Use of Curves for Prediction of Maternal Blood Levels of Placenta-Specific Substances for Diagnosis of Placental Function

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In evaluating placental function in a large number of pregnant women visiting the obstetric outpatient clinic, it is necessary that the test is simple and inexpensive as much as possible and the judgment is easy and precise as well. In order to meet this requirement, placenta-specific substances appearing in the maternal peripheral blood have been determined predominantly, because of their easy sampling, to make their levels serve as a yardstick. But most of the substances showing their normal ranges outstandingly wide, it is often inadequate to judge the placental function merely on the basis of their maternal blood levels high or low. Having developed a favorable system of predicting individual levels of heat-stable alkaline phosphatase (HSAP) on curves, we tried in the present study to ascertain whether the same system could be applied favorably as well to other placenta-originating substances; placental function test; prediction curves
MATERIALS AND METHODS

The subjects were 40 pregnant women who were available for retrospective tracing of a normal course of pregnancy out of all the women seen in the Obstetric Outpatient Clinic, Nara University Hospital, during an 8-month period between January and August, 1977. In the 40 women, the blood was sampled 200 times in total, some 5 ml at a time in the morning between 10 a.m. and 12 a.m., in the third trimester from the 29th to the 42nd week of gestation. In each of these blood samples, serum was promptly isolated to a final total of 200 normal specimens, each being kept at -25°C. Immediately after its melting, the subject substances contained in it were determined before their activities underwent changes. The same procedure was also applied to six women of abnormal pregnancy seen during the same period to provide a normal-abnormal contrast in the test.

The substances determined were CAP, HPL and SP-1. Besides these, HSAP and leucine aminopeptidase (LAP) conventionally used in our routine tests were also measured at the same time.

Determinations were made for CAP by a modification of the method of Watson and Gibbard (1973) using CAP Test Pack® Kits (Sankyo Co.) with S-benzyl-l-cysteine-P-nitroanilide as the substrate, with the value expressed in terms of mU/ml; for HPL by 125I-labeled solid phase radioimmunoassay Kits (Green Cross Co.), the value expressed in μg/ml; and for SP-1 by the single radial immunodiffusion method on the M-Partigen plate (Hoechst-Japan Co.), the value expressed in mg/100 ml. The values of HSAP and LAP were determined on Kits (Ishizu Chem. Co.) and expressed in K-A unit and G-R unit, respectively.

The values of each substance were arranged to obtain their mean and standard deviation for each gestation week, and these were further put to regression analysis. The theoretical grounds for the calculation have been reported elsewhere (Yamaguchi and Shimozato 1978). The weeks of pregnancy were expressed in terms of decimal notation. The mean value for a week was used as the value on the middle day of the week.

RESULTS

Relations between pregnancy weeks and serum levels of CAP, HPL and SP-1

The values of CAP, HPL and SP-1 obtained in the blood of the normal women in the third trimester of pregnancy are presented in Tables 1–3. All these marker substances determined were CAP, HPL and SP-1. Besides these, HSAP and leucine aminopeptidase (LAP) conventionally used in our routine tests were also measured at the same time.

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<table>
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<th>Weeks of gestation</th>
<th>Number of cases</th>
<th>Mean values (mU/ml)</th>
<th>Standard deviation</th>
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substances gradually elevated their levels with the progress of gestation. But, similarly to the case of HSAP, their values individually varied intractably, refusing establishment of general ranges of normality. Drawing to term, however, their elevation in level invariably became dull, finally turning to decline. The start of deviation from exponential elevation (critical point), as identified on the calculations presented in the next section, occurred for CAP, HPL and SP–1 at 37.9, 34.4 and 38.2 weeks of gestation, respectively, while that for HSAP appeared as in the preceding report at 38.7 weeks.

**Expressions for predicted values of marker substances**

A review of the changes in blood level of the three marker substances as identified from the values presented in Tables 1–3 revealed a feature that all of the three had rates of elevation in level almost identical with one another, independent
of the differences in height of the level among them, and alike showed their levels attaining peaks, though somewhat dissimilar in time, then turning to decline.

Their rates of elevation in level can be indicated by applying the following general expressions of natural logarithms:

\[
CAP: \log_{e} y = 0.3345 + 0.0147a + 0.953x \\
HPL: \log_{e} y = 0.1749a - 0.5763 + 0.0676x \\
SP-1: \log_{e} y = 0.19475a - 1.59559 + 0.0677x \\
\]

For HSAP and LAP, the expressions were, as previously reported, \( \log_{e} y = 1.03a - 3.37 + 0.123x \), and \( \log_{e} y = (1.418 + 0.452a) + (0.02a - 0.053x) \), respectively. On the other hand, their deviations from exponential rise after the critical point can all be expressed by a parabolic curve formula, \( y = Y(1 + d/100) \). Of the denotations in these expressions, \( x \) represents gestation weeks in decimal notation, \( y \) the predicted value of the substance, \( a \) the constant inherent in each individual woman as decided by the values \( y \) in arbitrary weeks, \( Y \) the predicted level presumed to be rising after the critical point, and \( d \) the deviation from exponential rise exclusively applicable after the critical point. In the parabolic curve formula, \( d \) for CAP is \( -d = 1.82(x - 37.9)^2 \); for HPL, \( -d = 576.31 - 35.33x + 0.54x^2 \); and for SP-1, \( -d = 213.48 - 18.16x + 0.33x^2 \).

Fig. 1. Prediction curve of serum CAP.
To avoid intricacy of performing calculations each time for prediction of values based on the described expressions, an alternatively convenient and easily practicable step was introduced.

Starting with the 30th week of gestation, a few weeks' values of the marker substance were measured in each individual woman, and following their shifting trend, a curve was drawn and extended in advance to predict on it later levels as illustrated in Figs. 1-3. In the figures, the dotted line denotes the level assumed to be rising after the critical point. This is to be used to help check spurious prolonged pregnancy. In women proving pregnancy, delivery and newborn all normal, the largest deviation of prediction from measured values of the substances remained within an allowable error range of ±15% in 85-90% of the cases.

Curves for prediction of values of marker substances

For practical use of this chart, the values of the marker substance will be measured initially in the 30th through the 32nd week of gestation, then the measured values in a row will be plotted on the extended curve in the chart. That will be enough to identify predicted values at later weeks (full and decimal notation).
on the curve as far as the pregnancy remains normal. In abnormal pregnancy, as shown in the figures, the actually measured values excessively deviated from the predicted values.

**Discussion**

Various phenomena in pregnancy, variable with time, should be interpreted dynamically. Placental function, the most typical of those, can never be judged adequately by a single determination of any of placenta-specific substances in the maternal blood, because it is difficult to ascertain whether the value at a certain point indicates one on the elevating course or another in the declining process, not allowing of linking it to the state of the placenta. In addition, as described in our preceding report on HSAP, the values of placenta-originating substances individually vary so outstandingly in the maternal peripheral blood that their levels, high or low, could not be related to favorable or unfavorable placental function. Ensuring that the normal range of the values of a marker substances is in no way identifiable in general pregnancy, but it can be indicated on an individual basis in each pregnant woman, we have been insisting that the pattern of shift in the individual level of a marker substance in the maternal blood would be emphasized as
Diagnosis of Placental Function

significantly helpful to the diagnosis of individual placental function (Yamaguchi et al. 1968). In succession to the preceding study substantiating our view in the example of HSAP, we pursued in the present study the patterns of shift in the maternal blood levels of other placenta-specific substances, CAP, LAP and SP-1, to ascertain whether they could behave like HSAP.

As the result, it has been realized that our argument is alike favorable in these substances. Another feature worthy of note in the findings of the present experiment includes: the tested three marker substances along with HSAP shift in nearly identical patterns in their concentrations in the maternal blood as far as the pregnancy progresses normally, but their critical points (turn from rising to falling in level) are different from one another, and in abnormal pregnancy, these substances not always show changes in level in the same manner. Such phenomena can be considered rather natural in view of the possible difference in their location in the placenta and their dissimilar role in metabolism. But, from another point of view, the difference in behavior of these substances itself should rather be underscored as suggesting abnormal circumstances involving the placenta. The significance of the role of such marker substances in pregnancy mostly remains uncertain except for HPL. However, their dissociations of patterns of shift in maternal blood level in abnormal pregnancy were already pointed out by Aoba et al. (1973) and ourselves (Shimozato and Yamaguchi 1974). It is emphasized that in reference to the predicted curves, analytical investigations performed into the shifting patterns of marker substances and their dissociations along with their quantitation to help evaluate pathological changes of the placenta.

References