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Neuron Specific Enolase in Relation to Chitotriosidase and Heat Shock Protein 72: A Network of Integrated Predictive Biomarkers in Preeclampsia and Eclampsia

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Abstract

Background: Preeclampsia and eclampsia are life-threatening conditions with increasing incidence. Since, Neuron specific enolase (NSE) is a vital biomarker for neuronal damage, our study aimed to investigate its role in relation to macrophage activation, angiogenesis, heat shock protein 72 and antioxidant biomarkers in preeclampsia and eclampsia patients.

Methods: This study included 45 pregnant women; divided into 3 groups. Group I included 15 healthy pregnant women (control group), Group II included 15 preeclampsia patients and Group III included 15 eclampsia patients. They were subjected to measurement of plasma of NSE, insulin growth factor-1 (IGF-1), nitric oxide (NO), heat shock protein 72 (HSP72) levels and chitotriosidase activity in addition to serum paraoxonase activity.

Results: The study showed significant increased levels of NSE, IGF-1, HSP72, and chitotriosidase activity in group II and III when compared to control group with the highest levels in group III. On the other hand, it showed significant decrease in NO level and paraoxonase activity in group II and III when compared to control group with lowest level in group III.

Conclusion: Based on our findings, NSE can be used as a reliable biomarker to predict the outcome of preeclampsia and early prediction of eclapmsia to avoid serious complications.

Keywords: Neuron specific enolase; Chitotriosidase; Heat shock protein 72; Insulin growth factor-1; Preeclampsia

Introduction

Preeclampsia is a common diagnosis in developed countries and remains a high cause of maternal and fetal morbidity and mortality in the developing world [1]. The conventional risk factors for preeclampsia include nulliparity, obesity, diabetes, hypertension, multi-fetal gestations and history of preeclampsia [2]. Neonatal complications associated with preeclampsia include intrauterine growth restriction, low birth weight and perinatal death [3].

Moreover, eclampsia can occur without prior history of proteinuria or hypertension and may be the first manifestation of the disease. Eclampsia increases the prevalence of perinatal mortality and/or morbidity [4]. The pathogenesis behind preeclampsia and eclampsia is not completely understood but theories are based on cerebral vasoconstriction or edema predominately in the parieto-occipital regions in the brain [5].

Neuron specific enolase (NSE) is a specific biomarker for neurons and peripheral neuroendocrine cells as it has been proved that NSE provides quantitative measures for brain damage and it can improve the diagnosis and prognosis of intracranial hemorrhage, ischemic stroke and seizures [6]. Additionally, in perinatology the significant use of neuronal markers as NSE in amniotic fluid, cerebrospinal fluid, cord and neonatal blood has been proved to predict brain damage in the newborn especially after preterm labor [7].

It was reported that upon stimulation, NSE can pass to cell surface and contribute to different pathologies such as injury, inflammation, autoimmunity and cancer. Cell-surface expression of enolase can be detected on activated macrophages, causing extracellular matrix degradation, production of pro-inflammatory cytokines and invasion of inflammatory cells in the sites of inflammation [8].

Interestingly, abnormal placentation is one of the initial events of preeclampsia and macrophages are thought to play many important roles in the implantation of the placenta. Macrophage-induced apoptosis has been proved to limit endovascular trophoblast invasion in pre-eclamptic women [9].

Additionally, chitotriosidase, the human chitinase, is synthesized exclusively by activated macrophages. It has been detected as a biochemical marker of macrophage activation in several lysosomal diseases and is a particular important tool for following the treatment effects in Gaucher disease [10].

Moreover, inadequate vascular dilation and angiogenesis represent a crucial underlying defect of gravidic hypertension, denoting a failed response to the vasodilation and pro-angiogenic challenge imposed by pregnancy. Concomitantly, Insulin like Growth Factor-1 (IGF-1)

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produced by the cytotrophoblast cells *in situ* has been proved to be a key angiogenic player in the pathogenesis of many diseases related to pregnancy [11].

Furthermore, low levels of nitric oxide (NO) [12] and high levels of arginase (which degrades a precursor molecule in the nitric oxide synthase pathway) have been assessed in preeclampsia. A deficiency in NO has been correlated with metabolic derangements seen in preeclampsia [13].

It was demonstrated that innate immune cells, which are highly activated in preeclampsia, produce both oxidative stress and proinflammatory cytokines causing release of heat shock protein (HSP) [14]. Increased concentrations of heat shock protein 72 (HSP72) was observed in the syncytiotrophoblast. These pathological changes are concomitant with an ischemia-reperfusion injury and reflect those seen in preeclampsia [15].

Aim of the work

The present study aimed to link the neurological marker, NSE, with factors contributing to preeclampsia and to focus on its role as a possible prognostic biomarker for eclampsia.

Subjects and Methods

This study was carried out on 45 pregnant women after giving informed written consent; they were admitted to Department of Obstetrics and Gynecology of the Tanta University Hospital, between January 2016 and February 2017. The study protocol was approved by the Local Research Ethics Committee of the Faculty of Medicine, Tanta University, and was in accordance with the principles of the Declaration of Helsinki II. The pregnant women (in third trimester: 28 to 38 weeks gestation) were divided into three groups, (Group I) included 15 healthy pregnant women with normal blood pressure taken as a control group, (Group II) included 15 preeclampsia patients and (Group III) included 15 eclampsia patients (their age ranged between 25-40 years old).

All groups were subjected to routine measurement of: Blood pressure, body weight and body mass index (BMI). Proteinuria was detected by random dipstick urine determination and bilateral limb edema detected by examination. We collected information including maternal age, height, pre-pregnancy weight, reproductive and medical history.

Selection criteria

The diagnostic criteria of preeclampsia followed international classification systems [16] and the diagnosis was defined as a sustained increase of blood pressure after twenty weeks of gestation with a systolic pressure of 140 mmHg or higher or a diastolic pressure of 90 mmHg or higher together with proteinuria (>300 mg of protein over 24 hours, or a random dipstick urine determination of > +1 protein or >30 mg/dl). Blood pressure should be elevated on at least 2 occasions 6 hours apart.

Exclusion criteria

Gestational and pregestational diabetes, pre-existing chronic hypertension and epilepsy, we also excluded chronic renal or hepatic diseases.

Blood samples

Blood samples taken from participants were used for the analysis of biochemical markers. Blood samples collected in both EDTA 5% coated tubes and plain tubes and they immediately be centrifuged for 10 minutes at 3000 rpm to separate clear plasma and serum supernatant respectively and kept frozen at -20°C until time of assay.

Biochemical assessments

I-Assessment of neuronal damage marker: Plasma NSE level was assayed by ELISA technique performed according to manufacture instructions of commercial kits supplied by Chemux BioScience, Inc Company, USA (Catalog No.10111).

II-Assessment of macrophage activation: Plasma chitotriosidase activity by fluorometery [17] was measured by incubating 5 µl of EDTA plasma with 100 µl of 0.022 mM 4-methylumbelliferyl-fl-D-NN,N'-triacetylchitotriose (4 MU-chitotrioside; Sigma Chemical Co., St. Louis, MO) as substrate in citrate/phosphate buffer (0.1 /0.2 M), pH 5.2, at 37° C. Samples were diluted 50X in demineralized water before incubation. After 15 min the reaction was stopped with 2 ml of 0.3 M glycine/NaOH buffer, pH 10.6. A fluorimeter was used to assay the fluorescent 4-methylumbelliferone at 445 nm. Chitotriosidase activity was measured by incubating 10 µl of the sample with 100 µl of substrate mixture for 30 min. The enzyme activities were linear with time of incubation and amount of enzyme.

III-Assessment of angiogenic markers: Plasma IGF-1 level was assayed by ELISA technique performed according to manufacture instructions of commercial kits supplied by SUNRED Company, Changhai (Catalog No. 201-12-0104). Plasma NO level was measured according to the method described by Montgomery et al. [18] using nitric oxide colorimetric assay kit (CAT. No. NO 25 33) supplied by Biodiagnostic Company, Egypt. NO is oxidized to nitrite and nitrate that used to quantitate NO production. The first step converts nitrate to nitrite. The second step uses Griess Reagents to convert nitrite to a dark purple azo compound. The amount of the azochromophore reflects nitric oxide amount in samples. The absorbance was measured at 450 nm.

IV-Assessment of heat shock protein 72 level and antioxidant markers: Plasma HSP72 level was assayed by ELISA technique performed according to manufacture instructions of commercial kits supplied by Chongqing Biospes Co., Ltd Company, China Catalog No. BYEK1378). Serum paroxonase 1 (PON-1) activity was measured by adding 20 µl of serum to Tris buffer (100 mmol/l, pH 8.0) containing 2 mmol/l $CaCl_2$ and 1 mmol/l paraoxon (O, O-diethyl-Onitrophenyl phosphate (Sigma) The rate of generation of Pnitrophenol was determined at 405 nm, 37°C over 50 second after 1 minute lag time with the use of continuously recording spectrophotometer as described previously by Mackness et al. [19].

Statistical analysis

Results represented mean ± SD, multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukeys post hoc test. Correlations between variables were estimated by Pearson's correlation test. All calculations were made using the computer program SPSS 23 (SPSS, Chicago, Ill, USA).

The difference was considered statistically significant at P<0.05. Receiver Operating Characteristic (ROC) curve was constructed to calculate the optimized cut-off points.

Results

Table 1 shows demographic data of the studied groups.

Table 2 shows comparison of biochemical findings among the studied groups.

Neuronal damage marker

Plasma level of NSE showed statistically significant increase in Group II and III patients as compared to control group (P<0.05). Further, its level showed statistically significant increase in Group III when compared to Group II (Table 2).

Macrophage activation marker

Plasma Chitotriosidase activity showed statistically significant increase in Group II and III patients as compared to control group (P<0.05). Further, its activity showed statistically significant increase in Group III when compared to Group II (Table 2).

Angiogenic markers

Plasma level of IGF-1 showed statistically significant increase in Group II and III patients as compared to control group (P<0.05). Further, its level showed statistically significant increase in Group III when compared to Group II (Table 2).

On the other hand, plasma NO level was statistically significantly decreased in Group II and III patients when compared to control group with the lower levels reported for Group III (Table 2).

Heat shock protein 72 and antioxidant biomarkers

As shown in (Table 2), the levels of plasma HSP72 showed statistically significant increase in Group II and III patients when compared to control group (P<0.05). Further, their levels in Group III showed statistically significant increase when compared to Group II.

On the other hand, serum PON-1 activity was statistically significantly decreased in Group II and III patients when compared to control group with the lowest levels reported for Group III.

Figure 1 shows for Receiver operating characteristic (ROC) curve of the different markers for Preeclampsia.

Table 3 shows for sensitivity and specificity for NSE, Chitotriosidase, IGF 1 and HSP72.

Table 4 shows for correlations between Plasma NSE and different studied parameters among Group II and III patients (n=30).

Characteristics	Control (n=15)		Preeclampsia (n=15)		Eclampsia (n=15)		F	P-value
Age (years)	30.47 ± 3.85		31.13 ± 4.36		32.40 ± 4.33		0.698	0.503
Gestational age (weeks)	32.07 ± 3.35		31.73 ± 4.22		31.00 ± 2.95		0.355	0.703
Body mass index, kg/m^2	27.23 ± 2.30		27.63 ± 2.38		28.03 ± 2.47		0.423	0.658
Systolic blood pressure, mmHg	107.67 ± 8.84		157.33 ± 8.84		174.00 ± 15.49		135.25	<0.001*
Diastolic blood pressure, (mmHg)	74.67 ± 6.399		100.33 ± 8.96		109.67 ± 9.90		67.40	<0.001*
Parturitions n (%(	26.7%		53.3%		73.3%		Pearson Chi-Square	0.037*
Primiparous Multiparous	73.3%		46.7%		26.7%			
Proteinuria>2+	Yes	0%	Yes	100%	Yes	100%	6.58	--
	No	100%	No	0%	No	0%		
Family history of preeclampsia	Yes	0%	Yes	33. 3%	Yes	46.7%	--	--
	No	100%	No	66.7%	No	53. 3%		
Bilateral limb edema	Yes	0%	Yes	60.0%	Yes	73.3%	--	--
	No	100%	No	40.0%	No	26.7%		

Table 1: Demographic data of the studied groups.

Parameters / Groups	Group I n=15	Group II n=15	Group III n=15	F	P-value
Plasma (NSE) level ng/ml	6.36 ± 2.50	10.03 ± 3.45 [a]	14.04 ± 3.64 [a,b]	21.076	<0.001*
Plasma Chitotriosidase Activity nmol/hr/ml plasma	80.89 ± 12.52	104.11 ± 11.01 [a]	120.55 ± 15.69 [a,b]	34.087	<0.001*
Plasma (IGF-1) level ng/ml	106.50 ± 9.54	131.51 ± 20.48 [a]	155.76 ± 26.48 [a,b]	22.525	<0.001*
Plasma (NO) µmol/l	34.37 ± 9.10	27.96 ± 6.21 [a]	21.76 ± 3.54 [a,b]	13.348	<0.00*
Plasma (HSP72) level ng/l	238.19 ± 8.18	253.83 ± 9.79 [a]	268.02 ± 14.70 [a,b]	26.435	<0.001*
Serum PON-1 Activity U/l	315.17 ± 22.24	282.30 ± 23.85 [a]	251.80 ± 23.82 [a,b]	27.716	<0.001*

Note: Data presented as mean ± SD, n: number of cases, [a] : Significantly different as compared with control group. [b] : Significant different as compared with Group II *P-value was considered significant at<0.05.Group I: control group, Group II: Preeclampsia patients Group III: Eclampsia patients. (NSE): Neuron specific enolase, (IGF-1): Insulin growth factor 1, (HSP72): Heat shock protein 72, (NO): Nitric oxide, (PON-1): Paraoxonase 1.

Table 2: Comparison of biochemical findings among the studied groups. Comparison between the studied groups was performed using one-way analysis of variance (ANOVA) with post hoc test.

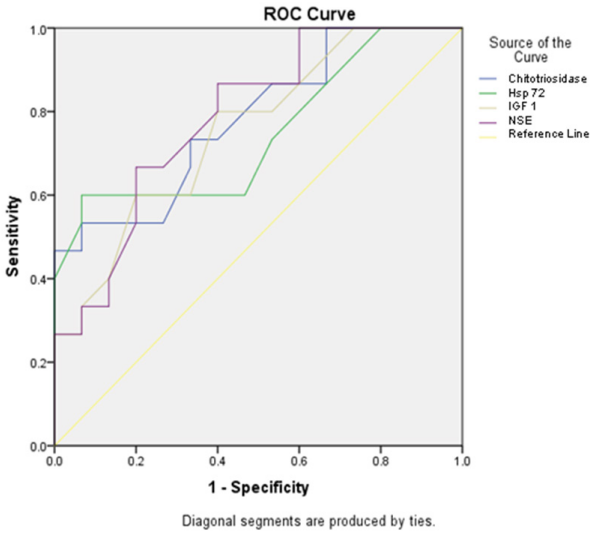

Figure 1: Receiver operating characteristic (ROC) curve of the different markers for preeclampsia.

Variables	AUC	p	95% C.I		Cut off	Sensitivity	Specificity
			LL	UL			
NSE	0.787	0.007*	0.624	0.949	≥10.66	86.7	60
Chitotriosidase	0.782	0.008*	0.619	0. 945	≥106.5	80	53.3
IGF-1	0.756	0.017*	0.584	0.927	≥137	80	53.3
HSP72	0.747	0.021*	0.566	0.927	≥255.25	60	53.3

Table 3: Agreement (sensitivity and specificity) for NSE, Chitotriosidase, IGF 1 and HSP72.

Correlations	Plasma NSE (ng/ml)	
	r	P-value
Plasma Chitotriosidase Activity (nmol/hr/ml plasma)	0.363	0.049*
Plasma IGF-1 level (ng/ml)	0.517	0.003*
Plasma NO level (µmol/l)	- 0.454	0.012*
Plasma HSP72 level (ng/l)	0.580	0.001*
Serum PON-1 Activity (U/l)	- 0.660	< 0.001*

Table 4: Correlations between plasma NSE and different studied parameters among group II and III patients (n=30).

Discussion

The pathogenesis of preeclampsia and eclampsia is not fully understood, but much progress was done in the last decades. Ischemic injury to the central nervous system leads to cellular activation and disintegration, leading to release of cell-type specific proteins such as NSE [20]. After neuronal damages, NSE can leak into extracellular compartment and bloodstream [21] making it an excellent serum biomarker of neuronal injury [22].

In the current study, plasma NSE level was significantly elevated in preeclampsia and eclampsia groups when compared to its level in healthy subjects with the highest level in eclampsia group. This aligned with results reported by Bergman et al. [23] who reported that in preeclampsia, the levels of NSE remained high throughout pregnancy and tended to decline in healthy women. The changes in NSE levels can reflect CNS tissue injury, this come in agreement with similar study that used NSE to predict neurological outcome after cardiac arrest [23].

Additionally, in the first trimester, the decidua normally experiences an influx of macrophages, which accumulate at the implantation site [24]. Furthermore, the removal of apoptotic cells by macrophages remodels the deciduas to facilitate trophoblast invasion and physiologic implantation; conversely, an excessive infiltration of macrophages seems to impair trophoblast invasion [25].

The decidua of preeclamptic women has been proved to contain abnormally elevated number of macrophages [26]. Chitotriosidase activity was assayed to assess its usefulness as an inflammatory marker in the etiology of pre-eclampsia and eclampsia as it has been reported to be a marker of macrophage activation [27].

The current study revealed that plasma chitotriosidase activity was significantly elevated in preeclampsia and eclampsia groups when compared to its level in healthy subjects with the highest level in eclampsia group. These results also agreed with [27] who demonstrated that maternal chitotriosidase activity was significantly higher in women with pre-eclampsia as chitotriosidase activity is related to the severity of atherosclerotic lesions knowing that inadequate trophoblastic invasion and acute atherosis of the spiral artery walls are two main features of pre-eclampsia, additionally macrophages within atherosclerotic plaques have been shown to produce high amounts of chitotriosidase [28].

The present results showed positive correlation between plasma NSE level and chitotriosidase activity and this came in agreement with the previous study revealed that patients presenting with ischemic brain injury has shown elevation in many biomarkers, including chitotriosidase [29].

From another aspect, IGF-1 has powerful proangiogenic and endothelial protective activities and it is implicated in vasodilation and microvascular organ sprouting [30]. It is considered as a key angiogenic player in the pathophysiology of several conditions related to pregnancy [31].

The current study revealed that plasma IGF-1 level was significantly elevated in preeclampsia and eclampsia groups when compared to its level in healthy subjects with the highest level in eclampsia group. These results agreed with a study that described higher serum IGF-1 and lower IGF binding protein-1 (IGFBP-1) levels in those developing preeclampsia than in controls this might account for the compensatory mechanism induced to overcome vascular abnormalities in trophoblastic invasion [32].

Noticeably, low IGFBP-1 is linked to low NO levels as proved in early gestation sera of women destined to develop preeclampsia. This resulted from the elevation of $Ca^{2+,}$ activation of Phopholipase C, PhosphoInositide-3 Kinase and Mitogen activated protein kinase leading to down regulation of inducible nitric oxide synthase [33].

The nitric oxide (NO)/nitric oxide synthase (NOS) system is also deranged in preeclampsia. NO is a powerful vasodilator that induces relaxation in vascular smooth muscle cells via cyclic guanosine monophosphate pathway [34] Decreased levels of NO have been reported in preeclampsia [12]. This also was proved in the current study.

Growing body of evidence documented that, up-regulation of HSP72 has been found in conditions of hypertension, rendering it a sensitive marker of ischemia-reperfusion injury. Therefore, the expression of HSP72 not only serves as a marker of oxidative stress, but also represents an adaptive response that prevents the aggregation of denatured proteins within the cytosol [15]. This coincided with other studies revealed that HSP72 may be released from dying cells that

have lysed during the response to stress or injury [35]. This protein is expressed at low levels under physiological conditions, but it is induced upon exposure to conditions that may cause cytosol protein misfolding as anoxia, ischemia [36].

The current study also revealed reduced activity of serum antioxidant enzyme PON-1 in preeclampsia and eclampsia groups in comparison to its level in control subjects. The results of the current study came in agreement with those who reported lower PON-1 activity in preeclamptic pregnant women compared to the control subjects [37]. This may be associated with increased oxidative stress and/or cytokine levels.

Conclusion

The use of neuron specific enolase as neurological marker in the blood, being linked to macrophage activation, oxidative stress and angiogenesis markers is an accessible reliable method to predict the outcome of preeclampsia and to determine the management strategy to prevent eclampsia and other neurological damage.

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