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Investigation of serum enzyme activities and oxidative stress markers in preeclampsia: a multiparameter analysis

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ABSTRACT

Objectives: Preeclampsia, a high cause of fetomaternal morbidity-mortality, remains a significant burden affecting 8% of all pregnancies. Environmental conditions induce disease development leading to endothelial dysfunction in genetically predisposed women. Our aim is to discuss oxidative stress as a well-established contributing factor to disease progression with being the first study to show new evidence about serum dehydrogenase enzyme levels (isocitrate, malate, glutamate dehydrogenase) with oxidative markers (myeloperoxidase, total antioxidant-oxidant status, oxidative stress index).

Material and Methods: Serum parameters were analyzed with photometric method (Abbott ARCHITECT c8000).

Results: The enzyme levels and oxidative markers were significantly higher in patients, supporting the redox imbalance in preeclampsia. According to ROC analysis, malate dehydrogenase showed an outstanding diagnostic ability with the highest AUC value of 0.9 and the cut-off value of 51.2 IU/L. Discriminant analysis including malate, isocitrate and glutamate dehydrogenase had predicted preeclampsia with an overall 87.9% accuracy.

Conclusions: Considering the above results, we propose that the enzyme levels increase with oxidative stress functioning as antioxidant defense factors. The unique finding of the study is that the serum levels of malate, isocitrate and glutamate dehydrogenase can be used both separately and combined in the early prediction of preeclampsia. As a novel approach, we also offer combining serum isocitrate and glutamate dehydrogenase levels with ALT, AST tests to state liver functions more reliably in patients. Still, larger sample-sized studies investigating enzyme expression levels are required to confirm the recent findings and to reveal underlying mechanisms.

Key words: isocitrate dehydrogenase; malate dehydrogenase; glutamate dehydrogenase; oxidative stress; preeclampsia

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INTRODUCTION

Preeclampsia (PE) is a common cause of fetomaternal morbidity-mortality and seen approximately 8% of all pregnancies worldwide. It is characterized with a first episode hypertension with proteinuria or with end-organ dysfunction after the 20th gestational week. PE development often requires multifactorial inputs and several predisposing factors have been described as placental-multiorgan ischemia, generalized vasoconstriction, microembolism, endothelial cell dysfunction, systemic inflammatory response syndrome and oxidative stress (OS) [1]. Our current study discusses OS as a well-established contributing factor in the development of PE and contributes to the literature by showing new evidence in support of this premise. The study brings novelty to the field by investigating two important Krebs cycle enzymes (isocitrate dehydrogenase, malate dehydrogenase) and glutamate dehydrogenase serum activity levels in PE patients with serum OS markers (myeloperoxidase, total antioxidant-oxidant status, oxidative stress index) and searching for a possible correlation between these parameters.

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Krebs cycle (Citric acid cycle) is the second stage of aerobic respiration and involves crucial reactions for production of macromolecules. Cycle enzyme mutations disrupt the normal respirational flow and lead to the cumulation of intermediate products. Both genetic alterations and environmental confounding factors may cause enzyme dysfunction and finally to various clinical outcomes such as cancer. Some of these enzymes have their cytosolic isoforms to function as a bridge between mitochondria and cytosol and so to maintain cardinal metabolite reserves. Studies showed that pathologies such as ischemia-reperfusion injury, hypertension and oxidative stress changes the levels of these enzymes [2]. Except from genetic polymorphisms, Krebs cycle enzymes are also found to be sensitive to the disruptive effects of reactive oxygen species and they may decline in oxidative stress-related diseases such as PE [3, 4].

One of the cycle enzymes, isocitrate dehydrogenase (IDH, EC:1.1.1.42), converts isocitrate to alpha-ketoglutarate (α-KG) while producing NAD(P)H from $NAD(P)^+$ and has three isoforms as IDH 1 (cytosolic, $NADP^+$-dependent), IDH 2 (mitochondrial, $NADP^+$-dependent), IDH 3 (mitochondrial, $NADH^+$ dependent). Due to the production of NADPH, cytosolic IDH has been described as an antioxidant enzyme for preventing cell environment from oxidative damage. With neomorphic activity, mutant IDH 1 and 2 catalyzes the conversion of α-KG to tumorigenic 2-hydroxyglutarate (2-HG) that has been shown to be responsible from various cancer types [5, 6].

Malate dehydrogenase (MDH, EC: 1.1.1.37) is the last enzyme of Krebs cycle converting malate to oxaloacetate in the presence of NAD^+ and has two isoforms as cytosolic and mitochondrial. Previous works reported that cytosolic MDH is regulated by redox state and is affected by increased OS [7].

Glutamate dehydrogenase (GDH, EC 1.4.1.2), previously known as a mitochondrial indicator, is an essential enzyme linking the catabolic-anabolic pathways. Its junction with the citric acid cycle is that both GDH and cycle enzyme alpha-ketoglutarate dehydrogenase use α-KG as a substrate, thus GDH can be regarded as a metabolic checkpoint of the cycle. GDH has its maximal specific activity in the liver tissue and some reports suggest the use of GDH serum levels as a reliable biomarker for liver cell necrosis [8].

Since certain part of patients display liver damage, defining serum GDH levels may have a value in the prediction or monitoring of PE. Myeloperoxidase (MPO), the marker of myeloid cells, is a heme-containing peroxidase that functions as microbicidal protein by producing hypochlorous acid during inflammatory active state. MPO has an important role in bridging the inflammatory processes to OS [9]. Therefore, both leucocyte and plasma levels of MPO is widely used to reflect oxidative status in a variety of clinical entities, including PE [10].

Total antioxidant status (TAS) and total oxidant status (TOS) are novel markers may be considered as the representative of the plasma total antioxidant defense capacity and OS state, respectively. There are several studies evaluating TAS and TOS levels with or without other biomarkers in PE [11]. While TOS levels are mostly found elevated in patient serums, TAS results are differed widely.

Objectives

The aim of this study is assessing IDH, MDH, GDH serum levels and their associations with oxidative parameters in PE patients and searching their potential use as a prediction tool. Testing the hypothesis of altered enzyme activities may be in relationship with oxidative markers, we evaluate MPO, TAS, TOS, OSI to assess oxidative status of participants and to look over any possible correlations with the enzyme levels. Current findings will be discussed in the context of OS presence in the pathogenesis of PE.

MATERIAL AND METHODS

Analysis with 80% power level revealed that a minimum number of 42 individuals for each group (total number of 84) should be included in the study (effect size of 0.62; p value of 0.05). From September to December 2020, the study included 43 preeclamptic women as the patient group and 45 healthy normotensive pregnant women as the control group. Enzyme activities were measured in all participants that were in their third trimester pregnancy (from week 28 to week 40). The study was conducted in compliance with the Declaration of Helsinki and was approved by the ethics committee of Bezmialem Vakıf University (27 October 2020). A written consent was obtained from each volunteer. Preeclampsia diagnosis has been made in reference to the criteria of American College of Obstetricians and Gynecologists ACOG Practice Bulletin [8].

Inclusion criteria

Preeclampsia diagnosis was confirmed if patients were normotensive before gestation and had ≥ 140/90 mmHg systolic blood pressure (more than one measurement with 6 hours interval) and proteinuria (≥ 300 mg in 24-hours urine) after 20^{th} gestational week. All participants were 18–45 years of age pregnant women in their third trimester pregnancy.

Exclusion criteria

Individuals with chronic diseases (including cardiovascular-renal diseases), diabetes, obesity, malignancies, genetic diseases, recent history of surgery or transfusion, history of fetal anomalies and fetal death in utero, active infection and regular drug/ alcohol/ cigarette usage were excluded from the study.

Methods

Samples were collected from volunteers in obstetrics and gynecology clinic of Istanbul Education Research Hospital and then analyzed in Bezmialem Vakıf University Biochemistry laboratory.

Serum IDH enzyme activity levels were assessed with commercially available Rel Assay $NADP^+$ -Dependent Isocitrate Dehydrogenase measurement kit (RL 0949, Gaziantep, Turkey) via photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer). The principle of the method depends on the reaction in which $NADP^+$ molecule is converted to its reduced form, $NADPH + H^+$, by the IDH enzyme in the presence of its substrate isocitrate. Increasing concentration of $NADPH + H^+$ over time generates absorbance change at 340 nm that is directly related to the enzymatic activity. The IDH enzyme activity then can be calculated by using the molar absorption coefficient of $NADPH + H^+$ ($6.3 \times 10^3\ M^{-1}\ cm^{-1}$).

Serum MDH enzyme activity levels were assessed with commercially available Rel Assay Malate Dehydrogenase measurement kit (RL 0925, Gaziantep, Turkey) via photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer). Principle of the method depends to the reaction in which $NADH + H^+$ molecule is converted to its oxidized form, NAD^+, by the MDH enzyme in the presence of its substrate oxaloacetate. Decreasing concentration of $NADH + H^+$ over time generates absorbance change at 340 nm that is directly related to the enzymatic activity. The MDH enzyme activity then can be calculated by using the molar absorption coefficient of $NADH + H^+$ ($6.3 \times 10^3\ M^{-1}\ cm^{-1}$).

Serum GDH enzyme activity levels were assessed with commercially available Rel Assay Glutamate Dehydrogenase measurement kit (RL 0932, Gaziantep, Turkey) via photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer). Principle of the method depends to the reaction in which $NADH + H^+$ molecule is converted to its oxidized form, NAD^+, by the GDH enzyme in the presence of its substrate alpha-ketoglutarate. Decreasing concentration of $NADH + H^+$ over time generates absorbance change at 340 nm that is directly proportional to the enzymatic activity (Abbott Architect c8000). The GDH enzyme activity then can be calculated by using the molar absorption coefficient of $NADH + H^+$ ($6.3 \times 10^3\ M^{-1}\ cm^{-1}$).

Serum TAS level is a fully automatic colorimetric method developed by Erel [12]. The principle of the method is based on measuring the amount of OH radical. The ferrous ion of o-diasidine with H_2O_2 reacts to the Fenton-type reaction to produce the OH radical, and color change occurs due to o-diasidine. Serum antioxidants neutralize oxidants and prevent color change. This method determines the antioxidant capacity against oxidative free radical reactions initiated by OH.

Serum TOS level was measured using a fully automatic photometric method developed by Erel [13]. The principle of the method, serum oxidants oxidize the ferrous ions of o-dianisidine into ferric ions. Ferric ions formed in an acidic environment with this oxidation take a visible color with xylenol orange. The resulting color density is correlated with the level of oxidants in the serum.

Serum OSI value is calculated by dividing the TOS value by the TAS value. OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/ /10 × TAS (mmol Trolox Eq/L).

The activity of MPO was determined by the method of Bradley et al. [14], based on the kinetic measurement of the formation rate belonging to the colorful product of the oxidation of o-dianisidine with MPO in the presence of H_2O_2, at 460 nm. MPO activity was expressed as units per liter of serum (IU/L).

Statistical analysis

The G Power software was used to calculate the sample size of the study. Shapiro wilk test was applied for the assessment of data distributions and data was found normally distributed ($p \geq 0.05$). MANOVA test was then assessed to match the two groups. Group differences were defined using independent sample t-test. Results have been shown as the mean ± standard deviation (SD) and p-value less than 0.05 was accepted statistically significant. Correlation analysis was carried out for each group. Receiver operating characteristic curve (ROC) analysis was performed to define the discrimination ability of the investigated enzymes for PE. Discriminant analysis was carried out to search the predictor effect of the enzymes (MDH, IDH, GDH). Analyzes were done with IBM SPSS version 27.0 software (IBM Corporation, Armonk, NY, USA).

RESULTS

Table 1 shows the demographical data and the mean values of clinical parameters. Age, gestational age (weeks) and body mass index (BMI) values were not significantly different between the groups ($p > 0.05$). Aspartate transaminase (AST) and alanine aminotransferase (ALT) mean values were proximate to the upper limit of the normal reference range in the PE patients with the values of 18.9 and 16.8 units per liter (IU/L) respectively (Tab. 1). ALT and AST levels were found significantly different in the groups ($p < 0.05$). MANOVA analysis evaluating the three enzymes (MDH, GDH, IDH) was performed and the group multivariate significance value indicated that enzyme levels can be interpreted as significant in both groups (Tab. 2, $p = 0.000$). Effect size calculations revealed that partial eta-squared values (η^2) were bigger than 0.14 for the enzymes ($\eta^2 = 0.48$ for MDH, 0.39 for GDH and 0.32 for IDH), referring to large effect size for the group mean differences (Tab. 2).

Table 1. Demographical data

Parameter	Control n = 45	Preeclampsia n = 43	p-value
Age [year]	27.2 ± 5.3	30.2 ± 6.8	ns
BMI [kg/m^2]	29.8 ± 3.9	29.5 ± 5.5	ns
Gestational week	35.8 ± 1.9	35.3 ± 3.3	ns
IDH [IU/L]	14.9 ± 2.1	17.9 ± 2.1	0.001***
MDH [IU/L]	46.6 ± 4.9	57.9 ± 6.7	0.001***
GDH [IU/L]	15.6 ± 3.3	21.1 ± 3.6	0.001***
MPO [IU/L]	26.9 ± 5.8	32.4 ± 6.2	0.001***
TAS [mmol Trolox Eq/L]	1.4 ± 0.2	1.8 ± 0.3	0.001***
TOS [μmol H_2O_2 Eq/L]	16.1 ± 1.5	16.9 ± 1.1	0.001***
OSI [arbitrary unit]	1.1 ± 0.2	0.9 ± 0.1	ns
ALT [IU/L]	12.9 ± 4.2	16.8 ± 7.4	0.011*
AST [IU/L]	11.9 ± 5.1	18.9 ± 10.9	0.001***

Values shown as Mean ± Standard Deviation; ns — not significant; BMI — body mass index; MDH — malate dehydrogenase; GDH — glutamate dehydrogenase; IDH — isocitrate dehydrogenase; MPO — myeloperoxidase; TAS – total antioxidant status; TOS — total oxidant status; OSI – OS index, ALT — aminotransferase; AST — aspartate transaminase; *0.01 < p 0.05; **0.001 < p < 0.01; ***p < 0.001

Table 2. MANOVA analysis showed the group differences of enzyme levels at the significance level of p < 0.001 and with large effect sizes (η^2 (partial eta-squared value) > 0.14 indicates large effect size)

Parameters	Sum of squares	F	sig.	η2 (partial)
MDH [IU/L]	2598.595	73.403	0.000	0.482
GDH [IU/L]	602.847	49.527	0.000	0.385
IDH [IU/L]	168.381	37.169	0.000	0.320

MDH — malate dehydrogenase; GDH — glutamate dehydrogenase; IDH — isocitrate dehydrogenase

Significantly higher activity levels of all enzymes were recorded in the patient group compared to controls (Fig. 1). The mean values of activities in the patient group were 17.9 IU/L for IDH, 57.9 IU/L for MDH and 21.1 IU/L for GDH (Tab. 1). The control group had significantly lower levels of IDH as 14.9 IU/L, MDH as 46.6 IU/L and GDH as 15.6 IU/L (Tab. 1). The correlation analysis revealed positive relationship between all three enzymes and MPO in patients as r = 0.48, p = 0.006 for IDH: r = 0.52, p = 0.001 for MPO with MDH and r = 0.36, p = 0.022 for MPO with GDH. MPO was also positively correlated with MDH (r = 0.59, p = 0.000) and GDH (r = 0.39, p = 0.027) in the control group. Between-enzyme results of correlation analysis showed MDH activity was in relationship with IDH (r = 0.76, p = 0.000) and GDH (r = 0.32, p = 0.044) in the patient group while only with GDH (r = 0.4, p = 0.02) in controls. Other significant correlation finding of the patient group was MDH with TAS levels (r = 0.38, p = 0.016) and of the control group was GDH with TOS levels (r = 0.42, p = 0.015).

Receiver operating characteristic analysis results have been shown in Figure 2 and Table 3. Among the three enzymes, MDH had the highest AUC value of 0.9, which is accepted as an outstanding discrimination factor for PE and the cut-off value was recorded as 51.2 IU/L (with 84% sensitivity and 92% specificity). IDH and GDH enzymes followed this with the AUC values of 0.84 and 0.86 and with the cut-off values of 16.6 and 19.9 IU/L, respectively, being as excellent discrimination parameters for PE (78% sensitivity and 81% specificity for IDH and 62% sensitivity and 92% specificity for GDH). Oxidative marker MPO had an acceptable yet not strong AUC value of 0.74. Next, the canonical liver function markers ALT and AST were analyzed to compare their AUC numbers with the investigated enzymes. The AUC values were 0.66 and 0.71 respectively, showing only AST had an acceptable diagnostic ability for PE (for AUC values, Tab. 3).

Discriminant analysis including the three enzymes (MDH, GDH, IDH) showed that the enzyme combination correctly distinguished PE patients from controls with 87.9% accuracy (with 86.1% sensitivity and 91.1% specificity, Tab. 4).

The MPO levels were investigated to assess oxidative status of individuals enrolled in the study. Significantly higher levels were recorded in patients with the mean value of 55.4 IU/L (Tab. 1, Fig. 1). The control group had the mean val-

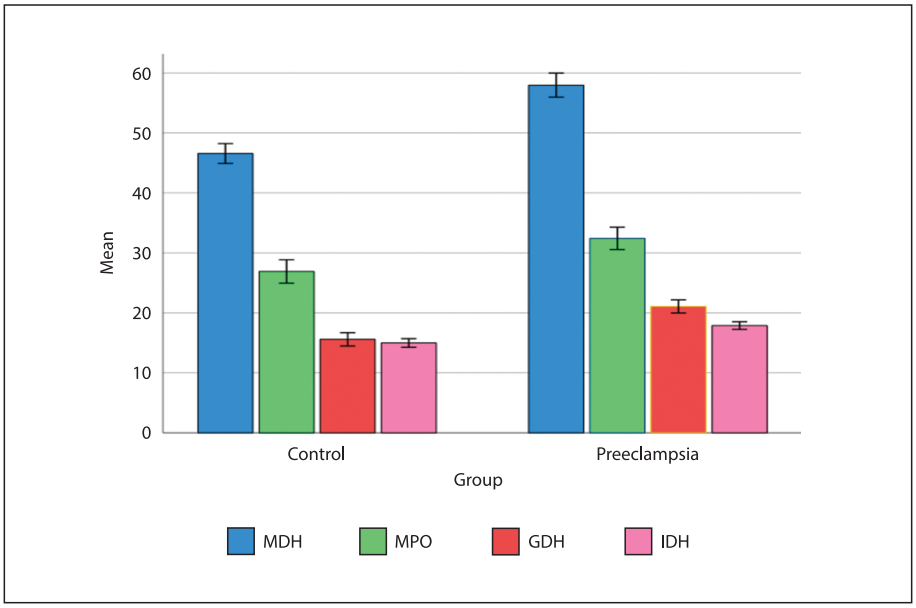

Figure 1. The figure shows the two different group levels of IDH, MDH, GDH, MPO enzymes. All parameters had significantly higher mean values in the preeclampsia group ($p < 0.05$); MDH — malate dehydrogenase; MPO — myeloperoxidase; GDH — glutamate dehydrogenase; IDH — isocitrate dehydrogenase

Table 3. Area under the curve values of malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH) enzymes and alanine aminotransferase (ALT), aspartate transaminase (AST) parameters

Area under the curve ??	
Test Result Variable(s)	**Area**
MDH	0.90
GDH	0.86
IDH	0.84
ALT	0.66
AST	0.71

ue of MPO as 24.7 IU/L (Tab. 1). MPO levels were found correlated with all three enzymes in two groups except with IDH activity in controls. Other oxidative markers, TAS ($r = 0.52$, $p = 0.001$ for patients and $r = 0.45$, $p = 0.009$ for controls) and TOS ($r = 0.47$, $p = 0.002$ for patients and $r = 0.46$, $p = 0.007$ for controls) were also found correlated with the MPO levels.

The patient group had significantly higher levels of both TAS and TOS levels with the mean values of 1.78 mmol/L and 7.9 µmol/L respectively ($p < 0.05$, Tab. 1). The control group had significantly lower levels with the mean values of 1.44 mmol/L for TAS and 5,9 µmol/L for TOS (Tab. 1). OSI was not statistically different between the groups and was found decreased in patients ($p > 0.05$, Tab. 1). TAS was also in relationship with MDH levels in patients while TOS was with GDH in controls ($r = 0.38$, $p = 0.016$ and $r = 0.42$, $p = 0.015$, respectively).

DISCUSSION

As current results showed, IDH, MDH, GDH activities were found significantly higher in the patient group and the enzyme activities were positively correlated with MPO levels in both groups (except the IDH with MPO in controls). Correlation analysis of MDH revealed a relationship with IDH and GDH in patients while only with GDH in controls. ROC analysis resulted with the highest AUC value for MDH which followed by GDH and IDH. Among the traditional liver function tests, only AST displayed an acceptable AUC value for predicting PE. Discriminant analysis combining the three enzymes (MDH, GDH, IDH) showed 87.9% accuracy for segregating PE patients from controls. Oxidative stress markers MPO, TAS, TOS levels displayed significantly higher levels in patients while OSI was not found statistically different between the groups.

Serum measurements of IDH indicated that the cytoplasmic IDH 1 originating from liver tissue has been found in elevated levels may be accepted as a sensitive biomarker of parenchymal liver disease [15]. Current finding of higher IDH levels in PE group supports this premise since liver functions may be affected in PE if serious complications, such as HELLP syndrome, occurs. Except, IDH is a member of antioxidant defense system providing $NADP^+$ to cell environment and so enhanced OS may induce the expression of IDH as an antioxidant enzyme. In support of this, a study displayed increased activity and expression of $NADP^+$-isocitrate dehydrogenase to favor NADPH production and limit NADH synthesis in the presence of OS

Table 4. Results display that when malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH) enzymes are included, the combination had 86.1% sensitivity and 91.1% specificity with total accuracy of 87.9% for estimating preeclampsia (PE)

Classification Results a			
Group	**Preeclampsia**	**Control**	**Total**
Preeclampsia	37	6	43
Control	4	41	45
Preeclampsia	86.1	13.9	100
Control	8.9	91.1	100
87.9% of original grouped cases correctly classified			

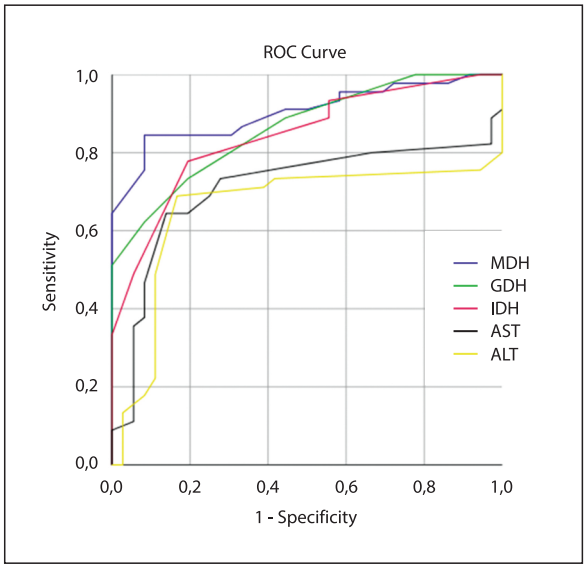

Figure 2. Demonstration of the Receiver operating characteristic (ROC) curve lines of investigated parameters; MDH — malate dehydrogenase; GDH — glutamate dehydrogenase; IDH — isocitrate dehydrogenase; AST — aspartate transaminase; ALT — alanine aminotransferase

[16]. IDH also provides substrate to the enzyme complex named alpha-ketoglutarate-dependent dioxygenases. The metabolic need of IDH for the enzyme family may have promoted the enzyme levels as well. Newly introduced by current study, IDH serum levels proved to be an excellent early diagnostic tool for PE having distinguished patients with higher degree than ALT and AST parameters. Thus, investigating serum IDH levels may have a great potential in the use of PE prediction and monitoring liver function of patients. Our results should be confirmed with prospective works explaining the suggested mechanisms responsible from elevated serum levels.

Malate dehydrogenase in eucaryotic cells has two isoforms (cytosolic and mitochondrial) and converts malate to oxaloacetate, an important intermediate functioning both in Krebs cycle and gluconeogenesis. Malate dehydrogenase replenishes the oxaloacetate reserve and ensures the energy production by oxidative phosphorylation in mitochondria. Cytosolic MDH 1 otherwise functions in the malate-aspartate shuttle to exchange reducing equivalents and to transform malate into oxaloacetate so that malate can be used on the other cellular processes. Malate dehydrogenase 1 has been found amplified and associated with poor diagnosis in human tumors, providing NAD equivalent alternatively to lactate dehydrogenase when advanced utilization of glucose raised the need of NAD regeneration [17]. In addition, few studies searched the association of OS with MDH activity demonstrated that increased OS upregulated the MDH 2 activity (mitochondrial form) via miR-743a and oxidant therapy reduced the enzyme activity [18]. Besides, the product of malate dehydrogenase, oxaloacetate, has been shown to be a crucial neuroprotective antioxidant molecule and a scavenger for removing H_2O_2 from cell culture media [19]. Consistent with these findings, we assume that our elevated serum activities of MDH in patients may be the result of an increased expression levels to balance enhanced OS and perpetuate antioxidant reserve by providing oxaloacetate. Since serum levels originates mainly from cytosolic MDH 1, our finding may also refer to the cell damage that led to leakage of MDH 1 from cytosol to serum, possibly from the dysfunctional endothelial tissue. Apart from the elevated serum levels, the most valuable finding upon MDH was that the highest AUC value which is considered as an outstanding discrimination factor in disease prediction. Presenting the cut-off value for MDH serum levels, we assume that the enzyme levels may be used in the early diagnosis and provide a new target in the PE research. Results are needed to be replicated within larger population and confirmed with further research specially looking for the tissue source of elevated serum MDH.

Glutamate dehydrogenase proved to be a substantial enzyme due to its antioxidant functions such as producing a precursor to glutathione and metabolizing the antioxidant key player alpha-ketoglutarate. Alpha-ketoglutarate dehydrogenase and glutamate dehydrogenase enzymes have been found to be working synchronically to regulate α-ketoglutarate levels under OS [20]. Redox imbalance

leads to promoted production of $NADP^+$ instead of $NADH^+$ (due to inhibition of alpha-ketoglutarate dehydrogenase activity) through activated GDH [21]. GDH was shown to enable cells to remain intact during metabolic stress (when the mitochondrial pyruvate carrier protein was inhibited) maintaining pyruvate levels with the aid of malic enzyme [22]. According to former works, GDH serum level is implied to show the liver tissue damage [8] and considering the localization of the enzyme, it shows the mitochondrial dysfunction in particular [23]. With these, we hypothesize that our finding of elevated serum GDH activities in patients may stem from the increased expression of GDH in mitochondria (as a compensatory factor to fight against OS) and its escape into cytoplasm where it is then released into the serum via cell damage. Like IDH, GDH levels displayed excellent AUC values as well, implying that the serum enzyme levels can predict patients before the onset of PE with high accuracy and carry high potential for further studies.

The traditionally accepted markers of liver function, ALT and AST, have been found significantly higher in patients with still being in the normal reference range. The normal range ALT and AST values in certain patients may obscure the hidden liver damage and we assume that combining GDH serum levels with these function tests may form a credible way to show liver function.

Myeloperoxidase is a powerful microbicidal found mainly in neutrophils to conduct inflammatory response. Its catalytic activity results with increased reactive oxygen radicals that react with biomolecules, leading finally to cell damage. It also promotes OS by oxidizing low-density lipoproteins and consuming endothelial nitric oxide (NO). Therefore, elevated serum levels of MPO may have a value as an indicator for diseases that display OS related pathogenesis. Considering PE as one of that diseases, MPO levels are expected to be higher in patients. Consistent with the previous works, MPO levels have been found significantly higher than controls in our study [24]. Supporting our finding and that MPO plays a role in promoting the local OS, MPO expression in placental tissue was found significantly higher in PE patients than healthy pregnant women [25]. However, conflicted results have also been reported for the serum levels. MPO levels were not different in PE patients compared to controls [26]. Another recent study that had three groups as mild, severe PE patients and controls also have not found any difference in plasma MPO levels [27]. Our study showed that the MPO levels in patients have been found correlated with IDH, GDH and MDH. This indicates that OS may alter the mitochondrial function and impair the Krebs cycle metabolism considering MPO as a potent indicator of OS. The control group also had correlated MPO levels with MDH and GDH, but the mean values were significantly lower compared to patients. MPO levels had shown correlation with TAS, TOS levels not only in patients but in healthy group as well. This result may refer to that serum MPO level is a sensitive signal for tracking the metabolic changes. Though, to acknowledge that MPO can be used as a biomarker in PE, randomized studies with larger sample sizes are still needed.

Several studies that investigated TAS, TOS levels differed widely in their group population (severe-mild or early onset-late onset PE patients) but most of them reported decreased serum levels of TAS and increased levels of TOS [28]. A study showed decreased levels of TAS in the placental tissue while increased levels were found in plasma [29]. This may imply the presence of an intact systemic response to OS but locally disrupted response in the placenta. Elevated levels of TAS and TOS have been also introduced by few studies [30]. Other findings of one of these studies were that TAS levels were significantly correlated with the adverse perinatal outcomes and as ROC analysis showed, TAS may have a value for the prediction of PE [30]. Our work showed elevated levels of both TAS and TOS and is consistent with the idea that serum TAS levels are increased as well as oxidative markers in PE patients as an antioxidant defense response. TAS levels were correlated with two enzyme activities, MDH and GDH, and with MPO levels in PE patients. Correlation analysis of control group revealed that only MPO levels were in relationship with TAS levels. TOS levels on the other hand were found correlated with MPO in both patient and control group. The correlation profile of the patient group (correlation of TAS, TOS levels with three enzymes and MPO) may have been attributed to changed redox balance considering that the enzymes and MPO are the markers of OS. Another marker, OSI, has been found not significantly different in the two groups with a decreased value in patients which is contradictory to the previous reports. The finding may be resulted from the increased serum TAS levels in patients. Contradictory results discussed here point to the need of further investigation of especially the serum TAS levels in PE patients.

Liver function tests (ALT and AST) have been widely investigated in PE to be used as an early diagnostic tool. A variety of studies revealed that ALT and AST levels may predict PE before the onset and that the enzyme levels may have a value for distinction of the severity of the disease [31]. Although the mean values of ALT and AST were in the normal range, our finding of significantly higher levels in patients compared to controls coincides with the most previous works [31]. With these findings, we support the premise that ALT, AST tests are valuable being one of the serum indicants of PE. We also offer combining serum IDH and GDH activity levels with the traditional ALT, AST parameters to state liver functions more precisely in PE patients.

CONCLUSIONS

Serum IDH, MDH and GDH levels have been investigated for the first time in PE and were found to be higher in patients compared to controls. Enzymes were also positively correlated with the serum myeloperoxidase (MPO) levels. MPO levels were significantly higher in patients reflecting redox imbalance in favor of oxidative processes. OS markers total oxidant-antioxidant status (TAS and TOS) and OS index (OSI) were also searched. TOS levels were higher in the patient group as expected while the TAS levels were higher in patients as contradictory some of the previous reports. Taken together, our current study was first to show higher serum dehydrogenase enzyme activities (IDH, MDH, GDH) and their relationships with the serum oxidative markers in preeclampsia patients. According to our findings, we suggest that the IDH, MDH, GDH enzyme activities may advance with the increased OS as an antioxidant response. Above all, we introduce a novel parameter for the early prediction of PE: the combination of MDH, GDH, IDH was able to segregate patients from controls with 87.9% accuracy. We also assume that combining IDH and GDH serum levels with traditional liver function tests (ALT, AST) may form more precise platform to show liver damage and in particular, mitochondrial dysfunction in PE. Still, further research investigating both expression levels of the enzymes and their metabolites is required to confirm our recent findings.

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Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the clinical research ethics committee of Bezmialem Vakıf University (No. 54022451-050.05.04).

Data availability

The datasets are available from the corresponding author upon request.

Conflict of interest

All authors declare no conflict of interest

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