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Polyamine Oxidase Activity in Women with Preeclampsia-Eclampsia

[1]José D. Méndez, [2]Montserrat Aguilar-Hernández and [2]Verna Méndez-Valenzuela

[1]Medical Research Unit in Metabolic Diseases, National Medical Center,
Mexican Institute of Social Security, P.O. Box A-047, México City, 06703 D.F., México
[2]Endocrinology Department, Specialities Hospital, National Medical Center,
Mexican Institute of Social Security, México City, México

Abstract: This study compares the polyamine oxidase activity in serum of preeclamptic patients with that of normal pregnant women. 40 women were included; 20 with normal pregnancy and 20 with severe preeclampsia and eclampsia. Clinical parameters were registered in both groups of patients. Serum polyamine oxidase activity was measured by the radiochemical method of Okuyama and Kobayashi. The enzyme activity was higher in preeclamptic patients compared to normal pregnant women (8.48±3.23 vs. 6.91±2.53 Picomoles of Δ^1-pyrroline/mg of protein/hour, Mean±S.D., respectively). The incidence for developing preeclampsia was significantly higher between weeks 39 and 41 of gestation. A tendency toward haemoconcentration was observed in preeclamptic women. There was not significant difference at birth weight in infants of preeclamptic and those of normal pregnant women. The relevance of our findings is that increased activity of polyamine oxidase in preeclamptic patients helps to formulate new hypothesis about the pathophysiology of preeclampsia in which polyamine derivates may be involved, however, this remain to be demonstrated.

Key words: Preeclampsia-eclampsia · Polyamines · Polyamine Oxidase · Acrolein · Urea end Products · Polyamine end Products

INTRODUCTION

According to WHO information, four major direct causes of maternal death are haemorraghe, infection, eclampsia and obstructed labor. Hypertensive disorders of pregnancy (pre-eclampsia and eclampsia) are the cause of 12% of maternal deaths around the world [1]. Preeclampsia, also named gestosis or toxemia usually appears in the woman after the 24th week of pregnancy[2]. Preeclamptic patients show hypertension concomitant with severe alterations in vascular structure, proteinuria and edema [2]. The polyamines putrescine, spermidine and spermine are highly charged, low molecular weight and polycationic substances that are essential for cell growth and differentiation [3-5]. During normal pregnancy, spermine levels increase while putrescine and spermidine levels decrease [6]. Polyamine oxidase is one of the key enzymes involved in polyamine catabolism because it catalyzes oxidative cleavage of polyamines spermidine or spermine to produce diaminopropane, H2O2 and an aminoaldehyde derivative [7].

In normal pregnancy, polyamine oxidase activity showed a progressive increase in gestational age to 21 weeks, or beyond, declining to very low levels in the 3 to 4 days postpartum [8-11]. It is known that polyamine oxidase activity is highest in the intervillous circulation where the first and closest contact between fetal and maternal surfaces takes place [11-12]. This led to the hypothesis that polyamine oxidase has a protective function in normal pregnancy and that amine oxidase at elevated levels protects mother and fetus f rom biogenic amines at high concentrations.

Also it is known that the products of the interaction of polyamines and polyamine oxidase contribute to the protection of fetus against maternal immune rejection. Our purpose was to compare the activity of polyamine oxidase in maternal serum of preeclamptic patients and normal pregnant women. This paper provides evidence about the increased activity of polyamine oxidase in preeclamptic-eclamptic patients.

Corresponding Author: Dr. José D. Méndez, Medical Research Unit in Metabolic Diseases, National Medical Center. Mexican Institute of Social Security, P.O. Box A-047, México 06703, D.F.

MATERIAL AND METHODS

Reagents: Bovine serum albumin, putrescine dihydrochloride and Folin & Ciocalteu´s Phenol Reagent were purchased from Sigma (Sigma Chemical Co. St. Louis, MO, U.S.A.). Putrescine dihydrochloride [1,4 ^{14}C] was obtained from New England Nuclear, Boston, MA, U.S.A. Insta-Gel (Catalog No. 6013004) was purchased from United Technologies Packard Inc. (Illinois, USA). All other chemicals were from analytical grade.

Patients: The study protocol was registered and approved (No. 12/84) at the Jefatura de Enseñanza e Investigación at the Obstetric and Gynecology Hospital # 2, National Medical Center (IMSS). The sample size was constituted by 40 pregnant women; 20 were with normal pregnancy and 20 suffering severe preeclampsia seen at the Obstetric and Gynecology Service Outpatient Clinic and the Intensive Care Unit of the Hospital, examined and selected if they met the following inclusion criteria: 1) The criteria for considering that a pregnant woman suffered severe preeclampsia was arterial pressure 160/110 mmHg or higher for about 6 hours or more, general or abdominal edema and a urine protein concentration higher than 3 g/l. The cases of preeclampsia after childbirth were excluded. 2) Age between 17 and 25 years. 3) First pregnancy. 4) Normal childbirth or cesarean. 5) Presence of toxemia symptoms since the 35 weeks of gestation. 6) Same socioeconomic conditions. Some patients were excluded because of the following reasons: 1) renal or cardiovascular pathology background. 2) A pathology different from toxemia that can complicate the patient condition. 3) Patient pregnant with twins.

Blood collection: Blood samples from women with normal pregnancy were obtained during the prenatal consults and the blood samples of preeclamptic patients were obtained at the entrance to the Intensive Care Unit at the Hospital before they receive any kind of drug or intravenous serum treatment.

It was collected 7-8 ml of blood from each patient. Serum was obtained by centrifugation at 3500 rpm during 10 minutes

Protein Determination: Proteins were determined in the serum samples by the method of Lowry *et al*. [13]. The absorbance was registered at 540 nm using a 2 mg/ml bovine albumin solution (Fraction V, Sigma Chemical Co., Catalog No. A-4503) as standard.

Polyamine oxidase activity: The determination of polyamine oxidase activity in serum was carried out by the radiochemical assay first reported by Okuyama and Kobayashi [14]. Briefly, to a serum sample containing 30 mg of protein, phosphate buffer (0.1 M, pH = 7.6) to a final volume of 2 ml, 10 µl of substrate were added (492 µg of cold putrescine + 60 µl of putrescine-^{14}C + 950 µl of HCl 0.01N). Putrescine- ^{14}C (radioactivity 0.055 mCi/31 nanomol). After 60 minutes of incubation at 37°C, the reaction was stopped with the addition of 200 µg of sodium bicarbonate. For each sample it was prepared a blank (serum sample with protein concentration of 30 mg, phosphate buffer (0.1M, pH = 7.6) to a final volume of 2 ml, then boiled at 92°C during 5 minutes for denaturing the enzyme, then the substrate was added. After this the enzymatic reaction was stopped, the samples and its controls were processed as follows:

A volume of 10 ml of the scintillation liquid was added to each sample and was mixed vigorously in a vortex for 1 minute. Then the aqueous phases of the samples were freezed on dry ice and the organic phases of samples were decanted to another vial (Fraction 1). This procedure was repeated twice (Fraction 2 and Fraction 3). Each fraction was separately counted in the liquid scintillation counter (Packard 460 CD U.S.A). Total radioactivity was expressed as desintegrations per minute (DPM). Polyamine oxidase activity was expressed as picomoles of Δ^1-pyrroline/mg of protein/hour.

The percentage of radioactivity recovery from serum samples was: radioactive sample in insta-gel (100%), serum polyamine oxidase of normal pregnant and preeclamptic women in phosphate buffer (88.2%) and denatured polyamine oxidase (1.15%).

RESULTS

In Table 1, the polyamine oxidase activity in serum of preeclamptic women is compared to that of normal pregnant women. In spite of the difference is not significant, the activity was higher in serum of

Table 1: Polyamine oxidase activity in serum of normal pregnant women and preeclamptic women

	Picomoles of Δ^1-pyrroline/mg of protein/hour
Normal pregnant women (n = 20)	6.91±2.53*
Preeclamptic women (n = 20)	8.48±3.23*

* Average±S>D

Table 2: Clinical values obtained from women with normal pregnancy

Case No.	Pregnant week	Arterial Pressure (mm Hg)	Urine	Protein (g%)	Hb (g/dl.)	Ht (%)	Body weight product (g)
1	37	110/70	normal	7.90	12.70	39	3200
2	39	110/70	normal	9.32	-	-	2750
3	41	100/70	normal	6.15	11.50	27	3400
4	42	110/70	normal	7.59	10.30	39	3200
5	34	110/80	normal	6.29	-	33	2880
6	42	120/70	normal	8.63	-	-	2640
7	37	120/80	normal	6.59	12.00	37	-
8	43	110/70	normal	7.62	11.60	-	3520
9	37	110/70	normal	7.67	12.40	40	2980
10	40	110/70	normal	7.46	10.80	37	-
11	38	120/80	normal	7.24	11.60	36	-
12	39	110/70	normal	4.93	12.40	38	2650
13	37	100/60	normal	7.43	10.80	35	3200
14	38	110/70	normal	6.67	11.75	37	3400
15	39	110/70	normal	6.74	12.50	39	3520
16	40	110/70	normal	5.47	11.00	35	3100
17	36	110/70	normal	7.82	-	-	3180
18	35	120/80	normal	7.18	12.40	38	4100
19	36	100/60	normal	5.58	10.50	33	-
20	38	110/70	normal	7.53	10.00	33	-

Table 3: Clinical values obtained from preeclamptic patients

Case No.	Pregnant week	Arterial Pressure (mm Hg)	Edema	Protein (g%)	Hb (g/dl.)	Ht (%)	Body weight product (g)	Diagnostic	Haemo globinuria	Platelets (*1000/µl)
1	40	150/100	+	5.33	10.85	35	3600	ECL	+	120
2	41	150/100	++	7.58	11	37	3700	ST	-	-
3	40	150/100	++++	5.51	15.8	47	3000	ST	+	135
4	40	150/110	++	6.24	12.7	41	2700	ST	++++	187
5	40	150/105	++	7.08	11.7	38	2700	ST	+++	139
6	40	150/110	no	6.61	14.1	45	2460	ST	-	97
7	40	150/110	++	7.12	13.6	42	2900	ST	++++	120
8	36	170/120	++	5.8	11.4	36	3000	ST	++	139
9	35	160/115	++	7.66	13	41	2900	ECL	++	150
10	36	150/100	++	7.35	10.2	32	2400	ST	+++	150
11	40	170/120	+++	8	14.5	48	3000	ST	Traces	140
12	36	180/120	+	9.86	12.4	39	3400	ST	-	103
13	36	200/150	+	6.61	14.1	44	1800	ST	+	148
14	34	150/110	+	4.44	12.9	40	1600	ECL	Traces	103
15	36	210/120	++	7.51	12.1	39	2500	ST	++++	266
16	39	150/110	++	6.68	12.4	38	2200	ST	-	92
17	43	170/110	++++	6.32	15.8	47		ST	-	90
18	40	150/110	++	7.95	10.2	34	3600	ST	+	183
19	40	140/100	++	6.74	8.65	32	2900	ST	-	75
20	41	140/100	no	7.8	13.2	42	3100	ST	-	-

*ST = Severe toxemia

preeclamptic women (8.48±3.23 vs. 6.91±2.53 Picomoles of Δ^1-pyrroline/mg of protein/hour, Mean±S.D., respectively). Table 2 shows values of hemoglobin, haematocrit, arterial pressure, general urine, child birth weight and protein concentration obtained from the 20 normal pregnant women tested in this study and Table 3 shows values of hemoglobin, haematocrit, arterial pressure, child birth weight, protein concentration, haemoglobinuria and platelets obtained from preeclamptic and eclamptic patients. Table 4 shows the mean of some clinical values from Table 2 and Table 3 and also shows normal values for non-pregnant women, normal pregnant women and preeclamptic women.

Table 4: Mean±S.D. of clinical parameters obtained from normal pregnant women and preeclamptic patients

Case	Arterial pressure (mm Hg)	Haemoglobin (g/dl.)	Haematocrit (%)	Body weight product (g)	Platelets (*1000/µl)	Protein (g%)
Experimental values						
Normal pregnancy	110/70	11.43±1.25	36.0±3.31	3181.3±384.4		7.09±1.06
Preeclamptic patient	160.5/111	12.53±1.86	39.8±4.8	2813.7±571.2	135 389±44 891	6.9±1.17
Normal values						
Normal women[a]	110/70	13.5 - 17.0	36 - 38	-	200- 400	6 - 8
Normal pregnancy[b]	110/70	12.5	37	2700 - 3200	200 - 400	6 - 8
Preeclamptic patient[b]	160/110	13.5 - 14.0	40 - 41	Variable[c]	Variable[d]	5 - 7

a: Non-pregnant women

b: Theoretical average values of women with 38 weeks of gestation (Obstetric and Gynecology Hospital No.2, IMSS).

c: According to the severity of toxemia it could be variable even a 66 % less.

d: According to the severity of toxemia and time of gestation it could be variable.

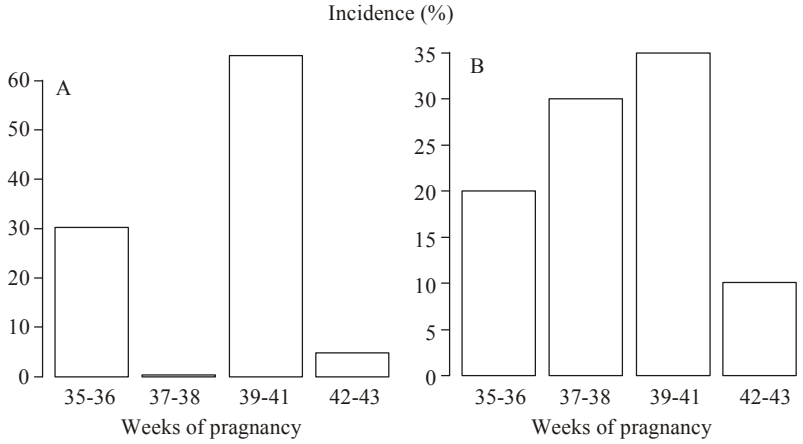

Fig. 1: Weeks of highest incidence for developing preeclampsia and hypertension in preeclamptic women (A) and normal pregnant women (B), respectively

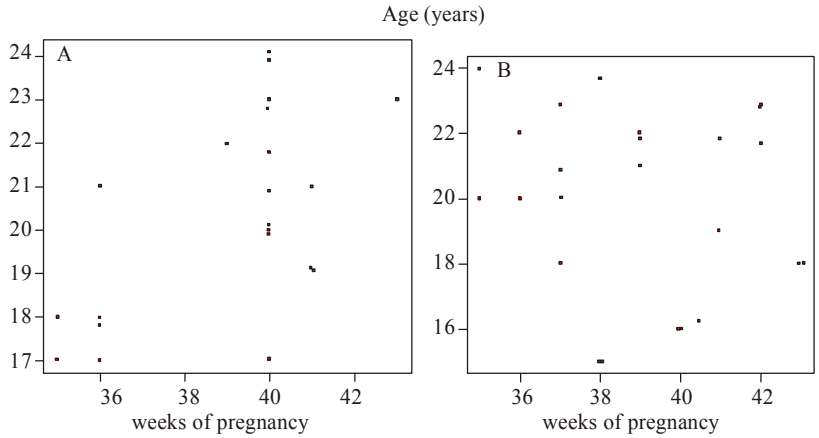

Fig. 2: Weeks of highest incidence for developing preeclampsia and hypertension in preeclamptic women (A) and normal pregnant women (B), respectively, between 17 and 24 years of age

Fig. 1 shows the pregnancy weeks of highest risk for developing preeclampsia (A) and hypertension (B) in preeclamptic and normal pregnant women, respectively.

Fig. 2 shows the weeks of pregnancy with the highest risk for developing preeclampsia (A) and hypertension (B) in mothers of age between 17 and 24 years.

DISCUSSION

It is remarkable the high incidence of preeclampsia between weeks 39 and 41 of gestation (Fig.1).

The data shown here demonstrate that preeclamptic patients have increased polyamine oxidase activity compared to normal pregnant women Table I). In normal pregnancy, hypertension appears uniformly during the third trimester. The clinical values from preeclamptic women demonstrate a tendency toward haemoconcentration (high hemoglobin concentration) that cause high blood viscosity, which results in both compromised oxygen delivery to tissues and cerebrovascular complications [15]. We did not found a significant difference between the infants weight of preeclamptic women and normal pregnant women, but it is known to be variable because of the severity of preeclampsia, time of gestation and mothers physiological response. In literature it is reported that the birth weight of infants with preeclamptic mothers can be 66% less than infants with healthy mothers.

It is known that alteration of glomerular endothelial cell morphologic features is the most consistent pathologic abnormality found in preeclamptic patients [16]. A study revealed that acrolein is a major toxic compound produced from spermine and spermidine by polyamine oxidase and that acrolein is accumulated in plasma of patients with chronic renal failure [17]. Furthermore, it was found that the main amine oxidase producing acrolein from spermine and spermidine is polyamine oxidase [17]. These results suggest that if preeclamptic women had increased polyamine oxidase levels and renal abnormalities, they may accumulate acrolein and possibly developed uraemia. Acrolein also could be capable of reacting with amino groups of proteins which can then rearrange to form polyamine end products (PEs) structurally similar to advanced glycation end products (AGEs) [18]. This hypothesis is supported in a study that shows that AGE´s accumulate markedly in the plasma and collagenous tissues in normoglycaemic uraemic patients [19-20]. Also shows that serum pentosidine and N∈-(carboxymethyl)-L-lysine, were elevated in haemodialysis patients several times above those of normal subjects and non-uraemic diabetic patients [20].

It is known that normal level of urea helps maintain the metabolic equilibrium of nitrogen in human body [21]. Recently, it was suggested by our Laboratory that urea can be used as a glycation protector [22]. Due to the renal abnormalities in preeclampsia, the urea levels in blood may be increased, this is known as azotemia [23]. It is also known that urea could be capable of reacting with aldehydes forming urea end products (UEs), similar compounds to AGEs [22]. Based on the information that uraemic sera contain either unknown precursor (s) and/or catalyst (s) for the Maillard reaction [24], we can suspect on the formation of polyamine end products (PEs) and UEs in preeclampsia, however, it remains to be demonstrated.

In summary, the relevance of our findings is that increased activity of polyamine oxidase in preeclamptic patients helps to formulate new hypothesis about the pathophysiology of preeclampsia. We consider that more research is urged to confirm this information.

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