their own or the representation of their families. Age, height, and weight were reported. Body mass index (BMI) was established in kilograms per square meter (kg/m^2). Hypertension was determined by taking a blood pressure reading that was >140/90 mmHg or by using an antihypertensive

medication before becoming pregnant. The history of participators has been documented, including vascular complications, nephropathy history, and history and state of retinopathy. Also, diabetes including the length of the disease.

Sampling

Venous fresh blood was taken from each patient and the control group participated in our study. Then, the drawing blood samples were kept in plain tubes allowed to clot and centrifuged for 10 minutes at 3000 rpm for separation of the serum then stored in a freezer at -20 °C to use for evaluation of the immunological tests.

Oral epithelial cell micronucleus assay

The patients underwent tests in the clinic to make sure that the oral tissues were in good health under powerful light and standard settings. According to Alhamadany *et al*. [19], the exfoliated cells from scraping the oral mucosa gather in the following ways: To lessen debris, a simple mouthwash made with distilled water was used. The internal surfaces of the right and left cheeks were then lightly scraped with a wooden spatula that had been dipped in water. Two glass slides were covered with the samples, which were then air-dried at room temperature. After that, slides were fixed with 100% methanol, let dry by air, and then stained with May Granwald-Giemsa stain.

Biochemical analysis

Estimation of the level of hepcidin hormone ferritin, apelin, and galectin-3

In the study, the quantity of hepcidin hormone was estimated using an assay kit created by the Chinese business SUNLONG employing the Sandwich type enzyme-linked immunosorbent assay (ELISA) technology. Also, the ELISA test technique is used for the estimation of ferritin levels [20]. Thermo-Scientific, USA provided an ELISA kit that was used to assess the levels of Gal-3. Apelin levels were determined using an ELISA kit that was obtained from Cloud-Clone Corp, USA.

Estimation of iron, TIBC, UIBC, transferrin, and transferrin saturation percentage

Tietz measured the total iron-related capacity (TIBC), non-iron binding capacity (UIBC), total iron-related capacity saturation [21], total iron-related capacity saturation [21] (Tietz, 1999), transferrin concentration calculation [22], and transferrin saturation percentage [21].

Statistical analysis: Data was collected, processed, and statistically analyzed using SPSS statistical program version 27 to produce cross-tabs and reach pertinent findings. An independent t-test and one-way ANOVA were used to tabulate and assess the variable groups based on the observed results. When the t-test (p) value was 0.05, it was significant, and when it was >0.05, it was non-significant. The effects of continuous variables were expressed using the mean and standard deviation.

Results

Table 1 shows the results of the Micronucleus test (MN) in oral epithelial cells among study groups (Healthy controls, Healthy pregnant, and preeclampsia patients with SARS-COV2). The results show that the highest MN were in preeclampsia patients with SARS-CoV-2 (3.51± 0.471) followed by Healthy pregnant as (2.96± 0.109) compared to healthy controls (0.27±0.071). With BN, the results revealed that BN was higher in preeclampsia patients with SARS-CoV-2 (1.36± 0.094) followed by Healthy pregnant as (0.31± 0.052) compared to healthy controls (0.36±0.049). see table 1. With PN, the results revealed that PN in Healthy pregnant was higher (2.68± 0.273) than in preeclampsia patients with SARS-CoV-2 (1.24± 0.852) compared to healthy controls (0.64±0.119). With KR, the results show that KR was lower in preeclampsia patients with SARS-CoV-2 (2.97±0.591) than Healthy pregnant as (4.71±0.153) compared to healthy controls (4.08±0.973). moreover, the results revealed that KL was higher in preeclampsia patients with SARS-COV2 (4.58± 0.934) while the healthy pregnant was lower (1.62±0.095) than healthy controls (1.82±0.741) (Table 1).

Table 1: compares the study groups' oral epithelial cells using the micronucleus test.

Study groups \ Tests		Total mn	BN	PN	KR	KL	DIF
		%					
Healthy controls	Mean	0.27	0.36	0.64	4.08	1.82	92.83
	Std. Deviation	0.071	0.049	0.119	0.973	0.741	4.683
	NO.	60	60	60	60	60	60
Healthy pregnant	Mean	2.96*	0.31	2.68*	4.71	1.62	87.72
	Std. Deviation	0.109	0.052	0.273	0.153	0.095	4.781

	NO.	60	60	60	60	60	60
preeclampsia patients with SARS-COV2	Mean	3.51*	1.36*	1.24*	2.97	4.58	86.34
	Std. Deviation	0.471	0.094	0.852	0.591	0.934	3.826
	NO.	58	58	58	58	58	58

*** The mean difference (S.E.) is significant at the 0.05 level (t-test); The total micronucleus, or mn, PN stands for pyrknotic nucleus, BN for binucleated, KR for keratorrhexis, KL for kerolytic cell, and DIF for normal differentiated cell.**

Table 2 in the present study shows the levels of Hepcidin, apelin, and galectin-3 among preeclampsia patients with SARS-CoV-2, healthy pregnant, and healthy control groups. The results revealed that the Hepcidin level of the preeclampsia patients with SARS-COV2 (152.82±14.18 ng\ml) significantly ($p < 0.05$) was higher than that of the healthy control group (118.94±12.63 ng\ml). while the healthy pregnant was lower (109.67±10.59 ng\ml) than of the healthy controls. Also, the results show that apelin level was lower in preeclampsia patients with SARS-CoV-2 (0.47±0.16 ng\ml) followed by healthy pregnant(0.52±0.14 ng\ml) when compared to the healthy control group (0.81±0.19ng\ml). with galectin-3, the results revealed that galectin-3 was higher in that preeclampsia patients with SARS-CoV-2 (22.76±3.39ng\ml) than healthy pregnant(20.43±3.17ng\ml) when compared to healthy controls group (14.94±2.06 ng\ml) (table 2).

Table2: Comparison of Hepcidin, Apelin, and galectin-3 levels among study groups

Study groups \ Tests		Hepcidin	Apelin	galectin-3
		ng/ml		
Healthy controls	Mean	118.94	0.81	14.94
	Std. Deviation	12.63	0.19	2.06

	NO.	60	60	60
Healthy pregnant	Mean	109.67*	0.52*	20.43*
	Std. Deviation	10.59	0.14	3.17
	NO.	60	60	60
preeclampsia patients with SARS-COV2	Mean	152.82*	0.47*	22.76*
	Std. Deviation	14.18	0.16	3.39
	NO.	58	58	58

The results of Iron, Ferritin, TIBC, UIBC, Transferrin, and TSAT levels among preeclampsia patients with SARS-CoV-2, healthy pregnant, and healthy controls were summarized in Table 3. The results revealed that Iron μmol/L was lower in the preeclampsia patients with SARS-CoV-2 group (14.86 ± 2.73) than healthy pregnant (18.04±3.96) when compared to healthy controls (19.96±4.07). with Ferritin, the present study shows that ferritin (ng/ml) level was higher in preeclampsia patients with SARS-CoV-2 (37.81±8.09) than healthy controls (18.03±3.01). while the ferritin (ng/ml) level was lower in healthy pregnant (15,05±2,78) than in healthy controls (18.03±3.01). Also, the current study shows that TIBC (μmol/L), UIBC (μmol/L), and Transferrin (μmol/L) levels were significantly higher in that the preeclampsia patients with SARS-CoV-2 group (60.74±4.57, 41.65±5.16, 42.57±4.38) respectively than healthy pregnant (57.83±5.66, 38.71±4.78, 37.57± 5.12) respectively when compared to healthy controls (46.81±4.96, 29.76±3.95, 31.86±4.08) respectively. while the present study shows that TSAT (%) was lower in that the preeclampsia patients with SARS-COV2 group (23.63±4.92) than the healthy pregnant group (28.97±4.59) when compared to the healthy control group (42.79±5.28). See Table 3.

Table 3: The levels of Iron, Ferritin, TIBC, UIBC, Transferrin, and TSAT among study groups

Study groups \ Tests		Iron µmol/L	Ferritin ng/ml	TIBC µmol/L	UIBC µmol/L	Transferrin µmol/L	TSAT %
Healthy controls	Mean	19.96	18.03	46.81	29.76	31.86	42.79
	Std. Deviation	4.07	3.01	4.96	3.95	4.08	5.28
	NO.	60	60	60	60	60	60
Healthy pregnant	Mean	18.04*	15,05*	57.83*	38.71*	37.57*	28.97*
	Std. Deviation	3.96	2,78	5.66	4.78	5.12	4.59
	NO.	60	60	60	60	60	60
preeclampsia patients with SARS-CoV-2	Mean	14.86*	37.81*	60.74*	41.65*	42.57*	23.63*
	Std. Deviation	2.73	8.09	4.57	5.16	4.38	4.92
	NO.	58	58	58	58	58	58

*: mean significant at $p \leq 0.05$

The results presented in Table 4 show a positive correlation between transferrin TIBC and UIBC in the healthy pregnant group and preeclampsia patients with the SARS-COV2 group. The values of the correlation coefficient of TIBC and UIBC in healthy pregnant group and preeclampsia patients with SARS-COV2 group, respectively, were (r = 0.938), (r = 0.983), (r = 0.968), and (r = 0.952) respectively, while preeclampsia patients with SARS-COV2 group and healthy pregnant group did not correlate positively with transferrin and TSAT%, with r values of (-0.672, -0.639) respectively (Table 4).

Table 4: correlation of transferrin with TIBC and UIBC among study groups

Tests	Transferrin	
	Healthy pregnant	preeclampsia patients with SARS-COV2
TIBC	0.983	0.968
UIBC	0.965	0.952
TSAT %	-0.672	-0.639

Discussion

In the current study, fifty-eight preeclamptic patients were included to evaluate the genotoxicity and biochemical parameter alterations in these patients to those of healthy pregnant and control subjects. Preeclampsia risk may be correlated with SARS-CoV-2 infection during pregnancy, according to research that has emerged during the COVID-19 pandemic. While some systematic reviews [23] discovered an increased risk when combining data from several cohorts, other research has shown that COVID-19 infection during pregnancy does not raise the risk of preeclampsia [24].

Although it is not yet clear if one causes the other, preeclampsia is more frequently linked to severe COVID-19 [25]. Increased levels of pro-inflammatory cytokines in the bloodstream and endothelial dysfunction are characteristics of both preeclampsia and COVID-19, indicating potential shared pathways [25]. This discovery merits additional exploration since it appears to be related to the activation of many of the same biological pathways, including endothelial dysfunction and angiogenesis, and there may be a dose-response connection. According to research by Dap and Morel [26], preeclampsia a common pregnancy condition marked by

hypertension and proteinuria linked to placental dysfunction may have been present in this case. Liu *et al*. [27] referred to the finding that proteinuria is more prevalent in non-pregnant COVID-19 patients compared to healthy controls (28.57 % Vs 11.11%; $p < 0.05$). Proteinuria does appear to correlate with Covid-19 severity [27]. Di Mascio et al. found that 16.2% of all their coronavirus-affected pregnancies resulted in preeclampsia [28] which is much more common than the 2-8% seen in the general population [26]. The first hypothesis that can be suggested is that proteinuria is indeed associated with infection rather than being a false positive for the diagnosis of preeclampsia as proposed by Mendoza et al. [29]. The second theory that could be considered is if the infection is causing greater placental compromise due to intravascular inflammation that may create a prothrombotic state both in the blood and in the placenta. Shanes et al. reported that placentas from SARS-CoV-2-positive women had an increased prevalence of maternal vascular malperfusion characteristics [30]. We are not aware of any studies that evaluate apelin levels in pregnant women with COVID-19, but Van Mieghem et al. reported that preeclamptic women had decreased apelin levels compared to prior studies [31].

Molvarec et al. [32] reported higher plasma apelin levels and lower placental apelin levels in women with preeclampsia in comparison to control women. Apelindecrease is primarily induced by proteinuria, according to a study by Al-Hakeim and Ali [33], who found that apelin had decreased as a result of increased proteinuria in preeclamptic patients. However, proteinuria has more implications for PE patients. Consequently, PE patients have more proteinuria and therefore aggravated protein loss compared to the normal group. The high level of apelin expression in preeclampsia placentas indicates that apelin might be involved in the development of preeclampsia

and that it likely inhibits endothelial repair in early placental invasion [34]. Suzuki *et al*. [35] demonstrated that high apelin could protect the heart from obesity-related myocardial dysfunction; thus, a decrease in apelin could be an unfavorable sign for cardiovascular health. In this study, Gal-3 levels in studies using a C57BL/6 mouse model to evaluate Galectin-3's role in the development of preeclampsia, Gal-3 immunostaining can be used to assess the potential function of Gal-3 in preeclampsia. between patients with PE and those in the control group showed a higher level of Galectin-3 compared to the control group. Studies have suggested that galectins expressed on the maternal-fetal interface may play vital roles in the crosstalk between mother and fetus. Therefore, studies of galectins could help predict, prevent, diagnose, and treat gestational disturbances [34].

Another study is necessary to establish if the immunological basis for higher levels of galectin-3 in the preeclampsia group than in the control group. Also, research by Venkatraman et al. shows that it might be a prognostic marker and therapeutic target for cardiovascular diseases (CVD) [36]. For example, Pang et al. correlated galectin-3 with inflammation and obesity among females [37]. In contrast, this study used samples from pregnant women with PE only. Riise et al.'s other important study indicated that preeclampsia was associated with a two-fold increased risk of CHD as well as a fourfold increment in subsequent incidence of HF [38].

Conclusion

According to the evidence produced by the researchers, we could conclude that the majority of preeclampsia patients have no children. The

COVID-19 virus is related to preeclampsia. My findings and other research found that apelin, hepcidin, as well as Gal-3, are linked to preeclampsia. In patients with COVID-19 infection suffering from preeclampsia, a study showed increased Hepcidin and Galectin-3 and reduced Apelin levels in their blood. Such severe cases had it worse.

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