

Comparison between umbilical artery and vein endogenous digoxin-like immuno-active factor levels in normal and pre-eclamptic patients

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Summary

Recent studies have pointed to the existence of an endogenous digoxin-like immuno-active factor (DLIF), which may be associated with hypertension and pre-eclampsia. The DLIF levels in the umbilical venous and umbilical arterial blood of neonates, as well as the maternal serum of primigravidas and multigravidas with and without pre-eclampsia, were determined by means of a commercially available radioimmunoassay kit, which is cross-reactive with DLIF, in 44 mothers and their babies in search for a possible placental, fetal or maternal origin of the DLIF. The mean placental and neonatal masses were significantly lower in the pre-eclampsia group than in the control group ($P < 0,01$). However, the DLIF levels in the maternal serum, umbilical cord venous and umbilical cord arterial serum were statistically significantly higher in the pre-eclampsia group than in the control pregnant group ($P < 0,05$). A very strong correlation was found between umbilical cord venous and arterial DLIF levels ($r = 0,90$; $P = 0,001$, Spearman rank-correlation coefficient). Although the mean DLIF level in cord arterial serum was lower than that of cord venous serum, statistical significance was not reached if the Bonferroni adjustment was applied to the P value.

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Recent studies have indicated the existence of an endogenous digoxin-like immuno-reactive factor (DLIF).^{1,2} There are interesting indications that this DLIF could be involved in the pathophysiology of essential hypertension³ and it may also be associated with pre-eclampsia.⁴

The presence of a DLIF has been demonstrated during oral salt loading⁵ in patients with renal impairment, in newborn infants, during the third trimester of pregnancy,² and also in patients with active acromegaly.⁶

DLIFs in plasma from hypertensive patients have been shown to inhibit the sodium-potassium adenosine triphosphatase (ATPase) of normal blood vessels, red cells and white cells.⁷ Inhibition of sodium-potassium ATPase and the sodium-potassium pump has also been reported at tissue and cellular levels in patients with low-renin hypertension.^{7,8}

Various tissues have been reported to contain DLIF, e.g. extracts of mammalian brain,⁹ rat adrenal tissue,¹⁰ and placental tissue.¹¹

The hypothalamus, pituitary and possibly the adrenal glands, under the influence of the hypothalamus, have been postulated as being the source of the DLIF.^{6,8,12}

In this study, DLIF levels in the umbilical vein, umbilical artery and maternal serum in patients with and without pre-eclampsia at delivery were measured in a search for a possible placental, fetal or maternal origin of the DLIF. This is the first study, to the authors' knowledge, investigating both the venous and arterial umbilical cord blood levels of DLIF.

Subjects and methods

From the patients who delivered at Tygerberg Hospital, 44 were selected at random for this study approximately proportional to the prevalence of pre-eclampsia in the primi- and multigravida populations. The study was approved by the Ethical Committee of the University of Stellenbosch and informed consent was obtained from all subjects. Blood samples were obtained by catheterising the umbilical vein and artery separately at delivery, and from a peripheral maternal vein immediately after delivery. All samples were collected in glass tubes and refrigerated. Serum digoxin levels were determined within 72 hours after collection.

According to clinical data collected during labour and from their antenatal records, patients were categorised into four groups, viz.: primigravidas with and without pre-eclampsia and multigravidas with and without pre-eclampsia. For some comparisons, the mothers without pre-eclampsia were regarded as a reference or 'control' group, and those with pre-eclampsia as the 'pre-eclampsia' group.

In order to make the diagnosis of pre-eclampsia, the blood pressure had to be 140/90 mmHg or more on two occasions at least 6 hours apart and accompanied by 150 mg/l proteinuria or more and/or oedema. None of the patients were on digoxin therapy, and except for those who were diagnosed as having pre-eclampsia, all were considered to be healthy.

A commercially available radio-immunoassay from Clinical Assays (Cambridge, Massachusetts, USA) was performed in accordance with the manufacturer's instructions as described in local studies done previously on the different available commercial kits.^{4,11} A Packard Autogamma counter was used to determine the radio-activity of the iodine-125-labelled digoxin. Control sera were used to prepare standard curves from which digoxin levels ranging from 0 ng/ml to 4 ng/ml could be determined automatically by the microprocessor of the gamma counter. None of the patients had been receiving digoxin, and the determined digoxin level was accepted to be caused by the immunoreactive cross-reacting substance.

Statistical methods

Since it is known that fetal DLIF levels correlate inversely with gestational age and birth weight,¹⁸ the DLIF levels in maternal serum, cord venous and cord arterial blood were adjusted for gestational age by using the 50th percentile of the birth-weight charts for gestational age, sex and primi- or multigravidas of Keen and Pearse,¹³ and Jaroszewicz *et al.*¹⁴

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Dividing the three DLIF variables by the median weight for the gestational age gave DLIF levels/g median weight for gestational age.

The Spearman correlation coefficient was used to test for association between pairs of continuous variables. The Wilcoxon rank-sum test was used to test for differences between mean values of two groups while the paired *t*-test was used in the case of paired data. The Bonferroni adjustment was applied to the *P* value when multiple comparisons were made.

Results

Clinical data

The results obtained are shown in Table I. Pre-eclampsia occurred in 11 patients. As was to be expected, gestational age at birth was significantly lower in the pre-eclampsia group ($34,5 \pm 5,2$ weeks) than in the control group ($38,9 \pm 2,8$ weeks) (Table II).

DLIF data

Although the mean mass of the babies (1994 ± 913 g v. 2993 ± 651 g) as well as the placental mass (410 ± 115 g v. 529 ± 142 g) in the pre-eclampsia group were significantly lower than in the control group, the DLIF levels in the maternal serum, cord venous and cord arterial blood were significantly higher in the pre-eclampsia group when adjusted for gestational age of baby (Table III).

A moderate correlation was found between maternal venous and cord venous DLIF levels ($r = 0,4784$; $P = 0,001$), while cord venous and cord arterial DLIF levels showed a strong association ($r = 0,89756$; $P = 0,0001$ Spearman rank-correlation coefficient).

Although the mean DLIF levels in cord arterial serum were lower than that of cord venous serum (Table III), statistically significant differences could not be demonstrated when the Bonferroni adjustment was applied to statistically significant results obtained using Student's paired *t*-test.

TABLE I. CLINICAL MEASUREMENTS AND DLIF LEVELS (MEAN \pm SD) IN THE FOUR GROUPS

	Group I (N = 13)	Group II (N = 7)	Group III (N = 20)	Group IV (N = 4)
Maternal age (yrs)	20,1 \pm 2,6	19,3 \pm 2,3	27,2 \pm 4,02	30,25 \pm 7,6
Gestational age (wks)	38,0 \pm 4,1	36,3 \pm 3,6	39,4 \pm 1,4	31,3 \pm 6,4
Systolic blood pressure (mmHg)	128 \pm 10	160 \pm 12	112 \pm 10	155 \pm 17
Diastolic blood pressure (mmHg)	74 \pm 8	106 \pm 11	70 \pm 9	110 \pm 12
Mass of baby (g)	2 925 \pm 702	2 187 \pm 722	3 037 \pm 631	1 655 \pm 1223
Placental mass (g)	530 \pm 126	500 \pm 61,9	528 \pm 154	288 \pm 70
DLIF level of mother (ng/ml)	0,66 \pm 0,28	0,81 \pm 0,17	0,81 \pm 0,71	0,71 \pm 0,48
Cord venous DLIF (ng/ml)	1,25 \pm 0,26	1,49 \pm 0,46	1,43 \pm 0,44	2,02 \pm 0,45
Cord arterial DLIF (ng/ml)	1,19 \pm 0,30	1,44 \pm 0,55	1,44 \pm 0,48	1,90 \pm 0,56

Group I = primigravidas without pre-eclampsia; group II = primigravidas with pre-eclampsia; group III = multigravidas without pre-eclampsia; group IV = multigravidas with pre-eclampsia.

TABLE II. CLINICAL MEASUREMENTS (MEAN \pm SD) IN CONTROL AND PRE-ECLAMPSIA GROUPS

Measurement	Control (N = 33)	Pre-eclampsia (N = 11)	<i>P</i> -value (Wilcoxon rank sum test)
Maternal age (yrs)	24,4 \pm 5,0	23,3 \pm 7,0	NS
Gestational age (wks)	38,9 \pm 2,8	34,5 \pm 5,2	0,016
Systolic blood pressure (mmHg)	118,0 \pm 13,0	158,0 \pm 13,0	0,001
Mass of baby (g)	2 993,0 \pm 651,0	1 994,0 \pm 913,0	0,002
Mass of placenta (g)	529,0 \pm 142,0	410,0 \pm 115,0	0,009

Control = primi- and multigravidas without pre-eclampsia; pre-eclampsia = primi- and multigravidas with pre-eclampsia.

TABLE III. DLIF LEVELS (MEAN \pm SD) IN CONTROL AND PRE-ECLAMPSIA GROUPS

	Control (N = 33)	Pre-eclampsia (N = 11)	<i>P</i> -value for values adjusted for gestational age
Maternal serum DLIF (ng/ml)	0,75 \pm 0,58	0,78 \pm 0,30	0,0131
Cord venous DLIF (ng/ml)	1,36 \pm 0,39	1,69 \pm 0,51	0,0142
Cord arterial DLIF (ng/ml)	1,34 \pm 0,43	1,61 \pm 0,58	0,0463

Control = primi- and multigravidas without pre-eclampsia; pre-eclampsia = primi- and multigravidas with pre-eclampsia.

Discussion

The difference in maternal age between the primigravidas and multigravidas is easily explained. The gestational age in the pre-eclampsia group was significantly lower than in the control group, probably because these pregnancies had to be terminated earlier due to the pre-eclampsia.

It is known that DLIF levels in the fetus correlate inversely with gestational age and weight.¹⁵ In this study both the placentas and babies were smaller in the pre-eclampsia groups, but DLIF levels were higher in the pre-eclampsia group. This was compensated for by statistically adjusting for gestational age and mass of the baby. When this was done, maternal serum DLIF levels, cord venous and cord arterial levels were found to be statistically significantly higher in the pre-eclampsia group (Table III).

There is recent evidence for a circulating endogenous inhibitor of the sodium-potassium pump in the plasma of some subjects with low renin hypertension.^{2,3} Immunoreactive DLIF levels were found to be significantly increased in the cord blood of patients with pre-eclampsia, and the levels were related to the severity of the pre-eclampsia.⁴

Reduced sodium-potassium ATPase activity was found in erythrocytes of infants born to pre-eclamptic mothers. This could be due to a substance that suppresses sodium-potassium ATPase activity.¹⁶

These observations suggest the presence of another system besides the renin-angiotensin aldosterone, antidiuretic hormone and atrial natriuretic peptide systems for the regulation of plasma volume and arterial pressure. This has led to a hypothesis regarding the genesis of low-renin volume expanded hypertension. Increased intravascular volume due to impaired excretion of sodium and water by the kidney is sensed by the central circulation and results in the release of a DLIF. Like the cardiac glycosides, this substance increases intracellular sodium, which leads to a diminished calcium efflux from the cells. This increases cardiac contractility, constricts blood vessels and reduces the renal tubular absorption of sodium. The increased blood pressure and reduced sodium reabsorption results in excretion of the excess volume. An agent with this combination of properties would represent an effective way to rid the body of extra salt and water. The increase in blood pressure would deliver more salt and water to the renal tubule, from which they would be excreted.

This agent would also help to explain why populations prone to low-renin hypertension (e.g. blacks and elderly people), who excrete a sodium load more slowly than normal when they are normotensive, excrete the load more rapidly than normal when they are hypertensive.¹⁵

There is evidence for hypothalamopituitary-adrenal regulation of body fluid balance. A study in patients with active acromegaly showed return of very high levels of sodium pump inhibitor to control values after adenomectomy.⁶ Experimental lesions of the hypothalamus decrease DLIF levels in the blood.³ Data recently published¹⁷ indicate that linoleic and oleic acids act as endogenous sodium-potassium ATPase inhibitors. These compounds were isolated and identified from

porcine plasma after the pigs had undergone volume-expansion experiments.

In our study, the other possible source of DLIF would be the placenta, as supernates from homogenates of human placental tissue at term have been shown¹⁸ to contain almost three times as much DLIF/g protein as normal human serum. Measuring umbilical cord arterial and venous DLIF levels in our study, we could not demonstrate any consistent difference between the DLIF levels in the serum originating from the fetus and placenta, respectively. It is therefore impossible to suggest a predominant fetal or placental source of origin.

The precise structure of DLIF is not yet known but the substance seems to be associated with pre-eclampsia, and hypotheses about the source and action of DLIF can be tested by further studies.

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REFERENCES

- Valdes R jun. Endogenous digoxin-immunoreactive substance measured in several patient populations. *Prog Clin Biol Res* 1985; **192**: 221-228.
- Valdes R jun. Endogenous digoxin-immunoreactive factor in human subjects. *Fed Proc* 1985; **44**: 2800-2805.
- Haddy FJ, Pamnani MB. Evidence for a circulating endogenous Na⁺-K⁺ pump inhibitor in low-renin hypertension. *Fed Proc* 1985; **44**: 2789-2794.
- Odendaal HJ, Beyers AD, Van Heyningen CF, Spruyt LL, Kotze TJVW, Van Jaarsveld PP. Immunoreactive digitalis-like substance in pre-eclampsia. *S Afr Med J* 1986; **70**: 535-537.
- Klingmuller D, Weiler E, Kramer HJ. Digoxin-like natriuretic activity in the urine of salt-loaded healthy subjects. *Klin Wochenschr* 1982; **60**: 1249-1253.
- Deray G, Rieu M, Devynck MA *et al.* Evidence of an endogenous digitalis-like factor in the plasma of patients with acromegaly. *N Engl J Med* 1987; **316**: 575-580.
- Pamnani MB, Clough DL, Huot SJ, Haddy FJ. Sodium-potassium pump activity in experimental hypertension. In: Vanhoutte PM, Leusen I, eds. *Vasodilatation*. New York: Raven Press, 1981: 391-403.
- De Wardener HE, Clarkson EM. Concept of natriuretic hormone. *Physiol Rev* 1985; **65**: 658-759.
- Lichtstein SD, Samuelov S. Membrane potential changes induced by the ouabain-like compound extracted from mammalian brain. *Proc Natl Acad Sci USA* 1982; **79**: 1453-1456.
- Schreiber V, Stepan J, Gregorova I, Krejciikova J. Crossed digoxin immunoreactivity in chromatographic fractions of rat adrenal extract. *Biochem Pharmacol* 1981; **30**: 805-806.
- Beyers AD, Spruyt LL, Seifart HI, Kriegler A, Parkin DP, Van Jaarsveld PP. Endogenous immunoreactive digitalis-like substance in neonatal serum and placental extracts. *S Afr Med J* 1984; **65**: 878-882.
- Wijedicks EFM, Vermeulen M, Van Brummelen P, Den Boer NC, Van Gijn J. Digoxin-like immunoreactive substance in patients with aneurysmal subarachnoid haemorrhage. *Br Med J* 1987; **294**: 729-732.
- Keen DV, Pearse RG. Birthweight between 14 and 42 weeks' gestation. *Arch Dis Child* 1985; **60**: 440-446.
- Jaroszewicz AM, Schuman DEW, Keet MP. Intra-uterine groeistandaarde van Kaapse kleurlingbabas. *S Afr Med J* 1975; **49**: 568-572.
- Haddy FJ. Endogenous digoxin-like factor or factors. *N Engl J Med* 1987; **316**: 621-622.
- Kuhnert BR, Kuhnert PM, Murray BA, Sokol RJ. Na/K and Mg-ATPase activity in the placenta and in maternal and cord erythrocytes of pre-eclamptic patients. *Am J Obstet Gynecol* 1977; **127**: 55-60.
- Tamura M, Kuwano H, Kinoshita T, Inagami T. Identification of linoleic and oleic acids as endogenous Na⁺, K⁺-ATPase inhibitors from acute volume-expanded hog plasma. *J Biol Chem* 1985; **260**: 9672-9677.
- Valdes R jun. Endogenous digoxin-like immunoreactive factors: impact on digoxin measurements and potential physiological implications. *Clin Chem* 1985; **31**: 1525-1532.