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A New Method to Estimate Inhibition Percentage of Endogenous Digitalis in Patients with Pre-eclampsia

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Abstract

Background: Pre-eclampsia is an idiopathic pregnancy disorder characterized by appearance proteinuria and hypertension, with poorly understood etiology. It has been linked to a variety of system abnormalities, including ion transport disorders in neonatal, maternal, and placental cell lines. A new method was described to evaluate the inhibition percentage of endogenous digitalis in plasma of pre-eclampsia patients compared with normal pregnancies, with the estimation of sensitivity and specificity of the proposed test.

Methods: This was a case-control study consisting of 130 cases that were divided into three groups, 55 normal pregnancies (positive control), 30 non-pregnant women (negative control), and 45 pre-eclampsia (patients). The new method included the estimation of the percentage inhibition of endogenous digitalis by measuring specific enzyme activity of Na-K ATPase for the patient and positive control. The results were analyzed using the statistical package for social sciences (SPSS®) software version 26.0. A p-value of$\leq$ 0.05 was considered significant.

Results: In the pre-eclampsia patient, the specific activity of Na-K ATPase was significantly lower with mean= 0.239 mg/g±0.043 compared to the control group which was 0.397 mg/g±0.021, p< 0.001. While the result of inhibition percentage of endogenous digitalis showed significantly higher in the pre-eclampsia patient (mean= 35.852 mg/g %±2.692%) compared to the control group (mean= 17.964%±1.784), with a p< 0.001.

Conclusions: Pre-eclampsia is linked with lower erythrocyte sodium pump activity significantly in pre-eclampsia patients than in normal pregnancies. Also, results show the inhibited percentage of endogenous digitalis elevation in patients with pre-eclampsia compared with normal pregnancy.

Keywords: Endogenous Digitalis, Hypertension, Inhibition Percentage, Pre-eclampsia.

Introduction

Pre-eclampsia (PE) is a disorder of human pregnancy diagnosed by proteinuria more than 300 mg/day and hypertension. It is a serious disorder that may lead to mother and fetal morbidity and death. The condition starts after 20 weeks of pregnancy. The incidence of pre-eclampsia is 2% to 10% of pregnancies depending on the definition used and population studied (1,2). Pre-eclampsia is classified as mild or severe based on the severity of hypertension, the amount of proteinuria, and the extent to which other organ systems are impacted (3). In severe, illness there may be hemolytic anemia, thrombocytopenia, hepatocellular dysfunction, peripheral edema, diplopia, or respiratory distress due to pulmonary edema. It generally develops during the third trimester (4). Pre-eclampsia has been linked to a variety of system abnormalities, including ion transport disorders

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in neonatal, maternal, and placental cell lines (5). The existence of placental-derived endogenous digitalis-like factors in plasma has been proposed as a possible explanation for these anomalies in pre-eclampsia. These factors, which resemble or are identical to cardiotonic steroids, inhibit the sodium-potassium adenosine triphosphates (Na-K ATPase) enzyme complex transport, which functions as the sodium pump. Inhibition of this enzyme has several consequences, including an increase in vascular resistance and, as a result, some pre-eclampsia symptoms (6,7).

About half a century ago, it was believed that endogenous digitalis-like factors, also known as cardiotonic steroids, play a significant role in disease and health (8). Cardiotonic steroids are steroid hormones found in the blood and excreted in the urine. It is classified into two types based on their chemical structure: cardenolides (plant-derived) and bufadienolides (mainly of animal source) (9,10). Ouabain and marinobufagenin exist in humans, and an effector mechanism has been identified by which these hormones signal via the inhibition of Na-K ATPase (11).

Pregnancy is associated with plasma volume expansion because of fluid retention and salt retention in the kidneys (12). So, the role of cardiotonic steroids in pregnancy and pregnancy-related diseases is logical to investigate. Graves *et al*. were the first to show increased circulating levels of cardiotonic steroids during pregnancy and hypothesize that cardiotonic steroids play a role in pre-eclampsia pathogenesis (13). The role of cardiotonic steroids in pregnancy is still unknown (10).

This study aimed to describe a new method and measure the inhibition percentage of endogenous digitalis in the plasma of pre-eclampsia patients compared with normal pregnancies.

Materials and Methods

Ethics Issues

The present sampling procedure was approved by the research ethics committee of Babylon health directorate. This work was permitted from the scientific committee of maternity and children hospital in Babylon governorate, Hilla city. To gain verbal acceptance from participating women, the goals of this study were explained to all participants in the current study.

Study Design

This was a case-control study consisting of 130 cases, with mean ages of 26.9±7.3 years, and these cases were divided into three groups, 55 normal pregnancies (positive control), 30 non-pregnant women (negative control), and 45 pre-eclampsia (patients).

Chemicals

All chemical substances were obtained from commercially available sources.

Instruments

A spectrophotometer (Aple, Japan) was used for the measurement of absorbance.

Collection of Blood Samples

Venous blood samples were collected during the period of 11/2/2019 to 20/2/2020. A disposable syringe was used to draw blood from all women who participated in the present study. Three ml of blood were obtained from everyone by venepuncture, collected into the EDTA tube, and mixed gently. Blood was centrifuged at 2000 G for approximately 10 minutes. Then, the plasma was transferred to the plain tube and stored at -20 °C until the endogenous digitalis analysis.

Determination of Endogenous Digitals

A. Principle

Endogenous cardiotonic steroids (CTS) are a class of steroid hormones linking high salt consumption and elevated blood pressure. CTS work primarily by inhibiting the Na-K ATPase transport enzyme, which is found throughout the body. A portion of the Na-K ATPase does not appear to actively "pump" sodium or potassium, but it is closely linked to other important signalling pathways (14,15).

B. Preparation of ATPase Reagent

One hundred ml of ATPase reagent was prepared by mixing the following materials:

1. Tris – HCl (100 mM, 1.2 g).
2. $MgCl_2$ (10 mM, 0.095 g).
3. KCl (15 mM, 0.11 g).
4. NaCl (85 mM, 0.4 g).
5. Na_2-EDTA (1 mM, 0.036 g).
6. ATP (2 mM, 0.1 g).

All materials were dissolved in 100 ml of distilled water and its pH was adjusted at 7.4 by adding sodium hydroxide.

C. Preparation of Red Blood Cell Ghosts

Fresh blood from negative control was collected in EDTA tubes as an anticoagulant and centrifuged at 2000 G for 10 min. RBCs were obtained by taking 40 µl of red cell sediment after centrifugation. In one ml of pharmaceutically available normal saline, the 40 µl red cell sediment was added and washed three times with subsequent centrifugation and decanting the residual normal saline supernatant. Washed RBCs obtained by the above procedure were subjected to lysis by the addition of deionized distilled water by adding 500 ml DDW and centrifuge at 2000 G for 10 min.

D. Measurements of Endogenous Digitals

1. Ten microliters of red cell ghosts from negative control were mixed with 10 µL of serum from the patient and incubated in a water bath at 37 ºC for 10 min.
2. After incubation, 500 ml of ATPase reagent prepared above was added and incubated for 30 min, exactly.
3. The samples were centrifuged at 4000 RPM for 10 min. Then pulled 50 µl from the supernatant to determine inorganic phosphates in the patient.
4. The inorganic phosphates for the patient were determined spectrophotometrically according to the method of kit linear.
5. For standardization, protein concentration in red blood cell ghosts was estimated according to the standard Biuret method.
6. Enzyme activity for the patient was expressed as the inorganic phosphorus to red cell ghost protein concentration.
7. Enzyme activity for negative control was measured by determining the inorganic phosphorus while the protein concentration value was the same.
8. The percentage of inhibition for endogenous digitals was expressed as follows:

$$\%\ Inhibtion\ of\ Endogenous\ Digitals = \frac{Enzyme\ ActivityN.C. - Enzyme\ Activity\ P.}{Enzyme\ Activity\ of\ N.C.} * 100\%$$

Where N.C.=Negative Control, p= Patient

Statistical Analysis

The analysis of results was carried out using Statistical Package of the Social Sciences (SPSS®) version 26 to get on the variables as the mean, the standard error for the mean (SEM), confidence interval, T-test, and correlation coefficient. A p-value of$\leq$ 0.05 was considered significant. While the sensitivity and specificity were carried out by using Medcalc software version 20.

Results

The mean and SEM values of the clinical characteristics of the control and patient groups are shown in Table 1.

The results of the inhibition present of endogenous digitalis in patients with pre-eclampsia were significantly higher than normal pregnant women, as shown in Table 2.

While the result of the correlation between specific enzyme activity of Na-K ATPase and inhibition present of endogenous digitalis are shown in Figure 1 and this result are shown an inverse relationship between specific enzyme activity and inhibition present of endogenous digitalis.

Additionally, the sensitivity and specialty for the new method were calculated by using MedCalc software and the results show in Table 3 and Figure 2. The result of this method shows more specificity than sensitivity.

Further, the result of the correlation between specific enzyme activity and criteria for digenesis pre-eclampsia are shown in Table 4.

Table 1. The Demographic Characteristics of Studied Groups.

Variables	Control Mean±SEM	Patient Mean±SEM	T- Test	p- Value
Albumin in Urine	0.225±0.100	2.806±0.242	-9.817	0.000*
Diastolic BP (mmHg)	77.105±1.219	98.205±1.936	- 9.167	0.000*
Systolic BP (mmHg)	117.948±1.690	149.512±1.708	-13.132	0.000*
Gestational Age (Weeks)	36.105±0.321	34.846±0.518	2.062	0.043*
Weight of Baby (gm)	3104.800±74.416	2726.30±144.044	2.335	0.025*

*p value< 0.05 was significant

Table 2. The Percent Inhibition of Endogenous Digitalis in Control Group Compared with Pre-eclampsia.

	Groups	Mean±SEM	95% Confidence Interval for Mean		p-value
			Lower	Upper	
Inhibition Percent of Endogenous Digitalis	**Patient**	35. 852±2.692	14.402	21.526	0.000*
	Control	17.964±1.784	30.428	41.275	

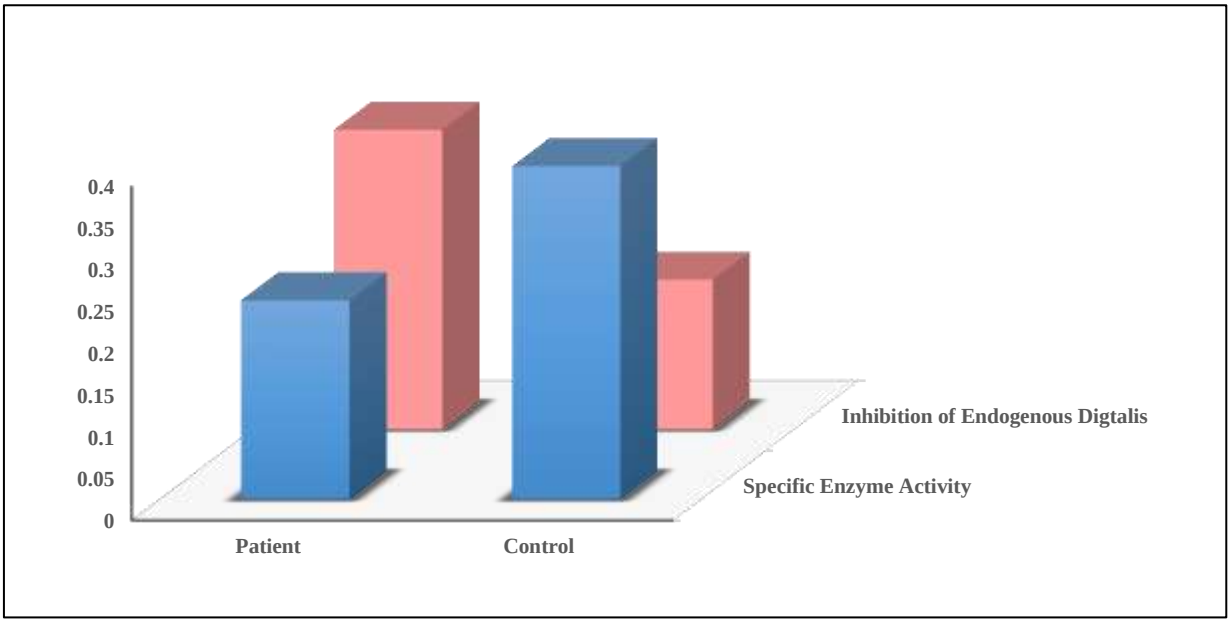

Fig. 1. Inhibitory Activity of Endogenous Digitalis in Pre-eclampsia and Control Group.

Table 3. The predicted cut-off value of inhibition percentage of endogenous digitalis in pre-eclamptic patients.

Variables	Cut-off Value	Sensitivity	Specificity	+ PV	-PV	AUC
Endogenous Digitals	31.65	52.2%	85.1%	54.2%	83.8%	75.8

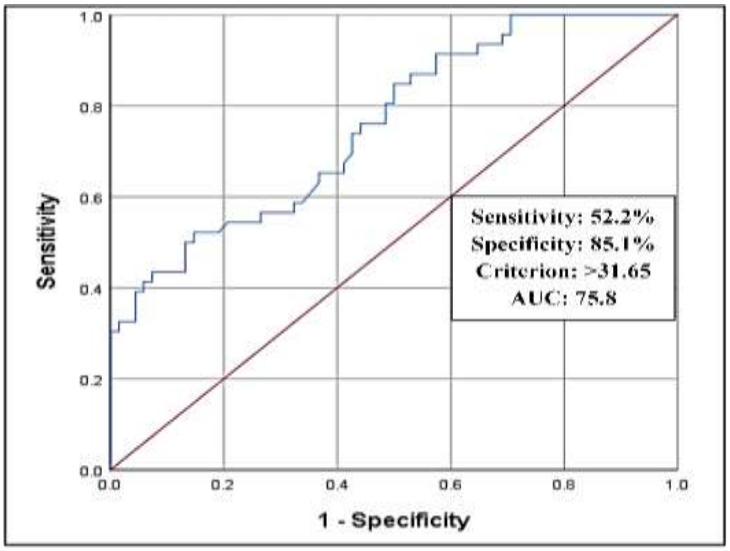

Fig. 2. The ROC Curve for Endogenous Digitalis Method. The Cut-Off Value, Sensitivity, Specificity, Positive, and Negative Predictive Values were 52.2%, 85.1%, 54.2%, and 83.8%, respectively.

Table 4. The Correlation among Specific Enzyme Activity, Inhibition Percent of Endogenous Digitalis and Criteria for Digenesis Pre-Eclampsia.

Variables	Systolic Pressure		Diastolic Pressure		Albumin in Urine	
	r	p-Value	r	p-Value	r	p-Value
Endogenous Digitalis	0.672**	0.000	0.588**	0.000	0.731**	0.000
Enzyme Activity	-0.290*	0.012	-0.313**	0.007	-0.163	0.206

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05

Discussion

Pre-eclampsia is a multisystem and multifactorial disease that affects both mother and fetus by intrauterine growth restriction and vascular dysfunction (16). Although there is much ongoing research in the field of pre-eclampsia the definitive cause of this condition is not yet known. Several causes of this syndrome have been postulated, including abnormal formation of the placenta, chemical imbalances, genetic factors, and immunological mechanisms (17). In this study, pre-eclampsia has been specifically dealt with because although it is a well-established threat to the woman and a much serious threat to her fetus, they are also considered as major contributors to maternal and perinatal morbidity (18).

The present study showed no statistically significant difference in maternal age between normal pregnancy and pre-eclampsia similar to the findings of other studies (19,20).

The mean systolic and mean diastolic pressures of the pre-eclampsia were significantly higher than that of the

normotensive pregnant women ($p< 0.001$). As well, the mean of the albumin in the urine of pre-eclampsia is significantly higher than that of the normotensive pregnant women ($p< 0.001$). This was however expected given the criteria used for the diagnosis of pre-eclampsia.

Clinically, pre-eclampsia presents as hypertension, proteinuria, with or without edema during pregnancy. Even with an adequate electrolyte and water content during pre-eclampsia, these are mainly located in the interstitial with a resulting decrease in intravascular circulating volume. This reduction in intravascular circulation volume results in the activation of baroreceptors and liberation of antidiuretic hormone (ADH) reason water retention and natriuretic (21).

The other clinical features include gestational age. The present study shows that there is a very highly significant difference ($p< 0.043$) in the mean weeks of gestation at labour which is lower in cases (34.846 weeks) than in controls (36.105 weeks). Also, the induction rate and caesarean section are more in cases than in controls. These differences are because delivery is the definitive treatment of PE and should be considered regardless of gestational age if any of the following "ominous" features are present such as severe persistent hypertension> 160/110 mmHg, deteriorating renal function, the sign of cerebral edema that may precede fit, severe HELLP syndrome, the sign of hepatic involvement, IUGR and, hemoconcentration (22).

In addition, so the results of baby weight appeared significantly lower in pre-eclampsia, than in normal pregnant women. For live births, comparing the birth weight of cases new-borns to that of controls new-borns (without adjustment for the gestational age) reveals a highly significant risk ($p= 0.025$) for cases to give birth to low weight babies birth. It is well known that mothers who have PE usually have smaller babies. This is partly the result of preterm birth or shortened gestational duration because early delivery is a consequence of PE, and it is the only effective treatment. This has masked the fact that most infants born to mothers with PE are not small when compared with infants born to mothers without PE at the same gestational age (23).

This study, using a new method to determine the inhibition present of endogenous digitalis by measuring specific enzyme activity in control and patients, and the results of the study show more percentage inhibition in patients compared with the control group.

While the result of the correlation between specific enzyme activity and criteria for digenesis pre-eclampsia are shown an inverse relationship between enzyme activity, systolic pressure, and diastolic pressure. This finding is particularly remarkable since the more was Na-K ATPase reduced, the more individuals presented rises diastolic blood pressure, which appeared by the significant negative correlation between these variables, which propose a possible role for Na-K ATPase lowering in blood pressure increase (24). Also, the result of the correlation between endogenous digitalis and criteria for digenesis pre-eclampsia is shown a positive relationship.

The lower levels of Na-K ATPase enzyme activity in the red blood cell membranes of the patients compared with control is linked inversely with inhibitory activity of endogenous digitalis in patients with pre-eclampsia. The new method for measurement inhibition percentage of endogenous digitalis is more specificity than sensitivity. The results of this study may supply new strategies for intervening in the evolution of pre-eclampsia in clinical practice.

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