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Plasma levels of digitalis-like substance in pregnancy-induced hypertension in Nigeria

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Summary

Digitalis-like substance or plasma inhibitor factor was measured by a competitive binding method in 56 subjects comprising:

(1) 12 normotensive normally menstruating non-pregnant women without a family history of hypertension;
(2) 13 normotensive normally menstruating non-pregnant women with a family history of hypertension;
(3) 15 normotensive pregnant subjects without a family history of hypertension; and
(4) 16 subjects with clinical features of pregnancy-induced hypertension.

Even though the mean value (1.14 ± 0.12) of the inhibitor factor (expressed as K_D ratios) in the non-pregnant women with family history was slightly higher than the mean value (0.95 ± 0.12) in the non-pregnant women without a family history, the difference did not attain statistical significance. The mean value (1.25 ± 0.24) of the inhibitor factor in the pregnant subjects without family history was, however, significantly elevated when compared with the non-pregnant subject without family history ($P < 0.05$). The inhibitor factor levels in the subjects with pregnancy-induced hypertension were generally lower than those of the normotensive pregnant subjects, although the difference was not statistically significant. The relevance of these results to the understanding of the pathophysiology of pregnancy-induced hypertension is discussed.

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Résumé

Une substance ressemblant le digitale ou le facteur inhibiteur de plasma était mesuré par une méthode de concurrence dans cinquante-six sujets y compris:

(1) 12 femmes avec les règles normales, une tension artérielle normale, sans grossesse et sans une histoire familiale de trouble de tension artérielle;
(2) 13 femmes avec les règles normales, une tension artérielle normale, sans grossesse, mais avec une histoire familiale de trouble de tension artérielle;
(3) 15 femmes enceintes avec une tension artérielle normale et sans une histoire familiale de trouble de tension artérielle; et
(4) 16 sujets avec les troubles de tension artérielle provoquée par la grossesse.

Bien que la valeur moyenne (1.14 ± 0.12) de facteur inhibiteur (exprimé comme K_v) dans les femmes sans grossesse mais avec une histoire familiale était un peu plus haut que la valeur moyenne (0.95 ± 0.12) des femmes sans grossesse et sans une histoire familiale, la différence n'a pas atteint la signification statistique. La valeur moyenne (1.25 ± 0.24) du facteur inhibiteur dans les sujets enceintes et sans d'histoire familiale était néanmoins d'une signification statistique en comparaison avec les sujets sans grossesse et sans histoire familiale ($P < 0.05$). La valeur moyenne du facteur inhibiteur dans les sujets avec les troubles de tension artérielle provoquée par la grossesse était généralement plus bas que celle des femmes enceintes avec les tensions normales bien que la différence n'était pas statistiquement importante. L'importance de ces résultats pour comprendre la pathophysiologie des

troubles de tension artérielle provoquée par la grossesse, est discutée.

Introduction

Pregnancy-induced hypertension (PIH) still constitutes a source of significant maternal morbidity and mortality in Nigeria [1–3]. The pathogenesis of this condition is said to be multifactorial and previous works have implicated immunological [4,5] and endocrinological factors [6,7]. Despite the impressive amount of literature that exists on the endocrine aspects of this disease, the direct contribution of mineralocorticoids and probable consequence of disturbances of fluid and electrolyte metabolism are still poorly defined.

Plasma Na^+–K^+ ATPase inhibitor activity has been shown to be a sensitive index of plasma volume, i.e. the index of activity is high in expanded states and a reduction in activity has been demonstrated in dynamic contraction of the plasma volume with treatment [8]. Furthermore, the change in fluid balance in this clinical syndrome is intriguing as evidence derived from direct measurement of plasma volume indicates a contraction in the extracellular fluid compartment [9,10]. Thus this study was designed to examine the role of volume factors as indicated by the Na^+–K^+ ATPase inhibitor levels in the pathogenesis of PIH.

Subjects and methods

Fifty-six female subjects were recruited into the study, comprising:

(1) 12 normotensive normally menstruating non-pregnant subjects without a family history of hypertension;
(2) 13 normotensive normally menstruating non-pregnant women with a family history of hypertension, i.e. one or both parents had received or was then receiving medical attention for essential hypertension;

(None of the patients in groups (1) and (2) was exposed then or in the recent past to steroidal contraception.)

(3) 15 normotensive pregnant subjects without a family history of hypertension; and
(4) 16 subjects with clinical features of PIH, i.e. increased blood pressure (diastolic BP 90 mmHg), pedal oedema and proteinuria (Albustics, Ames Co). (Only two of these had a family history of hypertension.)

Preparation of plasma samples

Venous blood was collected into ice-chilled heparinized tubes. The samples were centrifuged initially at 3000 *g* to separate the plasma; the red cells and buffy coat were discarded and the plasma was immediately boiled in a water bath for 15 min. The protein coagulum was broken into small fragments and the resulting mixture was again centrifuged at 5000 *g* for 15 min. The supernatant was separated and stored at −80°C for processing.

[^{3}H]Ouabain binding to erythrocytes

The binding experiments were performed according to the methods described by Devynck *et al.* [11], using fresh red blood cells obtained from subjects without a family history of hypertension. Such red cells were washed using a Tris–HCl buffer made up to an haematocrit of between 1 and 3%. The cells were then incubated for 5 h at 37°C in the presence of five different concentrations of [^{3}H]ouabain ranging from 2×10^{-9m} to 2.5×10^{-8m}. Non-specific (non-saturable) binding was also assessed by running parallel assays in the presence of an excess of unlabelled ouabain. Bound radioactivity was separated from free radioactivity using a Millipore filtration system (Whatman GFC filters). The filters were subsequently dried and the retained radioactivity counted.

Binding parameters, apparent affinity and number of binding sites were determined by computer analysis of Scatchard plots. In studying our subjects, binding experiments were carried out in the presence of plasma extracts and the K_D values were derived. The ability of the plasma extracts to compete with ouabain was quantified by the K_D1/K_D ratio where K_D1 and K_D represent the apparent affinities of ouabain for the sites in the presence and absence of plasma, respectively.

Determination of serum electrolytes and creatinine

Serum and urinary Na^+ were estimated by

routine flame photometric methods while serum bicarbonate and creatinine were measured by standard auto-analyser methods.

Statistical analysis

The difference between the means of the K_D ratios was determined by analysis of variance and the P values checked from standard statistical tables. The level of statistical significance was defined as $P < 0.05$. Results are expressed as means ± s.d. unless otherwise stated.

Results

The K_D ratios of the four groups of patients are as shown in Fig. 1. The mean of the K_D ratios in the non-pregnant females with a family history of hypertension was 1.14 ± 0.12 which was higher than in those without a family history (0.95 ± 0.12). The difference was, however, not statistically significant. The spread of the values in the normotensive pregnant subjects (mean 1.25 ± 0.24) was similar to that of non-pregnant subjects with a family history of hypertension and there was no statistical difference in their mean values. However, the mean of the values in the normotensive pregnant subjects was statistically higher than in the normotensive non-pregnant subjects without a family history of hypertension.

In the group of subjects with PIH there was a trend to low K_D ratio values and the mean of the values in these subjects was 1.16 ± 0.22. Even though the scatter was in the low range compared to the control groups, especially the pregnant subjects without PIH, the differences of the mean were not statistically significant.

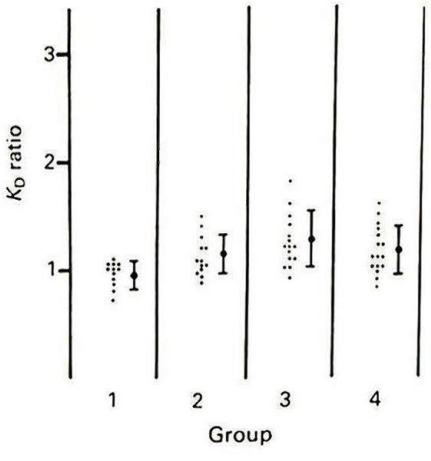

Fig. 1. Scattergrams representing the different groups of subjects studied (see text for details). The dots represent the values of the individual K_D ratios while the parallel line with a central dot stands for the mean and the standard deviation. On analysis of variance the only significant difference lies between Group 1 (non-pregnant normotensive subjects without family history) and Group 3 (pregnant normotensive subjects without family history) ($P < 0.05$).

Discussion

The progressive increase in plasma volume which is maximal in the second trimester of pregnancy is a well recognized phenomenon [12,13]. This marked increase in circulating fluid has been attributed in part to various factors, especially increases in the biosynthesis and release of aldosterone [14], deoxycorticosterone (DOC) and DOC sulphate (DOC-SO_4) [15].

Presumably the resultant dynamic changes in fluid and electrolyte balance are necessary in normal pregnancy. The expected K^+ wastage which should accompany hyperaldosteronism is probably prevented by the high levels of progesterone which are also secreted in large amounts during pregnancy [16]. In concert with these changes in the steroid metabolism are marked changes in the renin–angiotensin interaction [17]. There are massive increases in plasma renin activity reported during pregnancy [18]. Fortunately, the vessel reactivity to pressor agents, specifically angiotensin II [19], have been shown to be reduced which has the net effect of vasodilatation and consequent normotension.

Converse to the above changes which have been described in normal pregnancy, the observations in patients with PIH are different. The levels of aldosterone are found to be lower in PIH [20]. The role of circulating progesterone in the genesis of PIH is not clearly defined as existing literature conflicts. The studies by Klopper *et al.* [21] showed that there were significant differences in the levels of serum progesterone in PIH when compared to normal

pregnancy. However, the ability of the vessels to react to pressor agents is preserved in the patients, which to an extent might explain the increased peripheral resistance encountered in these patients with the consequences of hypertension. The relatively low aldosterone encountered in this situation compared to normal cyesis may also contribute to the contraction of plasma volume which has been reported in PIH. Direct plasma volume measurements in patients with PIH show that there is a relative decrease in plasma volume when compared to normotensive controls [9].

Previous attempts at measurement of inhibitory factor in subjects with PIH have produced conflicting results. The most recent report by Gudson *et al.* [22] seems to indicate an increase in the levels of PIF in these subjects. This is rather paradoxical, especially as it would seem from other studies that the inhibitory factor mirrors the state of the ECF and in the dynamic situation of rapid reduction of ECF there is also a demonstrable significant reduction in PIF [8].

In our present study the values of inhibitory factor in our subjects with PIH are not significantly different from those of our normotensive controls. We expect that the plasma volumes in our patients would be significantly lower than in the controls but this is not reflected in the PIF levels. The relative difference in the results obtained from different studies is probably a consequence of the different methods employed. However, the results seem to suggest a dissociation of the physiological relationship between the plasma volume and the inhibitory factor.

We speculate that longitudinal studies in patients with PIH would demonstrate that with effective treatment there would be predictable changes in inhibitory factor levels. This is currently being investigated and assessed as an adjunct for monitoring treatment in these patients.

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